IARC MONOGRAPHS

PERFLUOROOCTANOIC ACID (PFOA) AND PERFLUOROOCTANESULFONIC ACID (PFOS)

VOLUME 135

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

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VOLUME 135

This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met in Lyon, France, 7–14 November 2023

LYON, FRANCE - 2025

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

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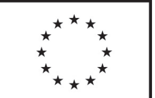
In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic hazard of chemicals to humans, involving the production of critically evaluated monographs on individual chemicals. The programme was subsequently expanded to include evaluations of carcinogenic hazards associated with exposures to complex mixtures, lifestyle factors and biological and physical agents, as well as those in specific occupations. The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for agents to which humans are known to be exposed and on specific exposure situations; to evaluate these data in terms of cancer hazard to humans with the help of international working groups of experts in carcinogenesis and related fields; and to identify gaps in evidence. The lists of IARC evaluations are regularly updated and are available on the internet at https://monographs.iarc.who.int/.

This programme has been supported since 1982 by Cooperative Agreement U01 CA33193 with the United States National Cancer Institute, Department of Health and Human Services. Additional support has been provided since 1986 by the European Commission Directorate-General for Employment, Social Affairs, and Inclusion, initially by the Unit of Health, Safety and Hygiene at Work, and since 2014 by the European Union Programme for Employment and Social Innovation "EaSI" (for further information please consult: https://ec.europa.eu/social/easi). Support has also been provided since 1992 by the United States National Institute of Environmental Health Sciences, Department of Health and Human Services. The contents of this volume are solely the responsibility of the Working Group and do not necessarily represent the official views of the United States National Cancer Institute, the United States National Institute of Environmental Health Sciences, the United States Department of Health and Human Services, or the European Commission.



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About the cover: Worker at a wastewater treatment plant. PFOA and PFOS are ubiquitous in the environment and may contaminate drinking-water.

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NOTE TO THE READER

The evaluations of carcinogenic hazard in the *IARC Monographs on the Identification of Carcinogenic Hazards to Humans* series are made by international working groups of independent scientists. The *IARC Monographs* classifications do not indicate the level of risk associated with a given level or circumstance of exposure. The *IARC Monographs* do not make recommendations for regulation or legislation.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic hazard of an agent to humans is encouraged to make this information available to the *IARC Monographs* programme, International Agency for Research on Cancer, 25 avenue Tony Garnier, CS 90627, 69366 Lyon Cedex 07, or via email at imo@iarc.who.int, in order that the agent may be considered for re-evaluation by a future Working Group.

Although every effort is made to prepare the monographs as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the *IARC Monographs* programme. Corrigenda are published online on the relevant webpage for the volume concerned (IARC Publications: https://publications.iarc.who.int/).

LIST OF PARTICIPANTS

Members [1]

Mohamed A-E. Abdallah

School of Geography, Earth and Environmental Sciences
University of Birmingham
Birmingham
UK

Chad R. Blystone

Division of Translational Toxicology
National Institute of Environmental Health Sciences
Morrisville, North Carolina
USA

Jens Peter Bonde (Subgroup Chair, Cancer in Humans)

University of Copenhagen
Copenhagen
Denmark

Miriam Calkins

United States Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health
Cincinnati, Ohio
USA

Weihsueh Albert Chiu (Subgroup Chair, Mechanistic Evidence)

School of Veterinary Medicine and Biomedical Sciences
Texas A&M University
College Station, Texas
USA

Guang-Hui Dong

School of Public Health
Sun Yat-sen University
Guangzhou
China

[1] Working Group Members and Invited Specialists serve in their individual capacities as scientists and not as representatives of their government or any organization with which they are affiliated. Affiliations are provided for identification purposes only. Invited Specialists do not serve as Meeting Chair or Subgroup Chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations. Each participant was asked to declare potentially relevant research, employment, and financial interests that are current or that have occurred during the past 4 years. Minimal interests are not disclosed here, and include stock valued at no more than US$ 1000 overall, grants that provide no more than 5% of the research budget of the expert's organization and that do not support the expert's research or position, and consulting or speaking on matters not before a court or government agency that does not exceed 2% of total professional time or compensation. All other non-publicly funded grants that support the expert's research or position and all consulting or speaking on behalf of an interested party on matters before a court or government agency are disclosed as potentially significant conflicts of interests.

David Dorman

Department of Molecular Biosciences
North Carolina State University
Raleigh, North Carolina
USA

Rebecca Fry

Gillings School of Public Health
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina
USA

Huan Guo [unable to attend]

Tongji Medical College
Huazhong University of Science and Technology
Wuhan
China

Line Småstuen Haug

Norwegian Institute of Public Health
Oslo
Norway

Jonathan Hofmann

Division of Cancer Epidemiology and Genetics
National Cancer Institute
Bethesda, Maryland
USA

Jane Hoppin (Subgroup Chair, Exposure Characterization)

North Carolina State University
Raleigh, North Carolina
USA

Motoki Iwasaki

Division of Epidemiology
National Cancer Center Institute for Cancer Control
Tokyo
Japan

Jun Kanno (Subgroup Chair, Cancer in Experimental Animals)

National Institute of Health Sciences and
Nissan Tamagawa Hospital
Tokyo
Japan

Miroslav Machala

Department of Pharmacology and Toxicology
Veterinary Research Institute
Brno
Czechia

Francesca Romana Mancini

Paris-Saclay University and
Institut national de la santé et de la recherche médicale (INSERM)
Paris
France

Peter Møller

Department of Public Health
University of Copenhagen
Copenhagen
Denmark

Jack Chakmeng Ng

Queensland Alliance for Environmental Health Sciences
The University of Queensland
Woolloongabba
Australia

Marc Pallardy

Université Paris-Saclay and
Institut national de la santé et de la recherche médicale (INSERM)
Paris
France

Gloria Post

New Jersey Department of Environmental Protection
Trenton, New Jersey
USA

Samira Salihovic

School of Medical Sciences
Örebro University
Örebro
Sweden

Jennifer Schlezinger

School of Public Health
Boston University
Boston, Massachusetts
USA

Anatoly Soshilov

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
Sacramento, California
USA

Kyle Steenland [not present for final evaluations]

Emory University
Atlanta, Georgia
USA

Inger-Lise K. Steffensen

Department of Food Safety
Norwegian Institute of Public Health
Oslo
Norway

Silvya Stuchi Maria Engler

School of Pharmaceutical Sciences
University of São Paulo
São Paulo
Brazil

Volodymyr Tryndyak

National Center for Toxicological Research
United States Food and Drug Administration
Jefferson, Arizona
USA

Alexandra White

National Institute of Environmental Health Sciences
National Institutes of Health
Durham, North Carolina
USA

Susan Woskie

Zuckerberg College of Health Sciences
University of Massachusetts Lowell
Lowell, Massachusetts
USA

Shelia Zahm [2] (Meeting Chair)

National Cancer Institute (retired)
Bangor, Maine
USA

[2] Dr Zahm is employed by Shelia K. Zahm Consulting, but the work conducted by this company does not involve competing interests for the topics covered by the present *IARC Monographs* meeting.

Invited Specialist

Antony Fletcher [3] [not present for final evaluations]

London School of Hygiene and Tropical Medicine
London
UK

Representatives

François Pouzaud

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES)
Maisons-Alfort
France

Andrea Winquist

National Center for Environmental Health
Centers for Disease Control and Prevention
Atlanta, Georgia
USA

Observers [4]

Amina Amadou

Centre Léon Bérard
Lyon
France

Valentin Thomas

Sciences Po Lyon (Institut d'études politiques de Lyon)
Lyon
France

Danny Whu [5]

International Association of Firefighters
Washington, District of Columbia
USA

IARC Secretariat

Nahid Ahmadi
Ayat Ahmadi *(Rapporteur, Cancer in Humans)*
Lamia Benbrahim-Tallaa *(Rapporteur, Cancer in Experimental Animals)*
Wendy Bijoux
Shirisha Chittiboyina *(Rapporteur, Cancer in Experimental Animals)*
Aline de Conti *(Rapporteur, Mechanistic Evidence)*
Caterina Facchin *(Rapporteur, Mechanistic Evidence)*

[3] Dr Fletcher reported providing expert opinion for plaintiffs in cases of exposure to PFOA in drinking-water in Italy and in the USA, and for the Government of Australia in defending against a class action lawsuit on behalf of communities seeking damages for drinking-water contaminated from use of aqueous film-forming foam (AFFF) on military airfields, and for the Government of Jersey in his capacity of member of the PFAS Scientific Advisory Panel.
[4] Each Observer agreed to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers did not serve as Working Group members, draft any part of a monograph, or participate in the evaluations. They also agreed not to contact participants before the meeting, not to lobby them at any time, not to send them written materials, and not to offer them meals or other favours. IARC asked and reminded Working Group members to report any contact or attempt to influence that they may have encountered, either before or during the meeting.
[5] Dr Whu reported receiving support for travel from the International Association of Firefighters.

Federica Madia *(Rapporteur, Mechanistic Evidence)*
Heidi Mattock *(Scientific Editor)*
Mira Merdas
Elisa Pasqual *(Rapporteur, Cancer in Humans)*
Mary Schubauer-Berigan *(Programme Head; Rapporteur, Cancer in Humans)*
Eero Suonio (*Rapporteur, Exposure Characterization)*
Susana Viegas *(Rapporteur, Exposure Characterization)*
Roland Wedekind *(Responsible Officer and Rapporteur, Exposure Characterization)* [6]
Ljubica Zupunski

Administrative and Technical Assistance

Noëmi Joncour
Jennifer Nicholson
Mathieu Rose
Sandrine Ruiz

Production Team

Niree Kraushaar
Solène Quennehen

Pre- and Post-Meeting Assistance

Scott Bartell
Liacine Bouaoun
Mark Cleasby
Nathan DeBono
Fatiha El Ghissassi
Yann Grosse
Misty Hein
John Kaldor
Simone Kühnle
Alison Lees
Karen Müller *(Managing Editor)*

[6] Dr Wedekind reports that his life partner is a salaried employee of the SEB group working for the Rowenta brand. Tefal, a former user of PFOA, is another brand of the SEB group. This was determined not to present a conflict of interest for the present *IARC Monographs* meeting, since Rowenta has no reported involvement with the agents under review, and Tefal ceased using PFOA more than a decade ago.

PREAMBLE

The Preamble to the *IARC Monographs* describes the objective and scope of the programme, general principles and procedures, and scientific review and evaluations. The *IARC Monographs* embody principles of scientific rigour, impartial evaluation, transparency, and consistency. The Preamble should be consulted when reading a *Monograph* or a summary of a *Monograph*'s evaluations. Separate Instructions for Authors describe the operational procedures for the preparation and publication of a volume of the *Monographs*.

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

Soon after the International Agency for Research on Cancer (IARC) was established in 1965, it started to receive frequent requests for advice on the carcinogenicity of chemicals, including requests for lists of established and suspected human carcinogens. In 1970, an IARC Advisory Committee on Environmental Carcinogenesis recommended "that a compendium on carcinogenic chemicals be prepared by experts. The biological activity and evaluation of practical importance to public health should be referenced and documented." The next year, the IARC Governing Council adopted a resolution that IARC should prepare "monographs on the evaluation of carcinogenic risk of chemicals to man", which became the initial title of the series.

In succeeding years, the scope of the programme broadened as *Monographs* were developed for complex mixtures, occupational exposures, physical agents, biological organisms, pharmaceuticals, and other exposures. In 1988, "of chemicals" was dropped from the title, and in 2019, "evaluation of carcinogenic risks" became "identification of carcinogenic hazards", in line with the objective of the programme.

Identifying the causes of human cancer is the first step in cancer prevention. The identification of a cancer hazard may have broad and profound implications. National and international authorities and organizations can and do use information on causes of cancer in support of actions to reduce exposure to carcinogens in the workplace, in the environment, and elsewhere. Cancer prevention is needed as much today as it was when IARC was established, because the global burden of cancer is high and continues to increase as a result of population growth and ageing and upward trends in some exposures, especially in low- and middle-income countries (https://publications.iarc.who.int/Non-Series-Publications/World-Cancer-Reports).

IARC's process for developing *Monographs*, which has evolved over several decades, involves

the engagement of international, interdisciplinary Working Groups of expert scientists, the transparent synthesis of different streams of evidence (exposure characterization, cancer in humans, cancer in experimental animals, and mechanisms of carcinogenesis), and the integration of these streams of evidence into an overall evaluation and classification according to criteria developed and refined by IARC. Since the *Monographs* programme was established, the understanding of carcinogenesis has greatly deepened. Scientific advances are incorporated into the evaluation methodology. In particular, strong mechanistic evidence has had an increasing role in the overall evaluations since 1991.

The Preamble is primarily a statement of the general principles and procedures used in developing a *Monograph*, to promote transparency and consistency across *Monographs* evaluations. In addition, IARC provides Instructions for Authors (https://monographs.iarc.who.int/preamble-instructions-for-authors/), which specify more detailed working procedures. IARC routinely updates these Instructions for Authors to reflect advances in methods for cancer hazard identification and accumulated experience, including input from experts.

2. Objective and scope

The objective of the programme is to prepare, with the engagement of international, interdisciplinary Working Groups of experts, scientific reviews and evaluations of evidence on the carcinogenicity of a wide range of agents.

The *Monographs* assess the strength of the available evidence that an agent can cause cancer in humans, based on three streams of evidence: on cancer in humans (see Part B, Section 2), on cancer in experimental animals (see Part B, Section 3), and on mechanistic evidence (see Part B, Section 4). In addition, the exposure to each agent is characterized (see Part B, Section 1). In this Preamble, the term “agent” refers to any chemical, physical, or biological entity or exposure circumstance (e.g. occupation as a painter) for which evidence on the carcinogenicity is evaluated.

A cancer *hazard* is an agent that is capable of causing cancer, whereas a cancer *risk* is an estimate of the probability that cancer will occur given some level of exposure to a cancer hazard. The *Monographs* assess the strength of evidence that an agent is a cancer hazard. The distinction between hazard and risk is fundamental. The *Monographs* identify cancer hazards even when risks appear to be low in some exposure scenarios. This is because the exposure may be widespread at low levels, and because exposure levels in many populations are not known or documented.

Although the *Monographs* programme has focused on hazard identification, some epidemiological studies used to identify a cancer hazard are also used to estimate an exposure–response relationship within the range of the available data. However, extrapolating exposure–response relationships beyond the available data (e.g. to lower exposures, or from experimental animals to humans) is outside the scope of *Monographs* Working Groups (IARC, 2014). In addition, the *Monographs* programme does not review quantitative risk characterizations developed by other health agencies.

The identification of a cancer hazard should trigger some action to protect public health, either directly as a result of the hazard identification or through the conduct of a risk assessment. Although such actions are outside the scope of the programme, the *Monographs* are used by national and international authorities and organizations to inform risk assessments, formulate decisions about preventive measures, motivate effective cancer control programmes, and choose among options for public health decisions. *Monographs* evaluations are only one part of the body of information on which decisions to control exposure to carcinogens may be based.

Options to prevent cancer vary from one situation to another and across geographical regions and take many factors into account, including different national priorities. Therefore, no recommendations are given in the *Monographs* with regard to regulation, legislation, or other policy approaches, which are the responsibility of individual governments or organizations. The *Monographs* programme also does not make research recommendations. However, it is important to note that *Monographs* contribute significantly to the science of carcinogenesis by synthesizing and integrating streams of evidence about carcinogenicity and pointing to critical gaps in knowledge.

3. Selection of agents for review

Since 1984, about every five years IARC convenes an international, interdisciplinary Advisory Group to recommend agents for review by the *Monographs* programme. IARC selects Advisory Group members who are knowledgeable about current research on carcinogens and public health priorities. Before an Advisory Group meets, IARC solicits nominations of agents from scientists and government agencies worldwide. Since 2003, IARC also invites nominations from the public. IARC charges each Advisory Group with reviewing nominations, evaluating exposure and hazard potential, and preparing a report that documents the Advisory Group's process for these activities and its rationale for the recommendations.

For each new volume of the *Monographs*, IARC selects the agents for review from those recommended by the most recent Advisory Group, considering the availability of pertinent research studies and current public health priorities. On occasion, IARC may select other agents if there is a need to rapidly evaluate an emerging carcinogenic hazard or an urgent need to re-evaluate a previous classification. All evaluations consider the full body of available evidence, not just information published after a previous review.

A *Monograph* may review:

(a) An agent not reviewed in a previous *Monograph*, if there is potential human exposure and there is evidence for assessing its carcinogenicity. A group of related agents (e.g. metal compounds) may be reviewed together if there is evidence for assessing carcinogenicity for one or more members of the group.

(b) An agent reviewed in a previous *Monograph*, if there is new evidence of cancer in humans or in experimental animals, or mechanistic evidence to warrant re-evaluation of the classification. In the interests of efficiency, the literature searches may build on previous comprehensive searches.

(c) An agent that has been established to be carcinogenic to humans and has been reviewed in a previous *Monograph*, if there is new evidence of cancer in humans that indicates new tumour sites where there might be a causal association. In the interests of efficiency, the review may focus on these new tumour sites.

4. The Working Group and other meeting participants

Five categories of participants can be present at *Monographs* meetings:

(i) *Working Group* members are responsible for all scientific reviews and evaluations developed in the volume of the *Monographs*. The Working Group is interdisciplinary and comprises subgroups of experts in the fields of (a) exposure characterization, (b) cancer in humans, (c) cancer in experimental animals, and (d) mechanistic evidence. IARC selects Working Group members on the basis of expertise related to the subject matter and relevant methodologies, and absence

of conflicts of interest. Consideration is also given to diversity in scientific approaches and views, as well as demographic composition. Working Group members generally have published research related to the exposure or carcinogenicity of the agents being reviewed, and IARC uses literature searches to identify most experts. Since 2006, IARC also has encouraged public nominations through its Call for Experts. IARC's reliance on experts with knowledge of the subject matter and/or expertise in methodological assessment is confirmed by decades of experience documenting that there is value in specialized expertise and that the overwhelming majority of Working Group members are committed to the objective evaluation of scientific evidence and not to the narrow advancement of their own research results or a pre-determined outcome (Wild and Cogliano, 2011). Working Group members are expected to serve the public health mission of IARC, and should refrain from consulting and other activities for financial gain that are related to the agents under review, or the use of inside information from the meeting, until the full volume of the *Monographs* is published.

IARC identifies, from among Working Group members, individuals to serve as Meeting Chair and Subgroup Chairs. At the opening of the meeting, the Working Group is asked to endorse the selection of the Meeting Chair, with the opportunity to propose alternatives. The Meeting Chair and Subgroup Chairs take a leading role at all stages of the review process (see Part A, Section 7), promote open scientific discussions that involve all Working Group members in accordance with normal committee procedures, and ensure adherence to the Preamble.

(ii) *Invited Specialists* are experts who have critical knowledge and experience but who also have a conflict of interest that warrants exclusion from developing or influencing the evaluations of carcinogenicity. Invited Specialists do not draft any section of the *Monograph* that pertains to the description or interpretation of cancer data, and they do not participate in the evaluations. These experts are invited in limited numbers when necessary to assist the Working Group by contributing their unique knowledge and experience to the discussions.

(iii) *Representatives of national and international health agencies* may attend because their agencies are interested in the subject of the meeting. They do not draft any section of the *Monograph* or participate in the evaluations.

(iv) *Observers* with relevant scientific credentials may be admitted in limited numbers. Attention is given to the balance of Observers from constituencies with differing perspectives. Observers are invited to observe the meeting and should not attempt to influence it, and they agree to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers do not draft any section of the *Monograph* or participate in the evaluations.

(v) The *IARC Secretariat* consists of scientists who are designated by IARC and who have relevant expertise. The IARC Secretariat coordinates and facilitates all aspects of the evaluation and ensures adherence to the Preamble throughout development of the scientific reviews and classifications (see Part A, Sections 5 and 6). The IARC Secretariat organizes and announces the meeting, identifies and recruits the Working Group members, and assesses the declared interests of all meeting participants. The IARC Secretariat supports the activities of the Working Group (see Part A, Section 7) by searching the literature and performing title and abstract screening, organizing conference calls to coordinate the development of pre-meeting

Table 1 Roles of participants at *IARC Monographs* meetings

Category of participant	Role			
	Prepare text, tables, and analyses	Participate in discussions	Participate in evaluations	Eligible to serve as Chair
Working Group members	✓	✓	✓	✓
Invited Specialists	✓[a]	✓		
Representatives of health agencies		✓[b]		
Observers		✓[b]		
IARC Secretariat	✓[c]	✓	✓[d]	

[a] Only for the section on exposure characterization.
[b] Only at times designated by the Meeting Chair and Subgroup Chairs.
[c] When needed or requested by the Meeting Chair and Subgroup Chairs.
[d] Only for clarifying or interpreting the Preamble.

drafts and discuss cross-cutting issues, and reviewing drafts before and during the meeting. Members of the IARC Secretariat serve as meeting rapporteurs, assist the Meeting Chair and Subgroup Chairs in facilitating all discussions, and may draft text or tables when designated by the Meeting Chair and Subgroup Chairs. Their participation in the evaluations is restricted to the role of clarifying or interpreting the Preamble.

All participants are listed, with their principal affiliations, in the front matter of the published volume of the *Monographs*. Working Group members and Invited Specialists serve as individual scientists and not as representatives of any organization, government, or industry (Cogliano et al., 2004).

The roles of the meeting participants are summarized in Table 1.

5. Working procedures

A separate Working Group is responsible for developing each volume of the *Monographs*. A volume contains one or more *Monographs*, which can cover either a single agent or several related agents. Approximately one year before the meeting of a Working Group, a preliminary list of agents to be reviewed, together with a Call for Data and a Call for Experts, is announced on the *Monographs* programme website (https://monographs.iarc.who.int/).

Before a meeting invitation is extended, each potential participant, including the IARC Secretariat, completes the WHO Declaration of Interests form to report financial interests, employment and consulting (including remuneration for serving as an expert witness), individual and institutional research support, and non-financial interests such as public statements and positions related to the subject of the meeting. IARC assesses the declared interests to determine whether there is a conflict that warrants any limitation on participation (see Table 2).

Approximately two months before a *Monographs* meeting, IARC publishes the names and affiliations of all meeting participants together with a summary of declared interests, in the interests of transparency and to provide an opportunity for undeclared conflicts of interest to be brought to IARC's attention. It is not acceptable for Observers or third parties to contact other participants before a meeting or to lobby them at any time. Meeting participants are asked to report all such contacts to IARC (Cogliano et al., 2005).

The Working Group meets at IARC for approximately eight days to discuss and finalize the scientific review and to develop summaries

Table 2 Public engagement during *Monographs* development

Approximate timeframe	Engagement
Every 5 years	IARC convenes an Advisory Group to recommend high-priority agents for future review
~1 year before a *Monographs* meeting	IARC selects agents for review in a new volume of the *Monographs* IARC posts on its website: Preliminary List of Agents to be reviewed Call for Data and Call for Experts Request for Observer Status WHO Declaration of Interests form
~8 months before a *Monographs* meeting	Call for Experts closes
~4 months before a *Monographs* meeting	Request for Observer Status closes
~2 months before a *Monographs* meeting	IARC posts the names of all meeting participants together with a summary of declared interests, and a statement discouraging contact of the Working Group by interested parties
~1 month before a *Monographs* meeting	Call for Data closes
~2–4 weeks after a *Monographs* meeting	IARC publishes a summary of evaluations and key supporting evidence
~9 months after a *Monographs* meeting	IARC Secretariat publishes the verified and edited master copy of plenary drafts as a *Monographs* volume

and evaluations. At the opening of the meeting, all participants update their Declaration of Interests forms, which are then reviewed by IARC. Declared interests related to the subject of the meeting are disclosed to the meeting participants during the meeting and in the published volume (Cogliano et al., 2004). The objectives of the meeting are peer review and consensus. During the first part of the meeting, subgroup sessions (covering exposure characterization, cancer in humans, cancer in experimental animals, and mechanistic evidence) review the pre-meeting drafts, develop a joint subgroup draft, and draft subgroup summaries. During the last part of the meeting, the Working Group meets in plenary session to review the subgroup drafts and summaries and to develop the consensus evaluations. As a result, the entire volume is the joint product of the Working Group, and there are no individually authored sections. After the meeting, the master copy is verified by the IARC Secretariat and is then edited and prepared for publication. The aim is to publish the volume within approximately nine months of the Working Group meeting. A summary of the evaluations and key supporting evidence is prepared for publication in a scientific journal or is made available on the *Monographs* programme website soon after the meeting.

In the interests of transparency, IARC engages with the public throughout the process, as summarized in Table 2.

6. Overview of the scientific review and evaluation process

The Working Group considers all pertinent epidemiological studies, cancer bioassays in experimental animals, and mechanistic evidence, as well as pertinent information on exposure in humans. In general, for cancer in humans, cancer in experimental animals, and mechanistic evidence, only studies that have been published or accepted for publication in the openly available scientific literature are reviewed. Under some circumstances, materials that are publicly available and whose content is final may be reviewed if there is sufficient information to permit an evaluation of the quality of the methods and results of the studies (see Step 1,

below). Such materials may include reports and databases publicly available from government agencies, as well as doctoral theses. The reliance on published and publicly available studies promotes transparency and protects against citation of premature information.

The principles of systematic review are applied to the identification, screening, synthesis, and evaluation of the evidence related to cancer in humans, cancer in experimental animals, and mechanistic evidence (as described in Part B, Sections 2–4 and as detailed in the Instructions for Authors). Each *Monograph* specifies or references information on the conduct of the literature searches, including search terms and inclusion/exclusion criteria that were used for each stream of evidence.

In brief, the steps of the review process are as follows:

Step 1. Comprehensive and transparent identification of the relevant information: The IARC Secretariat identifies relevant studies through initial comprehensive searches of literature contained in authoritative biomedical databases (e.g. PubMed, PubChem) and through a Call for Data. These literature searches, designed in consultation with a librarian and other technical experts, address whether the agent causes cancer in humans, causes cancer in experimental systems, and/or exhibits key characteristics of established human carcinogens (in humans or in experimental systems). The Working Group provides input and advice to IARC to refine the search strategies, and identifies literature through other searches (e.g. from reference lists of past *Monographs*, retrieved articles, and other authoritative reviews).

For certain types of agents (e.g. regulated pesticides and pharmaceuticals), IARC also provides an opportunity to relevant regulatory authorities, and regulated parties through such authorities, to make pertinent unpublished studies publicly available by the date specified in the Call for Data. Consideration of such studies by the Working Group is dependent on the public availability of sufficient information to permit an independent evaluation of (a) whether there has been selective reporting (e.g. on outcomes, or from a larger set of conducted studies); (b) study quality (e.g. design, methodology, and reporting of results), and (c) study results.

Step 2. Screening, selection, and organization of the studies: The IARC Secretariat screens the retrieved literature for inclusion based on title and abstract review, according to pre-defined exclusion criteria. For instance, studies may be excluded if they were not about the agent (or a metabolite of the agent), or if they reported no original data on epidemiological or toxicological end-points (e.g. review articles). The Working Group reviews the title and abstract screening done by IARC, and performs full-text review. Any reasons for exclusion are recorded, and included studies are organized according to factors pertinent to the considerations described in Part B, Sections 2–4 (e.g. design, species, and end-point). Inclusion of a study does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results.

Step 3. Evaluation of study quality: The Working Group evaluates the quality of the included studies based on the considerations (e.g. design, methodology, and reporting of results) described in Part B, Sections 2–4. Based on these considerations, the Working Group may accord greater weight to some of the included studies. Interpretation of the results and the strengths and limitations of a study are clearly outlined in square brackets at the end of study descriptions (see Part B).

Step 4: Report characteristics of included studies, including assessment of study

quality: Pertinent characteristics and results of included studies are reviewed and succinctly described, as detailed in Part B, Sections 1–4. Tabulation of data may facilitate this reporting. This step may be iterative with Step 3.

Step 5: *Synthesis and evaluation of strength of evidence*: The Working Group summarizes the overall strengths and limitations of the evidence from the individual streams of evidence (cancer in humans, cancer in experimental animals, and mechanistic evidence; see Part B, Section 5). The Working Group then evaluates the strength of evidence from each stream of evidence by using the transparent methods and defined descriptive terms given in Part B, Sections 6a–c. The Working Group then develops, and describes the rationale for, the consensus classification of carcinogenicity that integrates the conclusions about the strength of evidence from studies of cancer in humans, studies of cancer in experimental animals, and mechanistic evidence (see Part B, Section 6d).

7. Responsibilities of the Working Group

The Working Group is responsible for identifying and evaluating the relevant studies and developing the scientific reviews and evaluations for a volume of the *Monographs*. The IARC Secretariat supports these activities of the Working Group (see Part A, Section 4). Briefly, the Working Group's tasks in developing the evaluation are, in sequence:

(i) Before the meeting, the Working Group ascertains that all appropriate studies have been identified and selected, and assesses the methods and quality of each individual study, as outlined above (see Part A, Section 6). The Working Group members prepare pre-meeting working drafts that present accurate tabular or textual summaries of informative studies by extracting key elements of the study design and results, and highlighting notable strengths and limitations. They participate in conference calls organized by IARC to coordinate the development of working drafts and to discuss cross-cutting issues. Pre-meeting reviews of all working drafts are generally performed by two or more subgroup members who did not participate in study identification, data extraction, or study review for the draft. Each study summary is written or reviewed by someone who is not associated with the study.

(ii) At the meeting, within subgroups, the Working Group members critically review, discuss, and revise the pre-meeting drafts and adopt the revised versions as consensus subgroup drafts. Subgroup Chairs ensure that someone who is not associated with the study leads the discussion of each study summary. A proposed classification of the strength of the evidence reviewed in the subgroup using the *IARC Monographs* criteria (see Part B, Sections 6a–c) is then developed from the consensus subgroup drafts of the evidence summaries (see Part B, Section 5).

(iii) During the plenary session, each subgroup presents its drafts for scientific review and discussion to the other Working Group members, who did not participate in study identification, data extraction, or study review for the drafts. Subgroup Chairs ensure that someone who is not associated with the study leads the discussion of each study summary. After review, discussion, and revisions as needed, the subgroup drafts are adopted as a consensus Working Group product. The summaries and classifications of the strength of the evidence, developed in the subgroup in line with the *IARC Monographs* criteria

(see Part B, Sections 6a–c), are considered, revised as needed, and adopted by the full Working Group. The Meeting Chair proposes an overall evaluation using the guidance provided in Part B, Section 6d.

The Working Group strives to achieve consensus evaluations. Consensus reflects broad agreement among the Working Group, but not necessarily unanimity. The Meeting Chair may poll the Working Group to determine the diversity of scientific opinion on issues where consensus is not apparent.

Only the final product of the plenary session represents the views and expert opinions of the Working Group. The entire *Monographs* volume is the joint product of the Working Group and represents an extensive and thorough peer review of the body of evidence (individual studies, synthesis, and evaluation) by an interdisciplinary expert group. Initial working papers and subsequent revisions are not released, because they would give an incomplete and possibly misleading impression of the consensus developed by the Working Group over a full week of deliberation.

B. SCIENTIFIC REVIEW AND EVALUATION

This part of the Preamble discusses the types of evidence that are considered and summarized in each section of a *Monograph*, followed by the scientific criteria that guide the evaluations. In addition, a section of General Remarks at the front of the volume discusses the reasons the agents were scheduled for evaluation and any key issues encountered during the meeting.

1. Exposure characterization

This section identifies the agent and describes its occurrence, main uses, and production locations and volumes, where relevant. It also summarizes the prevalence, concentrations in relevant studies, and relevant routes of exposure in humans worldwide. Methods of exposure measurement and analysis are described, and methods of exposure assessment used in key epidemiological studies reviewed by the Working Group are described and evaluated.

Over the course of the *Monographs* programme, concepts of exposure and dose have evolved substantially with deepening understanding of the interactions of agents and biological systems. The concept of exposure has broadened and become more holistic, extending beyond chemical, physical, and biological agents to stressors as construed generally, including psychosocial stressors (National Research Council, 2012; National Academies of Sciences, Engineering, and Medicine, 2017). Overall, this broader conceptualization supports greater integration between exposure characterization and other sections of the *Monographs*. Concepts of absorption, distribution, metabolism, and excretion are considered in the first subsection of mechanistic evidence (see Part B, Section 4a), whereas validated biomarkers of internal exposure or metabolites that are routinely used for exposure assessment are reported on in this section (see Part B, Section 1b).

(a) Identification of the agent

The agent being evaluated is unambiguously identified. Details will vary depending on the type of agent but will generally include physical and chemical properties relevant to the agent's identification, occurrence, and biological activity. If the material that has been tested in experimental animals or in vitro systems is different from that to which humans are exposed, these differences are noted.

For chemical agents, the Chemical Abstracts Service Registry Number is provided, as well as the latest primary name and other names in common use, including important trade names,

along with available information on the composition of common mixtures or products containing the agent, and potentially toxic and/or carcinogenic impurities. Physical properties relevant to understanding the potential for human exposure and measures of exposure used in studies in humans are summarized. These might include physical state, volatility, aqueous and fat solubility, and half-life in the environment and/or in human tissues.

For biological agents, taxonomy and structure are described. Mode of replication, life-cycle, target cells, persistence, latency, and host responses, including morbidity and mortality through pathologies other than cancer, are also presented.

For foreign bodies, fibres and particles, composition, size range, relative dimensions, and accumulation, persistence, and clearance in target organs are summarized. Physical agents that are forms of radiation are described in terms of frequency spectrum and energy transmission.

Exposures may result from, or be influenced by, a diverse range of social and environmental factors, including components of diet, sleep, and physical activity patterns. In these instances, this section will include a description of the agent, its variability across human populations, and its composition or characteristics relevant to understanding its potential carcinogenic hazard to humans and to evaluating exposure assessments in epidemiological studies.

(b) Detection and analysis

Key methods of detection and quantification of the agent are presented, with an emphasis on those used most widely in surveillance, regulation, and epidemiological studies. Measurement methods for sample matrices that are deemed important sources of human exposure (e.g. air, drinking-water, food, residential dust) and for validated exposure biomarkers (e.g. the agent or its metabolites in human blood, urine, or saliva) are described. Information on detection and quantification limits is provided when it is available and is useful for interpreting studies in humans and in experimental animals. This is not an exhaustive treatise but is meant to help readers understand the strengths and limitations of the available exposure data and of the epidemiological studies that rely on these measurements.

(c) Production and use

Historical and geographical patterns and trends in production and use are included when they are available, to help readers understand the contexts in which exposures may occur, both within key epidemiological studies reviewed by the Working Group and in human populations generally. Industries that produce, use, or dispose of the agent are described, including their global distribution, when available. National or international listing as a high-production-volume chemical or similar classification may be included. Production processes with significant potential for occupational exposure or environmental pollution are indicated. Trends in global production volumes, technologies, and other data relevant to understanding exposure potential are summarized. Minor or historical uses with significant exposure potential or with particular relevance to key epidemiological studies are included. Particular effort may be directed towards finding data on production in low- and middle-income countries, where rapid economic development may lead to higher exposures than those in high-income countries.

(d) Exposure

A concise overview of quantitative information on sources, prevalence, and levels of exposure in humans is provided. Representative data from research studies, government reports and websites, online databases, and other citable, publicly available sources are tabulated. Data

from low- and middle-income countries are sought and included to the extent feasible; information gaps for key regions are noted. Naturally occurring sources of exposure, if any, are noted. Primary exposure routes (e.g. inhalation, ingestion, skin uptake) and other considerations relevant to understanding the potential for cancer hazard from exposure to the agent are reported.

For occupational settings, information on exposure prevalence and levels (e.g. in air or human tissues) is reported by industry, occupation, region, and other characteristics (e.g. process, task) where feasible. Information on historical exposure trends, protection measures to limit exposure, and potential co-exposures to other carcinogenic agents in workplaces is provided when available.

For non-occupational settings, the occurrence of the agent is described with environmental monitoring or surveillance data. Information on exposure prevalence and levels (e.g. concentrations in human tissues) as well as exposure from and/or concentrations in food and beverages, consumer products, consumption practices, and personal microenvironments is reported by region and other relevant characteristics. Particular importance is placed on describing exposures in life stages or in states of disease or nutrition that may involve greater exposure or susceptibility.

Current exposures are of primary interest; however, information on historical exposure trends is provided when available. Historical exposures may be relevant for interpreting epidemiological studies, and when agents are persistent or have long-term effects. Information gaps for important time periods are noted. Exposure data that are not deemed to have high relevance to human exposure are generally not considered.

(e) Regulations and guidelines

Regulations or guidelines that have been established for the agent (e.g. occupational exposure limits, maximum permitted levels in foods and water, pesticide registrations) are described in brief to provide context about government efforts to limit exposure; these may be tabulated if they are informative for the interpretation of existing or historical exposure levels. Information on applicable populations, specific agents concerned, basis for regulation (e.g. human health risk, environmental considerations), and timing of implementation may be noted. National and international bans on production, use, and trade are also indicated.

This section aims to include major or illustrative regulations and may not be comprehensive, because of the complexity and range of regulatory processes worldwide. An absence of information on regulatory status should not be taken to imply that a given country or region lacks exposure to, or regulations on exposure to, the agent.

(f) Critical review of exposure assessment in key epidemiological studies

Epidemiological studies evaluate cancer hazard by comparing outcomes across differently exposed groups. Therefore, the type and quality of the exposure assessment methods used are key considerations when interpreting study findings for hazard identification. This section summarizes and critically reviews the exposure assessment methods used in the individual epidemiological studies that contribute data relevant to the *Monographs* evaluation.

Although there is no standard set of criteria for evaluating the quality of exposure assessment methods across all possible agents, some concepts are universally relevant. Regardless of the agent, all exposures have two principal dimensions: intensity (sometimes defined as concentration or dose) and time. Time considerations include

duration (time from first to last exposure), pattern or frequency (whether continuous or intermittent), and windows of susceptibility. This section considers how each of the key epidemiological studies characterizes these dimensions. Interpretation of exposure information may also be informed by consideration of mechanistic evidence (e.g. as described in Part B, Section 4a), including the processes of absorption, distribution, metabolism, and excretion.

Exposure intensity and time in epidemiological studies can be characterized by using environmental or biological monitoring data, records from workplaces or other sources, expert assessments, modelled exposures, job-exposure matrices, and subject or proxy reports via questionnaires or interviews. Investigators use these data sources and methods individually or in combination to assign levels or values of an exposure metric (which may be quantitative, semi-quantitative, or qualitative) to members of the population under study.

In collaboration with the Working Group members reviewing human studies (of cancer and of mechanisms), key epidemiological studies are identified. For each selected study, the exposure assessment approach, along with its strengths and limitations, is summarized using text and tables. Working Group members identify concerns about exposure assessment methods and their impacts on overall quality for each study reviewed (see Part B, Sections 2d and 4d). In situations where the information provided in the study is inadequate to properly consider the exposure assessment, this is indicated. When adequate information is available, the likely direction of bias due to error in exposure measurement, including misclassification (overestimated effects, underestimated effects, or unknown) is discussed.

2. Studies of cancer in humans

This section includes all pertinent epidemiological studies (see Part B, Section 2b) that include cancer as an outcome. These studies encompass certain types of biomarker studies, for example, studies with biomarkers as exposure metrics (see Part B, Section 2) or those evaluating histological or tumour subtypes and molecular signatures in tumours consistent with a given exposure (Alexandrov et al., 2016). Studies that evaluate early biological effect biomarkers are reviewed in Part B, Section 4.

(a) *Types of study considered*

Several types of epidemiological studies contribute to the assessment of carcinogenicity in humans; they typically include cohort studies (including variants such as case–cohort and nested case–control studies), case–control studies, ecological studies, and intervention studies. Rarely, results from randomized trials may be available. Exceptionally, case reports and case series of cancer in humans may also be reviewed. In addition to these designs, innovations in epidemiology allow for many other variants that may be considered in any given *Monographs* evaluation.

Cohort and case–control studies typically have the capacity to relate individual exposures under study to the occurrence of cancer in individuals, and provide an estimate of effect (such as relative risk) as the main measure of association. Well-conducted cohort and case–control studies provide most of the evidence of cancer in humans evaluated by Working Groups. Intervention studies are much less common, but when available can provide strong evidence for making causal inferences.

In ecological studies, the units of investigation are usually whole populations (e.g. in particular geographical areas or at particular times), and cancer frequency is related to a summary

measure of the exposure in the population under study. In ecological studies, data on individual exposure and outcome are not available, which renders this type of study more prone to confounding and exposure misclassification. In some circumstances, however, ecological studies may be informative, especially when the unit of exposure is most accurately measured at the population level (see, for example, the *Monograph* on arsenic in drinking-water; IARC, 2004).

Exceptionally, case reports and case series may provide compelling evidence about the carcinogenicity of an agent. In fact, many of the early discoveries of occupational cancer hazards came about because of observations by workers and their clinicians, who noted a high frequency of cancer in workers who share a common occupation or exposure. Such observations may be the starting point for more structured investigations, but in exceptional circumstances, when the risk is high enough, the case series may in itself provide compelling evidence. This would be especially warranted in situations where the exposure circumstance is fairly unusual, as it was in the example of plants containing aristolochic acid (IARC, 2012a).

The uncertainties that surround the interpretation of case reports, case series, and ecological studies typically make them inadequate, except in rare instances as described above, to form the sole basis for inferring a causal relationship. However, when considered together with cohort and case–control studies, these types of study may support the judgement that a causal relationship exists.

Epidemiological studies of benign neoplasms, pre-neoplastic lesions, malignant precursors, and other end-points are also reviewed when they relate to the agents reviewed. On occasion they can strengthen inferences drawn from studies of cancer itself. For example, benign brain tumours may share common risk factors with those that are malignant, and benign neoplasms (or those of uncertain behaviour) may be part of the causal path to malignancies (e.g. myelodysplastic syndromes, which may progress to acute myeloid leukaemia).

(b) Identification of eligible studies of cancer in humans

Relevant studies of cancer in humans are identified by using systematic review principles as described in Part A, further elaborated in the Instructions for Authors, and as detailed below. Eligible studies include all studies in humans of exposure to the agent of interest with cancer as an outcome. Multiple publications on the same study population are identified so that the number of independent studies is accurately represented. Multiple publications may result, for example, from successive follow-ups of a single cohort, from analyses focused on different aspects of an exposure–disease association, or from inclusion of overlapping populations. Usually in such situations, only the most recent, most comprehensive, or most informative report is reviewed in detail.

(c) Assessment of study quality and informativeness

Epidemiological studies are potentially susceptible to several different sources of error, summarized briefly below. Qualities of individual studies that address these issues are also described below.

Study quality is assessed as part of the structured expert review process undertaken by the Working Group. A key aspect of quality assessment is consideration of the possible roles of chance and bias in the interpretation of epidemiological studies. Chance, which is also called random variation, can produce misleading study results. This variability in study results is strongly influenced by the sample size: smaller studies are more likely than larger studies to have effect estimates that are imprecise. Confidence intervals

around a study's point estimate of effect are used routinely to indicate the range of values of the estimate that could easily be produced by chance alone.

Bias is the effect of factors in study design or conduct that lead an association to erroneously appear stronger or weaker than the association that really exists between the agent and the disease. Biases that require consideration are varied but are usually categorized as selection bias, information bias (e.g. error in measurement of exposure and diseases), and confounding (or confounding bias) (Rothman et al., 2008). Selection bias in an epidemiological study occurs when inclusion of participants from the eligible population or their follow-up in the study is influenced by their exposure or their outcome (usually disease occurrence). Under these conditions, the measure of association found in the study will not accurately reflect the association that would otherwise have been found in the eligible population (Hernán et al., 2004). Information bias results from inaccuracy in exposure or outcome measurement. Both can cause an association between hypothesized cause and effect to appear stronger or weaker than it really is. Confounding is a mixing of extraneous effects with the effects of interest (Rothman et al., 2008). An association between the purported causal factor and another factor that is associated with an increase or decrease in incidence of disease can lead to a spurious association or absence of a real association of the presumed causal factor with the disease. When either of these occurs, confounding is present.

In assessing study quality, the Working Group consistently considers the following aspects:

- **Study description:** Clarity in describing the study design and its implementation, and the completeness of reporting of all other key information about the study and its results.
- **Study population:** Whether the study population was appropriate for evaluating the association between the agent and cancer. Whether the study was designed and carried out to minimize selection bias. Cancer cases in the study population must have been identified in a way that was independent of the exposure of interest, and exposure assessed in a way that was not related to disease (outcome) status. In these respects, completeness of recruitment into the study from the population of interest and completeness of follow-up for the outcome are essential measures.
- **Outcome measurement:** The appropriateness of the cancer outcome measure (e.g. mortality vs incidence) for the agent and cancer type under consideration, outcome ascertainment methodology, and the extent to which outcome misclassification may have led to bias in the measure(s) of association.
- **Exposure measurement:** The adequacy of the methods used to assess exposure to the agent, and the likelihood (and direction) of bias in the measure(s) of association due to error in exposure measurement, including misclassification (as described in Part B, Section 1f).
- **Assessment of potential confounding:** To what extent the authors took into account in the study design and analysis other variables (including co-exposures, as described in Part B, Section 1d) that can influence the risk of disease and may have been related to the exposure of interest. Important sources of potential confounding by such variables should have been addressed either in the design of the study, such as by matching or restriction, or in the analysis, by statistical adjustment. In some instances, where direct information on confounders is unavailable, use of indirect methods to evaluate the potential impact of confounding on exposure–disease associations is appropriate (e.g. Axelson and Steenland, 1988; Richardson et al., 2014).

- **Other potential sources of bias:** Each epidemiological study is unique in its study population, its design, its data collection, and, consequently, its potential biases. All possible sources of bias are considered for their possible impact on the results. The possibility of reporting bias (i.e. selective reporting of some results and the suppression of others) should be explored.
- **Statistical methodology:** Adequacy of the statistical methods used and their ability to obtain unbiased estimates of exposure–outcome associations, confidence intervals, and test statistics for the significance of measures of association. Appropriateness of methods used to investigate confounding, including adjusting for matching when necessary and avoiding treatment of probable mediating variables as confounders. Detailed analyses of cancer risks in relation to summary measures of exposure such as cumulative exposure, or temporal variables such as age at first exposure or time since first exposure, are reviewed and summarized when available.

For the sake of economy and simplicity, in this Preamble the list of possible sources of error is referred to with the phrase "chance, bias, and confounding", but it should be recognized that this phrase encompasses a comprehensive set of concerns pertaining to study quality.

These sources of error do not constitute and should not be used as a formal checklist of indicators of study quality. The judgement of experienced experts is critical in determining how much weight to assign to different issues in considering how all of these potential sources of error should be integrated and how to rate the potential for error related to each of these considerations.

The informativeness of a study is its ability to show a true association, if there is one, between the agent and cancer, and the lack of an association, if no association exists. Key determinants of informativeness include: having a study population of sufficient size to obtain precise estimates of effect; sufficient elapsed time from exposure to measurement of outcome for an effect, if present, to be observable; presence of an adequate exposure contrast (intensity, frequency, and/or duration); biologically relevant definitions of exposure; and relevant and well-defined time windows for exposure and outcome.

(d) Meta-analyses and pooled analyses

Independent epidemiological studies of the same agent may lead to inconsistent results that are difficult to interpret or reconcile. Combined analyses of data from multiple studies may be conducted as a means to address this ambiguity. There are two types of combined analysis. The first involves combining summary statistics such as relative risks from individual studies (meta-analysis), and the second involves a pooled analysis of the raw data from the individual studies (pooled analysis) (Greenland and O'Rourke, 2008).

The strengths of combined analyses are increased precision because of increased sample size and, in the case of pooled analyses, the opportunity to better control for potential confounders and to explore in more detail interactions and modifying effects that may explain heterogeneity among studies. A disadvantage of combined analyses is the possible lack of comparability of data from various studies, because of differences in population characteristics, subject recruitment, procedures of data collection, methods of measurement, and effects of unmeasured covariates that may differ among studies. These differences in study methods and quality can influence results of either meta-analyses or pooled analyses. If published meta-analyses are to be considered by the Working Group, their adequacy needs to be carefully evaluated, including the methods used to identify eligible studies

and the accuracy of data extracted from the individual studies.

The Working Group may conduct ad hoc meta-analyses during the course of a *Monographs* meeting, when there are sufficient studies of an exposure–outcome association to contribute to the Working Group's assessment of the association. The results of such unpublished original calculations, which would be specified in the text by presentation in square brackets, might involve updates of previously conducted analyses that incorporate the results of more recent studies, or de novo analyses.

Irrespective of the source of data for the meta-analyses and pooled analyses, the following key considerations apply: the same criteria for data quality must be applied as for individual studies; sources of heterogeneity among studies must be carefully considered; and the possibility of publication bias should be explored.

(e) Considerations in assessing the body of epidemiological evidence

The ability of the body of epidemiological evidence to inform the Working Group about the carcinogenicity of the agent is related to both the quantity and the quality of the evidence. There is no formulaic answer to the question of how many studies of cancer in humans are needed from which to draw inferences about causality, although more than a single study in a single population will almost always be needed. The number will depend on the considerations relating to evidence described below.

After the quality of individual epidemiological studies of cancer has been assessed and the informativeness of the various studies on the association between the agent and cancer has been evaluated, a judgement is made about the strength of evidence that the agent in question is carcinogenic to humans. In making its judgement, the Working Group considers several aspects of the body of evidence (e.g. Hill, 1965; Rothman et al., 2008; Vandenbroucke et al., 2016).

A strong association (e.g. a large relative risk) is more likely to indicate causality than is a weak association, because it is more difficult for confounding to falsely create a strong association. However, it is recognized that estimates of effect of small magnitude do not imply lack of causality and may have impact on public health if the disease or exposure is common. Estimates of effect of small magnitude could also contribute useful information to the assessment of causality if level of risk is commensurate with level of exposure when compared with risk estimates from populations with higher exposure (e.g. as seen in residential radon studies compared with studies of radon from uranium mining).

Associations that are consistently observed in several studies of the same design, or in studies that use different epidemiological approaches, or under different circumstances of exposure are more likely to indicate a causal relationship than are isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (e.g. differences in study informativeness because of latency, exposure levels, or assessment methods). Results of studies that are judged to be of high quality and informativeness are given more weight than those of studies judged to be methodologically less sound or less informative.

Temporality of the association is an essential consideration: that is, the exposure must precede the outcome.

An observation that cancer risk increases with increasing exposure is considered to be a strong indication of causality, although the absence of a graded response is not necessarily evidence against a causal relationship, and there are several reasons why the shape of the exposure–response association may be non-monotonic (e.g. Stayner et al., 2003). The demonstration of a decline in risk after cessation of or reduction in exposure

in individuals or in whole populations also supports a causal interpretation of the findings.

Confidence in a causal interpretation of the evidence from studies of cancer in humans is enhanced if it is coherent with physiological and biological knowledge, including information about exposure to the target organ, latency and timing of the exposure, and characteristics of tumour subtypes.

The Working Group considers whether there are subpopulations with increased susceptibility to cancer from the agent. For example, molecular epidemiology studies that identify associations between genetic polymorphisms and inter-individual differences in cancer susceptibility to the agent(s) being evaluated may contribute to the identification of carcinogenic hazards to humans. Such studies may be particularly informative if polymorphisms are found to be modifiers of the exposure–response association, because evaluation of polymorphisms may increase the ability to detect an effect in susceptible subpopulations.

When, in the process of evaluating the studies of cancer in humans, the Working Group identifies several high-quality, informative epidemiological studies that clearly show either no positive association or an inverse association between an exposure and a specific type of cancer, a judgement may be made that, in the aggregate, they suggest evidence of lack of carcinogenicity for that cancer type. Such a judgement requires, first, that the studies strictly meet the standards of design and analysis described above. Specifically, the possibility that bias, confounding, or misclassification of exposure or outcome could explain the observed results should be considered and ruled out with reasonable confidence. In addition, all studies that are judged to be methodologically sound should (a) be consistent with an estimate of relative effect of unity (or below unity) for any observed level of exposure, (b) when considered together, provide a combined estimate of relative risk that is at or below unity, and (c) have a narrow confidence interval. Moreover, neither any individual well-designed and well-conducted study nor the pooled results of all the studies should show any consistent tendency that the relative risk of cancer increases with increasing level of exposure. It must be noted that evidence of lack of carcinogenicity obtained from several epidemiological studies can apply only to the type(s) of cancer studied, to the exposure levels reported and the timing and route of exposure studied, to the intervals between first exposure and disease onset observed in these studies, and to the general population(s) studied (i.e. there may be susceptible subpopulations or life stages). Experience from studies of cancer in humans indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years; therefore, latency periods substantially shorter than about 30 years cannot provide evidence of lack of carcinogenicity. Furthermore, there may be critical windows of exposure, for example, as with diethylstilboestrol and clear cell adenocarcinoma of the cervix and vagina (IARC, 2012a).

3. Studies of cancer in experimental animals

Most human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species. For some agents, carcinogenicity in experimental animals was demonstrated before epidemiological studies identified their carcinogenicity in humans. Although this observation cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, it is biologically plausible that agents for which there is *sufficient evidence of carcinogenicity* in experimental animals (see Part B, Section 6b) present a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, such as strong evidence that a given agent causes cancer in

experimental animals through a species-specific mechanism that does not operate in humans (see Part B, Sections 4 and 6; Capen et al., 1999; IARC, 2003), these agents are considered to pose a potential carcinogenic hazard to humans. The inference of potential carcinogenic hazard to humans does not imply tumour site concordance across species (Baan et al., 2019).

(a) Types of studies considered

Relevant studies of cancer in experimental animals are identified by using systematic review principles as described in Part A, further elaborated in the Instructions for Authors, and as detailed below. Consideration is given to all available long-term studies of cancer in experimental animals with the agent under review (or possibly metabolites or derivatives of the agent) (see Part A, Section 7) after a thorough evaluation of the study features (see Part B, Section 3b). Those studies that are judged to be irrelevant to the evaluation or judged to be inadequate (e.g. too short a duration, too few animals, poor survival; see below) may be omitted. Guidelines for conducting long-term carcinogenicity experiments have been published (e.g. OECD, 2018).

In addition to conventional long-term bioassays, alternative studies (e.g. in genetically engineered mouse models) may be considered in assessing carcinogenicity in experimental animals, also after a critical evaluation of the study features. For studies of certain exposures, such as viruses that typically only infect humans, use of such specialized experimental animal models may be particularly important; models include genetically engineered mice with targeted expression of viral genes to tissues from which human cancers arise, as well as humanized mice implanted with the human cells usually infected by the virus.

Other types of studies can provide supportive evidence. These include: experiments in which the agent was administered in the presence of factors that modify carcinogenic effects (e.g. initiation–promotion studies); studies in which the end-point was not cancer but a defined precancerous lesion; and studies of cancer in non-laboratory animals (e.g. companion animals) exposed to the agent.

(b) Study evaluation

Considerations of importance in the interpretation and evaluation of a particular study include: (i) whether the agent was clearly characterized, including the nature and extent of impurities and contaminants and the stability of the agent, and, in the case of mixtures, whether the sample characterization was adequately reported; (ii) whether the dose was monitored adequately, particularly in inhalation experiments; (iii) whether the doses, duration and frequency of treatment, duration of observation, and route of exposure were appropriate; (iv) whether appropriate experimental animal species and strains were evaluated; (v) whether there were adequate numbers of animals per group; (vi) whether animals were allocated randomly to groups; (vii) whether the body weight, food and water consumption, and survival of treated animals were affected by any factors other than the test agent; (viii) whether the histopathology review was adequate; and (ix) whether the data were reported and analysed adequately.

(c) Outcomes and statistical analyses

An assessment of findings of carcinogenicity in experimental animals involves consideration of (i) study features such as route, doses, schedule and duration of exposure, species, strain (including genetic background where applicable), sex, age, and duration of follow-up; (ii) the spectrum of neoplastic response, from pre-neoplastic lesions and benign tumours to malignant neoplasms; (iii) the incidence, latency, severity, and multiplicity of neoplasms and pre-neoplastic

lesions; (iv) the consistency of the results for a specific target organ or organs across studies of similar design; and (v) the possible role of modifying factors (e.g. diet, infection, stress).

Key factors for statistical analysis include: (i) number of animals studied and number examined histologically, (ii) number of animals with a given tumour type or lesion, and (iii) duration of survival.

Benign tumours may be combined with malignant tumours in the assessment of tumour incidence when (a) they occur together with and originate from the same cell type as malignant tumours in an organ or tissue in a particular study and (b) they appear to represent a stage in the progression to malignancy (Huff et al., 1989). The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed.

Evidence of an increased incidence of neoplasms with increasing level of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms. The form of the dose–response relationship can vary widely, including non-linearity, depending on the particular agent under study and the target organ. The dose–response relationship can also be affected by differences in survival among the treatment groups.

The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose (Peto et al., 1980; Gart et al., 1986; Portier and Bailer, 1989; Bieler and Williams, 1993). The choice of the most appropriate statistical method requires consideration of whether there are differences in survival among the treatment groups; for example, reduced survival because of non-tumour-related mortality can preclude the occurrence of tumours later in life and a survival-adjusted analysis would be warranted. When detailed information on survival is not available, comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time that the first tumour was discovered) can be useful when significant differences in survival occur before tumours appear. The lethality of the tumour also requires consideration: for rapidly fatal tumours, the time of death provides an indication of the time of tumour onset and can be assessed using life-table methods; non-fatal or incidental tumours that do not affect survival can be assessed using methods such as the Mantel–Haenszel test for changes in tumour prevalence. Because tumour lethality is often difficult to determine, methods such as the poly-*k* test that do not require such information can also be used. When results are available on the number and size of tumours seen in experimental animals (e.g. papillomas on mouse skin, liver tumours observed through nuclear magnetic resonance tomography), other, more complicated statistical procedures may be needed (Sherman et al., 1994; Dunson et al., 2003).

The concurrent control group is generally the most appropriate comparison group for statistical analysis; however, for uncommon tumours, the analysis may be improved by considering historical control data, particularly when between-study variability is low. Historical controls should be selected to resemble the concurrent controls as closely as possible with respect to species, sex, and strain, as well as other factors, such as basal diet and general laboratory environment, which may affect tumour response rates in control animals (Haseman et al., 1984; Fung et al., 1996; Greim et al., 2003). It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls.

Meta-analyses and pooled analyses may be appropriate when the experimental protocols are sufficiently similar.

4. Mechanistic evidence

Mechanistic data may provide evidence of carcinogenicity and may also help in assessing the relevance and importance of findings of cancer in experimental animals and in humans (Guyton et al., 2009; Parkkinen et al., 2018) (see Part B, Section 6). Mechanistic studies have gained in prominence, increasing in their volume, diversity, and relevance to cancer hazard evaluation, whereas studies pertinent to other streams of evidence evaluated in the *Monographs* (i.e. studies of cancer in humans and lifetime cancer bioassays in rodents) may only be available for a fraction of agents to which humans are currently exposed (Guyton et al., 2009, 2018). Mechanistic studies and data are identified, screened, and evaluated for quality and importance to the evaluation by using systematic review principles as described in Part A, further elaborated in the Instructions for Authors, and as detailed below.

The Working Group's synthesis reflects the extent of available evidence, summarizing groups of included studies with an emphasis on characterizing consistencies or differences in results within and across experimental designs. Greater emphasis is given to informative mechanistic evidence from human-related studies than to that from other experimental test systems, and gaps are identified. Tabulation of data may facilitate this review. The specific topics addressed in the evidence synthesis are described below.

(a) Absorption, distribution, metabolism, and excretion

Studies of absorption, distribution, metabolism, and excretion in mammalian species are addressed in a summary fashion; exposure characterization is addressed in Part B, Section 1. The Working Group describes the metabolic fate of the agent in mammalian species, noting the metabolites that have been identified and their chemical reactivity. A metabolic schema may indicate the relevant metabolic pathways and products and whether supporting evidence is from studies in humans and/or studies in experimental animals. Evidence on other adverse effects that indirectly confirm absorption, distribution, and/or metabolism at tumour sites is briefly summarized when direct evidence is sparse.

(b) Evidence relevant to key characteristics of carcinogens

A review of Group 1 human carcinogens classified up to and including *IARC Monographs* Volume 100 revealed several issues relevant to improving the evaluation of mechanistic evidence for cancer hazard identification (Smith et al., 2016). First, it was noted that human carcinogens often share one or more characteristics that are related to the multiple mechanisms by which agents cause cancer. Second, different human carcinogens may exhibit a different spectrum of these key characteristics and operate through distinct mechanisms. Third, for many carcinogens evaluated before Volume 100, few data were available on some mechanisms of recognized importance in carcinogenesis, such as epigenetic alterations (Herceg et al., 2013). Fourth, there was no widely accepted method to search systematically for relevant mechanistic evidence, resulting in a lack of uniformity in the scope of mechanistic topics addressed across *IARC Monographs* evaluations.

To address these challenges, the key characteristics of human carcinogens were introduced to facilitate systematic consideration of mechanistic evidence in *IARC Monographs* evaluations (Smith et al., 2016; Guyton et al., 2018). The key characteristics described by Smith et al. (2016) (see Table 3), such as "is genotoxic", "is immunosuppressive", or "modulates receptor-mediated effects", are based on empirical observations of the chemical and biological properties associated with the human carcinogens identified by

Table 3 The key characteristics of carcinogens

	Ten key characteristics of carcinogens
1.	Is electrophilic or can be metabolically activated to an electrophile
2.	Is genotoxic
3.	Alters DNA repair or causes genomic instability
4.	Induces epigenetic alterations
5.	Induces oxidative stress
6.	Induces chronic inflammation
7.	Is immunosuppressive
8.	Modulates receptor-mediated effects
9.	Causes immortalization
10.	Alters cell proliferation, cell death, or nutrient supply

From Smith et al. (2016).

the *IARC Monographs* programme up to and including Volume 100. The list of key characteristics and associated end-points may evolve, based on the experience of their application and as new human carcinogens are identified. Key characteristics are distinct from the "hallmarks of cancer", which relate to the properties of cancer cells (Hanahan and Weinberg, 2000, 2011). Key characteristics are also distinct from hypothesized mechanistic pathways, which describe a sequence of biological events postulated to occur during carcinogenesis. As such, the evaluation approach based on key characteristics, outlined below, "avoids a narrow focus on specific pathways and hypotheses and provides for a broad, holistic consideration of the mechanistic evidence" (National Academies of Sciences, Engineering, and Medicine, 2017).

Studies in exposed humans and in human primary cells or tissues that incorporate end-points relevant to key characteristics of carcinogens are emphasized when available. For each key characteristic with adequate evidence for evaluation, studies are grouped according to whether they involve (a) humans or human primary cells or tissues or (b) experimental systems; further organization (as appropriate) is by end-point (e.g. DNA damage), duration, species, sex, strain, and target organ as well as strength of study design. Studies investigating susceptibility related to key characteristics of carcinogens (e.g. of genetic polymorphisms, or in genetically engineered animals) can be highlighted and may provide additional support for conclusions on the strength of evidence. Findings relevant to a specific tumour type may be noted.

(c) Other relevant evidence

Other informative evidence may be described when it is judged by the Working Group to be relevant to an evaluation of carcinogenicity and to be of sufficient importance to affect the overall evaluation. Quantitative structure–activity information, such as on specific chemical and/or biological features or activities (e.g. electrophilicity, molecular docking with receptors), may be informative. In addition, evidence that falls outside of the recognized key characteristics of carcinogens, reflecting emerging knowledge or important novel scientific developments on carcinogen mechanisms, may also be included. Available evidence relevant to criteria provided in authoritative publications (e.g. Capen et al., 1999; IARC, 2003) on thyroid, kidney, urinary bladder, or other tumours in experimental animals induced by mechanisms that do not operate in humans is also described.

(d) *Study quality and importance to the evaluation*

Based on formal considerations of the quality of the studies (e.g. design, methodology, and reporting of results), the Working Group may give greater weight to some included studies.

For observational and other studies in humans, the quality of study design, exposure assessment, and assay accuracy and precision are considered, in collaboration with the Working Group members reviewing exposure characterization and studies of cancer in humans, as are other important factors, including those described above for evaluation of epidemiological evidence (García-Closas et al., 2006, 2011; Vermeulen et al., 2018) (Part B, Sections 1 and 2).

In general, in experimental systems, studies of repeated doses and of chronic exposures are accorded greater importance than are studies of a single dose or time-point. Consideration is also given to factors such as the suitability of the dosing range, the extent of concurrent toxicity observed, and the completeness of reporting of the study (e.g. the source and purity of the agent, the analytical methods, and the results). Route of exposure is generally considered to be a less important factor in the evaluation of experimental studies, recognizing that the exposures and target tissues may vary across experimental models and in exposed human populations. Non-mammalian studies can be synthetically summarized when they are considered to be supportive of evidence in humans or higher organisms.

In vitro test systems can provide mechanistic insights, but important considerations include the limitations of the test system (e.g. in metabolic capabilities) as well as the suitability of a particular test article (i.e. because of physical and chemical characteristics) (Hopkins et al., 2004). For studies on some end-points, such as for traditional studies of mutations in bacteria and in mammalian cells, formal guidelines, including those from the Organisation for Economic Co-operation and Development, may be informative in conducting the quality review (OECD, 1997, 2016a, b). However, existing guidelines will not generally cover all relevant assays, even for genotoxicity. Possible considerations when evaluating the quality of in vitro studies encompass the methodology and design (e.g. the end-point and test method, the number of replicate samples, the suitability of the concentration range, the inclusion of positive and negative controls, and the assessment of cytotoxicity) as well as reporting (e.g. of the source and purity of the agent, and of the analytical methods and results). High-content and high-throughput in vitro data can serve as an additional or supportive source of mechanistic evidence (Chiu et al., 2018; Guyton et al., 2018), although large-scale screening programmes measuring a variety of end-points were designed to evaluate large chemical libraries in order to prioritize chemicals for additional toxicity testing rather than to identify the hazard of a specific chemical or chemical group.

The synthesis is focused on the evidence that is most informative for the overall evaluation. In this regard, it is of note that some human carcinogens exhibit a single or primary key characteristic, evidence of which has been influential in their cancer hazard classifications. For instance, ethylene oxide is genotoxic (IARC, 1994), 2,3,7,8-tetrachlorodibenzo-*para*-dioxin modulates receptor-mediated effects (IARC, 1997), and etoposide alters DNA repair (IARC, 2012a). Similarly, oncogenic viruses cause immortalization, and certain drugs are, by design, immunosuppressive (IARC, 2012a, b). Because non-carcinogens can also induce oxidative stress, this key characteristic should be interpreted with caution unless it is found in combination with other key characteristics (Guyton et al., 2018). Evidence for a group of key characteristics can strengthen mechanistic conclusions (e.g. "induces oxidative stress" together with "is electrophilic or can be metabolically activated to an

electrophile", "induces chronic inflammation", and "is immunosuppressive"); see, for example, 1-bromopropane (IARC, 2018).

5. Summary of data reported

(a) Exposure characterization

Exposure data are summarized to identify the agent and describe its production, use, and occurrence. Information on exposure prevalence and intensity in different settings, including geographical patterns and time trends, may be included. Exposure assessment methods used in key epidemiological studies reviewed by the Working Group are described and evaluated.

(b) Cancer in humans

Results of epidemiological studies pertinent to an evaluation of carcinogenicity in humans are summarized. The overall strengths and limitations of the epidemiological evidence base are highlighted to indicate how the evaluation was reached. The target organ(s) or tissue(s) in which a positive association between the agent and cancer was observed are identified. Exposure–response and other quantitative data may be summarized when available. When the available epidemiological studies pertain to a mixed exposure, process, occupation, or industry, the Working Group seeks to identify the specific agent considered to be most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data permit.

(c) Cancer in experimental animals

Results pertinent to an evaluation of carcinogenicity in experimental animals are summarized to indicate how the evaluation was reached. For each animal species, study design, and route of administration, there is a statement about whether an increased incidence, reduced latency, or increased severity or multiplicity of neoplasms or pre-neoplastic lesions was observed, and the tumour sites are indicated. Special conditions resulting in tumours, such as prenatal exposure or single-dose experiments, are mentioned. Negative findings, inverse relationships, dose–response patterns, and other quantitative data are also summarized.

(d) Mechanistic evidence

Results pertinent to an evaluation of the mechanistic evidence on carcinogenicity are summarized to indicate how the evaluation was reached. The summary encompasses the informative studies on absorption, distribution, metabolism, and excretion; on the key characteristics with adequate evidence for evaluation; and on any other aspects of sufficient importance to affect the overall evaluation, including on whether the agent belongs to a class of agents for which one or more members have been classified as carcinogenic or probably carcinogenic to humans, and on criteria with respect to tumours in experimental animals induced by mechanisms that do not operate in humans. For each topic addressed, the main supporting findings are highlighted from exposed humans, human cells or tissues, experimental animals, or in vitro systems. When mechanistic studies are available in exposed humans, the tumour type or target tissue studied may be specified. Gaps in the evidence are indicated (i.e. if no studies were available in exposed humans, in in vivo systems, etc.). Consistency or differences of effects across different experimental systems are emphasized.

6. Evaluation and rationale

Consensus evaluations of the strength of the evidence of cancer in humans, the evidence of cancer in experimental animals, and the mechanistic evidence are made using transparent criteria and defined descriptive terms. The Working Group then develops a consensus overall evaluation of the strength of the evidence of carcinogenicity for each agent under review.

An evaluation of the strength of the evidence is limited to the agents under review. When multiple agents being evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single and unified evaluation of the strength of the evidence.

The framework for these evaluations, described below, may not encompass all factors relevant to a particular evaluation of carcinogenicity. After considering all relevant scientific findings, the Working Group may exceptionally assign the agent to a different category than a strict application of the framework would indicate, while providing a clear rationale for the overall evaluation.

When there are substantial differences of scientific interpretation among the Working Group members, the overall evaluation will be based on the consensus of the Working Group. A summary of the alternative interpretations may be provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

The categories of the classification refer to the strength of the evidence that an exposure is carcinogenic and not to the risk of cancer from particular exposures. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used as descriptors of different strengths of evidence of carcinogenicity in humans; *probably carcinogenic* signifies a greater strength of evidence than *possibly carcinogenic*.

(a) Carcinogenicity in humans

Based on the principles outlined in Part B, Section 2, the evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

***Sufficient evidence of carcinogenicity*:** A causal association between exposure to the agent and human cancer has been established. That is, a positive association has been observed in the body of evidence on exposure to the agent and cancer in studies in which chance, bias, and confounding were ruled out with reasonable confidence.

***Limited evidence of carcinogenicity*:** A causal interpretation of the positive association observed in the body of evidence on exposure to the agent and cancer is credible, but chance, bias, or confounding could not be ruled out with reasonable confidence.

***Inadequate evidence regarding carcinogenicity*:** The available studies are of insufficient quality, consistency, or statistical precision to permit a conclusion to be drawn about the presence or the absence of a causal association between exposure and cancer, or no data on cancer in humans are available. Common findings that lead to a determination of inadequate evidence of carcinogenicity include: (a) there are no data available in humans; (b) there are data available in humans, but they are of poor quality or informativeness; and (c) there are studies of sufficient quality available in humans, but their results are inconsistent or otherwise inconclusive.

***Evidence suggesting lack of carcinogenicity*:** There are several high-quality studies covering the full range of levels of exposure that humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and the studied cancers at any observed level of exposure. The results from these studies

alone or combined should have narrow confidence intervals with an upper limit below or close to the null value (e.g. a relative risk of unity). Bias and confounding were ruled out with reasonable confidence, and the studies were considered informative. A conclusion of *evidence suggesting lack of carcinogenicity* is limited to the cancer sites, populations and life stages, conditions and levels of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

When there is *sufficient evidence*, a separate sentence identifies the target organ(s) or tissue(s) for which a causal interpretation has been established. When there is *limited evidence*, a separate sentence identifies the target organ(s) or tissue(s) for which a positive association between exposure to the agent and the cancer(s) was observed in humans. When there is *evidence suggesting lack of carcinogenicity*, a separate sentence identifies the target organ(s) or tissue(s) where evidence of lack of carcinogenicity was observed in humans. Identification of a specific target organ or tissue as having *sufficient evidence* or *limited evidence* or *evidence suggesting lack of carcinogenicity* does not preclude the possibility that the agent may cause cancer at other sites.

(b) Carcinogenicity in experimental animals

The evidence relevant to carcinogenicity from studies in experimental animals is classified into one of the following categories:

***Sufficient evidence of carcinogenicity*:** A causal relationship has been established between exposure to the agent and cancer in experimental animals based on an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories and/or under different protocols. An increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices (GLP), can also provide *sufficient evidence*.

Exceptionally, a single study in one species and sex may be considered to provide *sufficient evidence of carcinogenicity* when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour, or age at onset, or when there are marked findings of tumours at multiple sites.

***Limited evidence of carcinogenicity*:** The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, for example, (a) the evidence of carcinogenicity is restricted to a single experiment and does not meet the criteria for *sufficient evidence*; (b) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; (c) the agent increases tumour multiplicity or decreases tumour latency but does not increase tumour incidence; (d) the evidence of carcinogenicity is restricted to initiation–promotion studies; (e) the evidence of carcinogenicity is restricted to observational studies in non-laboratory animals (e.g. companion animals); or (f) there are unresolved questions about the adequacy of the design, conduct, or interpretation of the available studies.

***Inadequate evidence regarding carcinogenicity*:** The studies cannot be interpreted as showing either the presence or the absence of a carcinogenic effect because of major

qualitative or quantitative limitations, or no data are available on cancer in experimental animals.

***Evidence suggesting lack of carcinogenicity*:** Well-conducted studies (e.g. conducted under GLP) involving both sexes of at least two species are available showing that, within the limits of the tests used, the agent was not carcinogenic. The conclusion of *evidence suggesting lack of carcinogenicity* is limited to the species, tumour sites, age at exposure, and conditions and levels of exposure covered by the available studies.

(c) Mechanistic evidence

Based on the principles outlined in Part B, Section 4, the mechanistic evidence is classified into one of the following categories:

***Strong mechanistic evidence*:** Results in several different experimental systems are consistent, and the overall mechanistic database is coherent. Further support can be provided by studies that demonstrate experimentally that the suppression of key mechanistic processes leads to the suppression of tumour development. Typically, a substantial number of studies on a range of relevant end-points are available in one or more mammalian species. Quantitative structure–activity considerations, in vitro tests in non-human mammalian cells, and experiments in non-mammalian species may provide corroborating evidence but typically do not in themselves provide strong evidence. However, consistent findings across a number of different test systems in different species may provide strong evidence.

Of note, "strong" relates not to potency but to strength of evidence. The classification applies to three distinct topics:

(a) Strong evidence that the agent belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified as carcinogenic or probably carcinogenic to humans. The considerations can go beyond quantitative structure–activity relationships to incorporate similarities in biological activity relevant to common key characteristics across dissimilar chemicals (e.g. based on molecular docking, –omics data).

(b) Strong evidence that the agent exhibits key characteristics of carcinogens. In this case, three descriptors are possible:

1. The strong evidence is in exposed humans. Findings relevant to a specific tumour type may be informative in this determination.
2. The strong evidence is in human primary cells or tissues. Specifically, the strong findings are from biological specimens obtained from humans (e.g. ex vivo exposure), from human primary cells, and/or, in some cases, from other humanized systems (e.g. a human receptor or enzyme).
3. The strong evidence is in experimental systems. This may include one or a few studies in human primary cells and tissues.

(c) Strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans. Certain results in experimental animals (see Part B, Section 6b) would be discounted, according to relevant criteria and considerations in authoritative publications (e.g. Capen et al., 1999; IARC, 2003). Typically, this classification would not apply when there is strong mechanistic evidence that the agent exhibits key characteristics of carcinogens.

Limited mechanistic evidence: The evidence is suggestive, but, for example, (a) the studies cover a narrow range of experiments, relevant end-points, and/or species; (b) there are unexplained inconsistencies in the studies of similar design; and/or (c) there is unexplained incoherence across studies of different end-points or in different experimental systems.

Inadequate mechanistic evidence: Common findings that lead to a determination of inadequate mechanistic evidence include: (a) few or no data are available; (b) there are unresolved questions about the adequacy of the design, conduct, or interpretation of the studies; (c) the available results are negative.

(d) Overall evaluation

Finally, the bodies of evidence included within each stream of evidence are considered as a whole, in order to reach an overall evaluation of the carcinogenicity of the agent to humans. The three streams of evidence are integrated and the agent is classified into one of the following categories (see Table 4), indicating that the Working Group has established that:

The agent is carcinogenic to humans (Group 1)

This category applies whenever there is *sufficient evidence of carcinogenicity* in humans.

In addition, this category may apply when there is both *strong evidence in exposed humans that the agent exhibits key characteristics of carcinogens* and *sufficient evidence of carcinogenicity* in experimental animals.

The agent is probably carcinogenic to humans (Group 2A)

This category generally applies when the Working Group has made at least *two of the following* evaluations, *including at least one* that involves either exposed humans or human cells or tissues:

- *Limited evidence of carcinogenicity* in humans,
- *Sufficient evidence of carcinogenicity* in experimental animals,
- *Strong evidence that the agent exhibits key characteristics of carcinogens.*

If there is *inadequate evidence regarding carcinogenicity* in humans, there should be *strong evidence in human cells or tissues that the agent exhibits key characteristics of carcinogens.* If there is *limited evidence of carcinogenicity in humans*, then the second individual evaluation may be from experimental systems (i.e. *sufficient evidence of carcinogenicity* in experimental animals or *strong evidence in experimental systems that the agent exhibits key characteristics of carcinogens).*

Additional considerations apply when there is *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* for one or more tumour sites. Specifically, the remaining tumour sites should still support an evaluation of *sufficient evidence in experimental animals* in order for this evaluation to be used to support an overall classification in Group 2A.

Separately, this category generally applies if there is *strong evidence that the agent belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A.*

The agent is possibly carcinogenic to humans (Group 2B)

This category generally applies when only one of the following evaluations has been made by the Working Group:

- *Limited evidence of carcinogenicity* in humans,
- *Sufficient evidence of carcinogenicity* in experimental animals,

Table 4 Integration of streams of evidence in reaching overall classifications (the evidence in *bold italic* represents the basis of the overall evaluation)

Stream of evidence			Classification based on strength of evidence
Evidence of cancer in humans[a]	Evidence of cancer in experimental animals	Mechanistic evidence	
Sufficient	Not necessary	Not necessary	**Carcinogenic to humans (Group 1)**
Limited or Inadequate	***Sufficient***	***Strong (b)(1) (exposed humans)***	
Limited	***Sufficient***	Strong (b)(2–3), Limited, or Inadequate	**Probably carcinogenic to humans (Group 2A)**
Inadequate	***Sufficient***	***Strong (b)(2) (human cells or tissues)***	
Limited	Less than Sufficient	***Strong (b)(1–3)***	
Limited or Inadequate	Not necessary	***Strong (a) (mechanistic class)***	
Limited	Less than Sufficient	Limited or Inadequate	**Possibly carcinogenic to humans (Group 2B)**
Inadequate	***Sufficient***	Strong (b)(3), Limited, or Inadequate	
Inadequate	Less than Sufficient	***Strong (b)(1–3)***	
Limited	***Sufficient***	***Strong (c) (does not operate in humans)***[b]	
Inadequate	***Sufficient***	***Strong (c) (does not operate in humans)***[b]	**Not classifiable as to its carcinogenicity to humans (Group 3)**
All other situations not listed above			

[a] Human cancer(s) with highest evaluation.

[b] The *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* must specifically be for the tumour sites supporting the classification of *sufficient evidence in experimental animals*.

- *Strong evidence that the agent exhibits key characteristics of carcinogens.*

Because this category can be based on evidence from studies in experimental animals alone, there is **no** requirement that the strong mechanistic evidence be in exposed humans or in human cells or tissues. This category may be based on *strong evidence in experimental systems that the agent exhibits key characteristics of carcinogens.*

As with Group 2A, additional considerations apply when there is *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* for one or more tumour sites. Specifically, the remaining tumour sites should still support an evaluation of *sufficient evidence in experimental animals* in order for this evaluation to be used to support an overall classification in Group 2B.

The agent is not classifiable as to its carcinogenicity to humans (Group 3)

Agents that do not fall into any other group are generally placed in this category.

This includes the case when there is *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* for one or more tumour sites in experimental animals, the remaining tumour sites do not support an evaluation of *sufficient evidence in experimental animals*, and other categories are not supported by data from studies in humans and mechanistic studies.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that the agent is of unknown carcinogenic potential and that there are significant gaps in research.

If the evidence suggests that the agent exhibits no carcinogenic activity, either through *evidence suggesting lack of carcinogenicity* in both humans and experimental animals, or through *evidence suggesting lack of carcinogenicity* in

experimental animals complemented by strong negative mechanistic evidence in assays relevant to human cancer, then the Working Group may add a sentence to the evaluation to characterize the agent as well-studied and without evidence of carcinogenic activity.

(e) Rationale

The reasoning that the Working Group used to reach its evaluation is summarized so that the basis for the evaluation offered is transparent. This section integrates the major findings from studies of cancer in humans, cancer in experimental animals, and mechanistic evidence. It includes concise statements of the principal line(s) of argument that emerged in the deliberations of the Working Group, the conclusions of the Working Group on the strength of the evidence for each stream of evidence, an indication of the body of evidence that was pivotal to these conclusions, and an explanation of the reasoning of the Working Group in making its evaluation.

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GENERAL REMARKS

This one-hundred-and-thirty-fifth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of perfluoro-octanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), and their corresponding isomers and salts.

PFOA was considered previously by the *IARC Monographs* programme in 2014 (IARC, 2016), when it was evaluated as *possibly carcinogenic to humans* (Group 2B). PFOS has not been evaluated previously by the *IARC Monographs* programme.

The Advisory Group to Recommend Priorities for the *IARC Monographs* that met in 2019 recommended that some perfluorinated compounds, such as PFOA, be evaluated with high priority (IARC, 2019a; Marques et al., 2019), largely on the basis of emerging evidence of carcinogenicity in experimental animals and of mechanistic evidence related to the key characteristics of carcinogens (KCs). A summary of the findings of this volume appears in *The Lancet Oncology* (Zahm et al., 2023).

PFOA and PFOS in the context of the broader class of PFAS

PFOA and PFOS are part of a class of thousands of synthetic per- and polyfluoroalkyl substances (PFAS) that are used widely throughout the world. The Working Group noted that the carbon–fluorine bond is one of the strongest in organic chemistry and is responsible for the environmental and biological persistence, long-range environmental transport, as well as bioaccumulation and biomagnification of this class of chemicals. In the present monograph, the Working Group assessed the carcinogenic hazard of only two PFAS, the uses of both of which have restricted or eliminated under the Stockholm Convention to which more than 180 countries are parties (UNEP, 2023). Information is limited regarding exposure to precursors of PFOA and PFOS and to PFAS used to replace PFOA and PFOS, and few studies in humans have examined these substances as the primary exposure metric when evaluating health outcomes.

Lack of comprehensive exposure data for PFOA and PFOS

The Working Group noted major gaps in the existing literature that hampered the understanding of PFOA and PFOS exposure worldwide. When stratified by location or exposure source, including country (e.g. within the USA and Europe versus outside, as well as in communities with a known source of contamination versus those without), this gap in knowledge was exacerbated by the absence of surveillance initiatives.

Although many countries have phased-out the production and/or use of PFOA or PFOS, the Working Group identified studies indicating that certain precursor PFAS are known to break down or transform into PFOA and PFOS in the environment and biological systems, including in humans. This suggests that ongoing exposure may be expected, even if production and use of PFOA and PFOS compounds were to cease entirely around the world.

Although the workplace is often the source of highest exposure to PFAS, characterization of occupational exposure to PFOA and PFOS was limited to only a few occupations. The majority of studies focused on biomonitoring of fluorochemical-production workers (including perfluoroalkyl polymer-production workers) and first responders (especially firefighters), and other occupations that produce, use, or dispose of products that have been treated with or contain PFAS have been examined to a lesser degree (if at all). Female workers are largely absent in the available literature, limiting potential epidemiological analyses of occupational exposure sources among women. Additionally, the relative contribution of different exposure routes, namely dermal absorption versus inhalation, in these settings is poorly understood.

New evidence on cancer in humans published since the previous *IARC Monographs* evaluation

When PFOA was evaluated by the *IARC Monographs* programme in 2014, the epidemiological evidence consisted of studies on three occupationally exposed populations, one population exposed to drinking-water that was highly contaminated via a nearby industrial facility, and three case–control studies of members of the general population in communities without a PFOA pollution point source (this ambient exposure is referred to in the present monograph as "background" exposure). The present evaluations of PFOA and PFOS are based on 36 epidemiological studies, including further reports on the same three occupationally exposed populations, two additional populations in highly contaminated areas, and many case–control studies in the general population. The latter included nested case–control studies using prospectively collected biospecimens and less-informative, non-nested case–control studies using biospecimens collected after diagnosis of cancer and, in some instances, after treatment for cancer. Ecological studies, with the exception of one with an extremely high contrast in environmental exposure to PFOA relative to exposure to other PFAS, were excluded from the review. Despite these additional studies, data gaps and limitations remain, including low exposure contrasts in the studies of "background" exposure, potential healthy-worker survivor bias in most of the occupational studies, and, in case–control studies, uncertainties surrounding the measurement of PFOA and PFOS after diagnosis and, possibly, treatment for cancer. Additionally, there were few studies that addressed cancer subtypes defined by histology, genotype, receptor status, and other characteristics. Another data gap was the lack of studies among additional populations known to have occupational or substantial environmental exposure, such as workers in fluorochemical production or residents in communities with substantial pollution, e.g. in Italy, France, or Australia. Such studies might help address the data gaps noted above related to the carcinogenicity of PFOS and to the specific cancer types linked to PFOA and PFOS exposure in populations with high exposure contrast.

One challenge in the epidemiological literature is the difficulty in evaluating the effects of individual PFAS compounds, because there is widespread co-exposure to many highly correlated PFAS compounds. The evaluation of the cancer hazard resulting from exposure to

mixtures of PFAS compounds, although important, was beyond the scope of the present volume and may require the development of new statistical analytical approaches.

The Working Group conducted three new analyses of existing epidemiological data, which assisted in their evaluation (see Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans): (i) an analysis based on summary statistics for repeated serological measurements of PFOA that were available from subsets of participants in two nested case–control studies, which were used to evaluate the representativeness of serum PFOA measurements from a single time point as a surrogate for longer term levels; (ii) a meta-analysis of PFOA exposure and kidney cancer; and (iii) an ecological analysis of the correlation between serum concentration measurements of PFOA and the rates of orchiectomies (a strong correlate of testicular cancer incidence in this region) within 21 municipalities in the Veneto region of Italy where there had been industrial contamination of drinking-water with PFOA.

Extensive mechanistic evidence

Since the previous *IARC Monographs* evaluation of PFOA in 2014, by far the greatest increase in the amount of research available has occurred with respect to toxicokinetic data and mechanistic evidence, including data relevant to the KCs (see Section 4). Particularly noteworthy is the extent of evidence related to epigenetic alterations (Section 4.2.4) and immunosuppression (Section 4.2.7) in exposed humans. The Working Group noted that there are only a few agents evaluated by the *IARC Monographs* programme (e.g. occupational exposure as a firefighter) for which there are such extensive data from multiple studies in multiple populations supporting these KCs. These data, combined with the data from cancer bioassays in experimental animals, underpin the rationale for the evaluation of PFOA as *carcinogenic to humans*, Group 1. Moreover, this is the first time that mechanistic evidence from a variety of test systems specifically for these two KCs has supported a Group 1 evaluation, particularly in the absence of strong evidence in exposed humans for either genotoxicity (KC2) or modulation of receptor-mediated effects (KC8).

It should also be noted that the contribution of mechanistic evidence in exposed humans to a Group 1 evaluation does not require a PFOA-specific mechanism of carcinogenicity to be identified. Thus, although empirical data directly linking PFOA-specific effects on the epigenome and immune system to increased cancer risk in humans were not available, it was the judgement of the Working Group that the observed effects in exposed humans, supported by evidence in human primary cells and in experimental systems, were sufficiently linked to carcinogenic processes to support a Group 1 evaluation, in combination with the positive results in cancer bioassays in animals.

Challenges in using PFOA and PFOS to define a mechanistic class of carcinogens

For the present volume, the Working Group identified overall similar mechanistic evidence for PFOA and PFOS across the KCs on the basis of data obtained in exposed humans, in human primary cells, and in experimental systems; as reported above, this included consistent and coherent mechanistic evidence for the KCs “induces epigenetic alterations” (KC4) and “is immunosuppressive” (KC7). In addition, it was reported that both agents have long half-lives in humans and both bind to multiple relevant protein targets, including nuclear receptors,

membrane transporters, and carrier proteins. However, it remained unclear whether PFOA might represent a mechanistic class of carcinogens to which PFOS (or other PFAS compounds) may belong. Despite a rich mechanistic database (as reviewed in Section 4 of the present monograph), the Working Group could not identify a common specific mechanism by which exposure to PFOA and PFOS leads to carcinogenesis; it is possible or even likely that multiple mechanisms are in play.

It is worth noting that additional data streams that could be helpful in establishing a chemical class, including studies on the non-cancer-related toxicity of PFOA and PFOS independent of the KCs, relative potency considerations, and mixture effects, were beyond the scope of an *IARC Monographs* evaluation. Although both PFOA and PFOS appear to activate a similar suite of nuclear receptors in human primary cells and experimental systems in vivo (e.g. there was consistent and coherent evidence for activation of peroxisome proliferator-activated receptor alpha (PPARα), constitutive androstane receptor (CAR), and pregnane X receptor (PXR) and suggestive evidence for activation of PPARγ in human primary cells), the degree of activation differs between the receptors, potentially influencing the strength of receptor-driven carcinogenic effects. Additionally, technical challenges hinder the ascertainment of whether there is modulation of these receptor pathways in exposed humans, since accessible and specific biomarkers of these pathways are not readily available in humans. This data gap is compounded by the fact that there are species differences in the events associated with modulation of these pathways by PFOA and PFOS. As yet, no studies have been conducted in mice expressing human genes for these receptors.

Overall, the Working Group was not able to conclude whether PFOA could represent a mechanistic class to which PFOS (or other PFAS) belong, based on considerations described in the Preamble to the *IARC Monographs* (see present volume; IARC, 2019b).

Relevance to humans of PFOA and PFOS effects on altered lipid metabolism in rodents

The Working Group noted that nuclear receptor activation and deregulation of lipid metabolism are relevant KC-related end-points and mechanisms that might contribute to the hepatocarcinogenicity of PFOA and PFOS in rodents.

The activation of hepatic PPARα and CAR/PXR in rodents has been reported to: transiently increase the activity of liver enzymes such as acyl coenzyme A (CoA) oxidase, and cytochrome P450s CYP4A, CYP2B, and CYP3A; increase the liver proliferative index and decrease the liver apoptotic index; decrease the frequency of hepatocellular glycogen-induced vacuoles; increase the frequency of centrilobular hepatocellular hypertrophy (Elcombe et al., 2012a, b), but also cause alterations in plasma cholesterol level, and increase centrilobular hepatocellular hypertrophy. Also, induction of hepatic steatosis has been observed in mice after dietary exposure to PFOS (Bagley et al., 2017).

The molecular mechanisms by which PFOA or PFOS can cause hepatotoxicity (e.g. fatty liver disease and other hepatotoxic effects) have not been fully described either in experimental animals or in humans. However, an accumulation of fatty acids and triglycerides and deregulation of the expression of genes related to the metabolism of fatty acids and triglycerides has been reported in a series of in vitro studies (as well as several epidemiological studies) (e.g. Wan et al., 2012; Louisse et al., 2020). These and other effects independent of the PPARα receptor that cause deregulation of gene expression, resulting in a substantial shift from carbohydrate metabolism

to fatty acid oxidation and hepatic triglyceride accumulation, have been also observed in human and rat primary liver cells (Vanden Heuvel et al., 2006; Bjork et al., 2011; Das et al., 2017; Rosen et al., 2017; Behr et al., 2018).

Other mechanisms that could be related to PFOA/PFOS-induced hepatocarcinogenicity through alterations in lipid metabolism, identified in studies in human hepatoma cells, include the activation of specific endoplasmic reticulum stress (ERS)-response genes (e.g. ATF4, DDIT3, ATF3) and enzymes involved in lipid metabolism, e.g. cholesterol (HMGCR), upon PFOS exposure (Louisse et al., 2023). After exposure to PFOA, activation of the unfolded protein response (UPR) pathway, induction of steatosis and fibrosis and expression of TNFα and IL6 inflammatory markers, increased production of endogenous reactive oxygen species in liver cells (Qi et al., 2023), and deregulation of the genes controlling lipid homeostasis (Das et al., 2017) were observed. In addition, ERS/UPR stress was also induced by PFOA in pancreatic acinar cells (Hocevar et al., 2020); and induction of cell proliferation and migration and invasion upon exposure to PFOA or PFOS were reported for various in vitro models (Matkowskyj et al., 2014; Pierozan et al., 2020; Hu et al., 2022).

Consistent with a potential involvement of both PFOA and PFOS in metabolic alterations, the Working Group identified data from metabolomic analyses in exposed humans suggesting increased activities of glycolytic pathways. Transcriptomic analyses have also indicated alterations in cell proliferation and in lipid metabolism pathways in human primary and experimental systems, respectively (see Section 4.2.11).

Data gaps for PFOS and other PFAS

The evaluation of the carcinogenicity of PFOS was hampered by a relative paucity of studies of cancer in humans and also by the existence of only one study in experimental animals that complied with Good Laboratory Practice and gave positive results. However, the strength of the mechanistic evidence for PFOS, together with its relatively potent toxic effects, suggest that additional carcinogenicity studies may fill this data gap.

Many “novel” or emerging PFAS are currently used, but the toxic and carcinogenic characteristics of most of these have not been tested systematically. Some emerging PFAS have a chemical structure similar to that of PFOA and PFOS, and a similar pattern of effects has been reported for PFOA, PFOS, and several other PFAS, e.g. perfluorononanoic acid (PFNA). In view of the large number of PFAS in past and present use, it has been suggested that grouping these compounds on the basis of chemical structure or exposure levels would facilitate the choice of suitable candidates for future research on their impact on human health. However, there is a gap in data to support such potential groupings of PFAS. Similarly, the effects of typical mixtures of PFAS have not been characterized systematically and remain a subject of ongoing research.

Scope of the systematic review

Standardized searches of the PubMed database (NCBI, 2023) were conducted for PFOA and PFOS for each outcome (cancer in humans, cancer in experimental animals, and mechanistic evidence, including the KCs). For cancer in humans, searches were also conducted in the Web of Science (Clarivate, 2023) and Embase (Elsevier, 2023) databases. The literature tree for PFOA and PFOS, including the full set of search

terms for the agent name and each outcome type, is available online.[a]

As described in the current Preamble to the *IARC Monographs* (last revised in 2019; IARC, 2019b; see present volume), the Working Group reviews publicly available scientific data, such as peer-reviewed papers in the scientific literature, and may also review unpublished reports, if made available in their final form by governmental agencies and if they contain enough detail for critical review. A public Call for Data was opened on the *IARC Monographs* website 1 year ahead of the meeting for Volume 135. Eligible studies were only those published or accepted for publication in the openly available scientific literature by the time of the Working Group meeting.

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1. EXPOSURE CHARACTERIZATION

1.1 Identification of the agent

Because of their acidic nature, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) exist in the environment, and in aqueous solutions, in equilibrium with their conjugated bases, perfluorooctanoate and perfluorooctane sulfonate. Salts of PFOA and PFOS will dissociate in solution and in the human body (except the stomach) to produce the respective anions perfluorooctanoate and perfluorooctane sulfonate.

The terms "PFOA" and "PFOS" are used for both the acid and the deprotonated form in environmental or biological samples, if not otherwise specified.

All isomeric forms of PFOA and PFOS and their salts should be considered to be part of the definition of the agents considered in the present monograph.

1.1.1 Nomenclature and molecular information

(a) PFOA and its salts

The agents considered in the present monograph include PFOA and its salts (see Table 1.1 for a non-exhaustive list). PFOA and its salts exist as linear and branched isomers (see Fig. 1.1). Depending on the production method used, PFOA is present primarily as the linear isomer or as a mixture of linear (*n*-isomer) and branched isomers (see Section 1.2).

(b) PFOS and its salts

The agents considered in this present monograph include PFOS and its salts. Linear and branched isomers of PFOS and its salts exist (see Table 1.2). Depending on the production method, PFOS is present primarily as the linear isomer or as a mixture of linear and branched isomers (see Fig. 1.2).

1.1.2 Chemical and physical properties of the pure substances

Selected chemical and physical properties of PFOA and PFOS are presented in Table 1.3. [The Working Group noted that there is some inconsistency in the data reported for these agents. This may be attributed to a combination of factors, including the purity of the acid form used to conduct the measurement; the low water solubility of the pure acid forms; and their strong surface active properties, resulting in sorption to interfaces such as the water surface or the walls of a glass vessel to an extent that is unknown for other substances (Goss, 2008).] The salts of PFOA and PFOS are more soluble in water than are their acid forms. For example, the water solubility of PFOA is 9.5 g/L, whereas the water solubility of ammonium perfluorooctanoate (APFO) is

Table 1.1 Nomenclature and molecular information for PFOA isomers and selected salts

Chemical name	CAS No.	IUPAC name and synonyms	Molecular formula	Relative molecular mass
n-Perfluorooctanoic acid	335-67-1 (NCBI, 2023a)	2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Pentadecafluoro-octanoic acid PFOA; *n*-Perfluorooctanoic acid; Pentadecafluoro-1-octanoic acid; Pentadecafluoro-*n*-octanoic acid; Pentadecafluorooctanoic acid; Perfluorocaprylic acid; Perfluoroctanoic acid; Perfluoroheptanecarboxylic acid (NCBI, 2023a)	$C_8HF_{15}O_2$ (NCBI, 2023a)	414.07 (NCBI, 2023a)
branched-Perfluorooctanoic acid	207678-51-1 705240-04-6 1144512-18-4 909009-42-3 15166-06-0 1144512-35-5 1192593-79-5 1144512-36-6 1144512-34-4 35605-76-6 (Nielsen, 2012)	sb-Perfluorooctanoic acid (CDC, 2022), br-Perfluorooctanoic acid (e.g. Jin et al., 2020) [The Working Group noted that different sums of isomers have been used. The exact definition varies between studies and might include all or just some of the isomers.] (See Fig. 1.1 for the names of some isomers)	$C_8HF_{15}O_2$ (NCBI, 2023a)	414.07 (NCBI, 2023a)
Ammonium perfluorooctanoate	3825-26-1 207678-62-4 19742-57-5 13058-65-5 (Nielsen, 2012)	Ammonium perfluorocaprylate; Pentadecafluorooctanoic acid ammonium salt; Octanoic acid, pentadecafluoro-, ammonium salt, APFO (NCBI, 2023c)	$C_8H_4F_{15}NO_2$ (NCBI, 2023c)	431.10 (NCBI, 2023c)
Sodium perfluorooctanoate	335-95-5 207678-72-6 646-84-4 18017-22-6 1195164-59-0 (Nielsen, 2012)	Sodium perfluorocaprylate; Octanoic acid, pentadecafluoro-, sodium salt; Perfluorooctanoic acid sodium salt (NCBI, 2023d)	$C_8F_{15}NaO_2$ (NCBI, 2023d)	436.05 (NCBI, 2023d)
Potassium perfluorooctanoate	2395-00-8 207678-65-7 29457-73-6 (Nielsen, 2012)	Potassium, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-octanoate; Octanoic acid, pentadecafluoro-, potassium salt (NCBI, 2023e)	$C_8F_{15}KO_2$ (NCBI, 2023e)	452.16 (NCBI, 2023e)

br, branched; CAS, Chemical Abstracts Service Registry; IUPAC, International Union of Pure and Applied Chemistry; PFOA, perfluorooctanoic acid; sb, sum of branched isomers.

> 500 g/L, at 20 °C (OECD, 2008). [The Working Group noted that other properties of the salts might be different from those of the acid form, but data are lacking.]

[The Working Group noted that even though the data on the pK_a of PFOA and PFOS were inconsistent, the values were in the range of that for weak to strong acids. In aqueous samples of low concentrations (e.g. drinking-water, bio-specimen), it can be assumed that both agents are mainly present in the deprotonated form.]

Fig. 1.1 Main salts and isomers of PFOA

a. PFOA isomers

Structure of carbon chain	CAS No.
COOH	335-67-1 Linear PFOA
COOH	207678-51-1 Perfluoro-2-methyl-heptanoic acid
COOH	705240-04-6 Perfluoro-3-methyl-heptanoic acid
COOH	1144512-18-4 Perfluoro-4-methyl-heptanoic acid
COOH	909009-42-3 Perfluoro-5-methyl-heptanoic acid
COOH	15166-06-0 Perfluoro-6-methyl-heptanoic acid
COOH	1144512-35-5 Perfluoro-3,5-dimethyl-hexanoic acid
COOH	1192593-79-5 Perfluoro-4,4-dimethyl-hexanoic acid
COOH	1144512-36-6 Perfluoro-4,5-dimethyl-hexanoic acid
COOH	1144512-34-4 Perfluoro-5,5-dimethyl-hexanoic acid
COOH	35605-76-6 Perfluoro-2-ethyl-hexanoic acid

b. Ammonium salts of PFOA isomers (APFO)

Structure of carbon chain	CAS No.
$COO^{-}NH_4^{+}$	3825-26-1
$COO^{-}NH_4^{+}$	207678-62-4
$COO^{-}NH_4^{+}$	19742-57-5
$COO^{-}NH_4^{+}$	13058-06-5

c. Sodium salts of PFOA isomers

Structure of carbon chain	CAS No.
$COO^{-}Na^{+}$	335-95-5
$COO^{-}Na^{+}$	207678-72-6
$COO^{-}Na^{+}$	646-84-4
$COO^{-}Na^{+}$	18017-22-6
$COO^{-}Na^{+}$	1195164-59-0

d. Potassium salts of PFOA isomers

Structure of carbon chain	CAS No.
$COO^{-}K^{+}$	2395-00-8
$COO^{-}K^{+}$	207678-65-7
$COO^{-}K^{+}$	29457-73-6

APFO, ammonium perfluorooctanoate; CAS, Chemical Abstracts Service; PFOA, perfluorooctanoic acid.
From Nielsen (2012), as cited in IARC (2016).

Table 1.2 Nomenclature and molecular information for PFOS isomers and selected salts

Chemical name	CAS No.	IUPAC name and synonyms	Molecular formula	Relative molecular mass
n-Perfluorooctane-sulfonic acid	1763-23-1 (NCBI, 2023b)	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluorooctane-1-sulfonic acid PFOS, *n*PFOS; Heptadecafluorooctane-1-sulfonic acid; Perfluorooctane sulfonate; Perfluorooctane-1-sulfonic acid; Perfluorooctylsulfonic acid; Heptadecafluoro-1-octanesulfonic acid; Heptadecafluorooctane sulfonic acid; 1-Perfluorooctanesulfonic acid (NCBI, 2023b; Royal Society of Chemistry, 2023)	$C_8HF_{17}O_3S$ (NCBI, 2023b)	500.13 (NCBI, 2023b)
Branched-Perfluorooctane-sulfonic acid	255831-20-0 747385-21-3 775554-63-7 740777-79-1 765246-09-1 927670-12-0 950669-24-6 950669-23-5 950669-22-4 950669-21-3 927670-09-5 (CAS, 2023)	sm-Perfluorooctanesulfonic acid (CDC, 2022), br-Perfluorooctanesulfonic acid (EFSA Panel on Contaminants in the Food Chain, 2018) [The Working Group noted that different sums of isomers have been used. The exact definition varies between studies and might include all or just some of the isomers.] See Fig. 1.2 for the names of some isomers.	$C_8HF_{17}O_3S$ (NCBI, 2023b)	500.13 g/mol (NCBI, 2023b)
Ammonium perfluorooctane-sulfonate	29081-56-9 (NCBI, 2023f)	Ammonium heptadecafluoro-1-octanesulfonate (NCBI, 2023f)	$C_8H_4F_{17}NO_3S$ (NCBI, 2023f)	517.16 g/mol (NCBI, 2023f)
Potassium perfluorooctane-sulfonate	2795-39-3 (NCBI, 2023h)	Potassium heptadecafluoro-1-octanesulfonate 1-Octanesulfonic acid, heptadecafluoro-, potassium salt (NCBI, 2023h)	$C_8F_{17}KO_3S$ (NCBI, 2023h)	538.22 g/mol (NCBI, 2023h)
Lithium perfluorooctane-sulfonate	29457-72-5 (NCBI, 2023g)	Lithium heptadecafluorooctanesulfonate Heptadecafluoro-1-octanesulfonic acid lithium salt (NCBI, 2023g)	$C_8F_{17}LiO_3S$ (NCBI, 2023g)	506.10 g/mol (NCBI, 2023g)

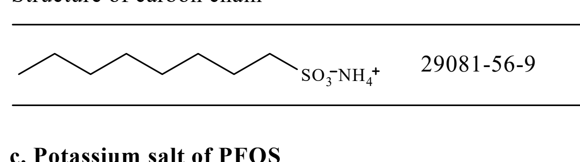
br, branched; CAS, Chemical Abstracts Service Registry; IUPAC, International Union of Pure and Applied Chemistry; PFOS, perfluorooctanesulfonic acid; sm, sum of perfluoromethylheptane sulfonate isomers.

Fig. 1.2 Main salts and isomers of PFOS

a. PFOS isomers

Structure of carbon chain	CAS No.
	1763-23-1 Linear PFOS
	255831-20-0 Perfluoro-6-methyl-heptanesulfonic acid
	747385-21-3 Perfluoro-5-methyl-heptanesulfonic acid
	775554-63-7 Perfluoro-4-methyl-heptanesulfonic acid
	740777-79-1 Perfluoro-3-methyl-heptanesulfonic acid
	765246-09-1 Perfluoro-2-methyl-heptanesulfonic acid
	927670-12-0 Perfluoro-1-methyl-heptanesulfonic acid
	950669-24-6 Perfluoro-1,1-dimethyl-hexanesulfonic acid
	950669-23-5 Perfluoro-2,2-dimethyl-hexanesulfonic acid
	950669-22-4 Perfluoro-3,3-dimethyl-hexanesulfonic acid
	950669-21-3 Perfluoro-4,4-dimethyl-hexanesulfonic acid
	927670-09-5 Perfluoro-5,5-dimethyl-hexanesulfonic acid

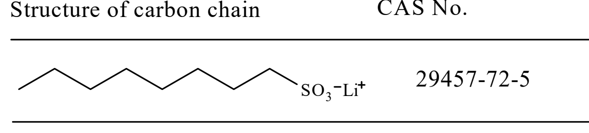
b. Ammonium salt of PFOS

Structure of carbon chain	CAS No.
	29081-56-9

c. Potassium salt of PFOS

Structure of carbon chain	CAS registry number
	2795-39-3

d. Lithium salt of PFOS

Structure of carbon chain	CAS No.
	29457-72-5

CAS, Chemical Abstracts Service; PFOS, perfluorooctanesulfonic acid.
From Langlois and Oehme (2006); Miralles-Marco and Harrad (2015).

Table 1.3 Chemical and physical properties of pure PFOA and PFOS in acid form

Property	PFOA	PFOS
Boiling-point	192 °C (US EPA, 2017a; NCBI, 2023a)	258–260 °C (US EPA, 2017a)
Melting-point	54.3 °C (IARC, 2016; ATSDR, 2021)	84 °C [The Working Group noted that these are predicted data (US EPA, 2023a)]
Vapour pressure	[0.0421 hPa] at 25 °C (ATSDR, 2021; NCBI, 2023a), [0.700 hPa] at 25 °C (US EPA, 2017a)	[0.003 hPa] at 25 °C (US EPA, 2017a; NCBI, 2023b)
Water solubility	9.5 g/L at 25 °C (IARC, 2016; US EPA, 2017a; ATSDR, 2021)	680 mg/L at 25 °C (US EPA, 2017a; NCBI, 2023b)
Density	1.8 g/cm³ at 20 °C (IARC, 2016; ATSDR, 2021)	1.84 g/cm³ [The Working Group noted that these are predicted data (US EPA, 2023a)]
log K_{ow} (octanol/water partition coefficient, *P*)	Not measurable, since PFOA forms multiple layers in an octanol/water mixture (ATSDR, 2021)	Not measurable, since PFOS forms multiple layers in an octanol/water mixture (ATSDR, 2021; NCBI, 2023b)
log K_{oc} (organic carbon/water partition coefficient)	2.06 (US EPA, 2017a)	2.57–3.14 (US EPA, 2017a; ATSDR, 2021)
Conversion factor	1 ppm = 16.94 mg/m³, 1 mg/m³ = 0.059 ppm, at 25 °C and 101 kPa	1 ppm = 20.45 mg/m³; 1 mg/m³ = 0.049 ppm, at 25 °C and 101 kPa
Physical description	White to off-white powder (ATSDR, 2021)	White powder (ACS, 2019); also reported as off-white to grey liquid (NCBI, 2023b)
Stability	When heated to decomposition, it emits toxic vapours of hydrogen fluoride. Perfluoroalkyl carboxylates are resistant to direct photolysis and reaction with acids, bases, oxidants, and reductants (IARC, 2016; ATSDR, 2021).	When heated to decomposition, it emits toxic vapours of sulfur oxides and fluorine (NCBI, 2023b). Perfluoroalkyl sulfonates are resistant to direct photolysis and reaction with acids, bases, oxidants, and reductants (ATSDR, 2021).

PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; ppm, parts per million.

1.1.3 Technical grade and impurities

(a) PFOA

Before 2002, PFOA, which was produced mainly by the electrochemical fluorination (ECF) method, was reported to have a consistent isomer composition of 78% ± 1.2% linear isomers and 22% ± 1.2% branched-chain isomers in 18 production lots tested over a 20-year period. PFOA produced by ECF was reported to contain the following impurities: perfluorohexanoate, 0.73%; perfluoroheptanoate, 3.7%; perfluorononanoate, 0.2%; perfluorodecanoate, 0.0005%; perfluoroundecanoate, 0.0008%; and perfluorododecanoate, 0.0008%. From 2002 onwards, PFOA, which is produced mainly by the telomerization method, is typically an isomerically pure, linear product (Benskin et al., 2010a; IARC, 2016).

(b) PFOS

PFOS and its salts are mainly produced by ECF. This ECF-produced PFOS comprises 11 different isomers, including the linear isomer (approximately 70%) and various branched isomers (approximately 30%) (Naile et al., 2010). Some of these isomers (specifically those with branched chains) are chiral, and the environmental fate and behaviour of PFOS may vary according to its isomeric and enantiomeric composition (Miralles-Marco and Harrad, 2015). The following impurities were reported in a commercial sample of potassium perfluorooctanesulfonate (purity, 86.9%): homologues with fewer carbons (C2–C7, predominantly C6), 9.38%; metals (calcium, magnesium, sodium, nickel, and iron), 1.45%; inorganic fluoride, 0.59%; molecules containing perfluorinated sulfur(VI) (sulfur hexafluoride), 0.68%; PFOA,

0.33%; nonafluoropentanoic acid, 0.28%; hydrocarbon sulfonate salts, 0.15%; terminal cyclopentyl PFOS, 0.11%; heptafluorobutyric acid, 0.1%; and trifluoroacetic acid, 0.015% (Seacat et al., 2003).

1.2 Production and uses

1.2.1 Production process

PFOA and PFOS have been manufactured by ECF and fluorotelomerization. During ECF, an organic acyl or sulfonyl fluoride backbone structure is dissolved in a solution of aqueous hydrogen fluoride (Buck et al., 2011; ATSDR, 2021; ITRC, 2022c). All the hydrogens on the molecule are then replaced with fluorines when a direct electrical current is passed through the solution. Perfluoroacyl fluorides produced by ECF are hydrolysed to form the perfluorocarboxylic acid, which is then separated via distillation. The ECF process results in a mixture of linear and branched isomers, with 78% and 70% linear forms of PFOA and PFOS, respectively (Buck et al., 2011; ATSDR, 2021; ITRC, 2022c).

Fluorotelomerization produces primarily linear perfluorocarboxylic acids with an even number of carbon atoms, which includes PFOA. The process begins with the preparation of pentafluoroiodoethane from tetrafluoroethene. Tetrafluoroethene is then added to the product at a molar ratio that gives a product of the desired chain length, before the product is oxidized to form the carboxylic acid (Buck et al., 2011; ATSDR, 2021; ITRC, 2022c).

1.2.2 Production volume

Production of perfluoroalkyl carboxylates began in 1947, initially by ECF. By 2000, ECF was still the leading process, accounting for the majority (80–90%) of the production of APFO – a salt of PFOA – worldwide, which was approximately 260 tonnes in 1999 (Prevedouros et al., 2006). Global production of perfluorooctane sulfonyl fluoride (POSF) – a production precursor of PFOS – was estimated to be 96 000 tonnes (or 122 500 tonnes, including wastes, largely disposed of through land farming/landfilling or incineration) between 1970 and 2002. One major company based in the United States of America (USA) manufactured most of the POSF, using ECF, accounting for about 78% of global production in 2000 (Paul et al., 2009). [The Working Group noted that data on production volumes were limited, particularly after 2002 (see below).]

In the USA, the manufacture and import of PFOA and PFOS has been phased out; however, some existing stocks may remain. PFOS was phased out of production by its primary manufacturer between 2000 and 2002 (US EPA, 2016) and was not reported in the 2006 or 2012 Chemical Data Reporting effort (US EPA, 2023e). Before 2006, production volume ranges in the USA were reported as follows: PFOA, [5–227] tonnes in 1986, 1994, 1998, and 2002; APFO, [5–227] tonnes in 1986, 1990, 1994, and 1998, and [227–454] tonnes in 2002; and PFOS, [5–227] tonnes in 1994 and 2002 (ATSDR, 2021). In 2006, the United States Environmental Protection Agency (US EPA) invited eight major leading companies manufacturing PFOA to join the 2010/2015 PFOA Stewardship Program. All participating companies reported meeting the goals of this programme, which included eliminating emissions by 2015 (US EPA, 2022). As of November 2016, PFOA and PFOS are no longer used in food contact applications sold in the USA (US FDA, 2023). For regulatory agency guidelines on the production and use of PFOA and PFOS that might explain changes over time, see Section 1.5.

Since 2002 there has been a geographical shift in industrial production (particularly fluoropolymer-production sites) from North America, Europe, and Japan to some countries in Asia, especially China (Wang et al., 2014). Zhang et al. (2012) report PFOS production in China of 247 tonnes

in 2006 and about 100 tonnes in 2008, with the majority used in metal plating (30–40 tonnes/year) and aqueous film-forming foam (AFFF) (25–35 tonnes/year), as well as the production of sulfluramid insecticides (4–8 tonnes/year). During 2004–2012, 480 tonnes of PFOA and its salts were produced in China using the ECF process (Li et al., 2015). China has also implemented a phase-out of PFOA and PFOS, with the Chinese Ministry of Environmental Protection restricting and banning different uses (OECD, 2023a). Brazilian imports of *N*-ethyl perfluorooctane sulfonamide (*N*-EtFOSA), a PFOS precursor, for the production of sulfluramid between 2005 and 2015 were almost exclusively from China; imports of *N*-EtFOSA peaked at > 1.3 tonnes in 2012, and exports increased to around 2 tonnes per year in 2012 (Löfstedt Gilljam et al., 2016). [The Working Group noted that these data were not for PFOS itself, but might give some indication of use or production in these geographical regions where data for PFOS itself are lacking.]

1.2.3 Uses

The unique properties of per- and polyfluoroalkyl substances (PFAS), including PFOA and PFOS, have led to extensive uses in a wide variety of diverse applications. These properties, including the "ability to lower the aqueous surface tension, high hydrophobicity, high oleophobicity, non-flammability, high capacity to dissolve gases, high stability, extremely low reactivity, high dielectric breakdown strength, good heat conductivity, low refractive index, low dielectric constant, ability to generate strong acids, operation at a wide temperature range, low volatility in vacuum, and impenetrability to radiation" (Glüge et al., 2020) facilitate nearly 300 different uses and functions. For the more than 1400 PFAS evaluated by Glüge et al. (2020), uses fell within 20 industry branches (e.g. chemical industry and electroplating) and 44 other use categories (e.g. cleaning compositions and personal care products). [However, the Working Group noted that the uses identified by Glüge et al. (2020) are summarized across all 1400 PFAS; some uses may not be applicable to PFOA and PFOS.] PFOA and PFOS may be present in industrial and consumer products as main ingredients, or as unreacted raw materials, undesired reaction by-products, or cross-contaminants along production and supply chains (OECD, 2015a; Glüge et al., 2020). PFOA and APFO are used in chemical manufacturing processes, industrial products and processes, and consumer products. As a processing aid, APFO has been used extensively to manufacture fluoropolymers, such as polytetrafluoroethylene (PTFE) (Buck et al., 2011). Applications for fluoropolymers containing PFOA, as well as direct uses for PFOA, include household products with non-stick coatings (e.g. cookware); textiles for outdoor or personal protection applications (e.g. firefighter turnout gear); personal care products (e.g. cosmetics, sunscreens, dental floss); seals and gaskets used in the aviation and aerospace industries; coatings for cables and wires; electronics, solar panels and electrolyte fuel cells; fluoropolymer fabrication materials used in food processing (e.g. liners for grills and ovens); carpets; cleaning and impregnating agents; construction materials (e.g. chipboard and oriented strand board); and surface coatings conferring stain-, oil- and water resistance on carpets, textiles, leather products, and paper or cardboard packaging used in food and feed contact paper and board (e.g. popcorn bags, pizza boxes, fast food containers) (Kotthoff et al., 2015; Bečanová et al., 2016; ATSDR, 2021; Ramírez Carnero et al., 2021; ITRC, 2022a). [The Working Group noted that the concentration of PFOA varied by application and product. For example, in fluoropolymer-based consumer products (e.g. non-stick cookware or textiles) PFOA may be present in a chemically bound form or at lower concentrations than in products in which PFOA is an intentionally added ingredient.]

With some applications that overlap those of PFOA, such as waxes (e.g. car, shoe, floor, ski), carpets, and packaging used for food and feed (Kotthoff et al., 2015; Nordic Council of Ministers, 2017), PFOS has additionally been used in the semiconductor industry; as a hydraulic fluid additive in the aviation and aerospace industries; as an etchant and antireflective coating in photolithography processes; and in the fabrication of imaging devices (e.g. cameras, mobile phones, and printers) (ITRC, 2022a). During electroplating processes in metal finishing and plating operations, PFOS has been used as a mist-suppressing agent to prevent workers' exposure to aerosols and mists; however, in the USA, the US EPA National Emissions Standards for Hazardous Air Pollutants (NESHAP) mandated that use of PFOS-based mist-suppressants in chromium electroplating be discontinued by 2015 (Office of the Federal Register, 2012). Similar phase-outs of PFOS for this application have occurred in other countries (Ramírez Carnero et al., 2021; ITRC, 2022a); however, this application is still permitted in the European Union (EU) (Swedish Chemicals Agency, 2020). PFOS is also present in a variety of building and construction materials, including paints and varnishes; insulation (phenolic foam); dyes and ink; and in wetting, levelling, and dispersing agents (ITRC, 2022a).

PFOS together with other PFAS have been used extensively in class B firefighting foams known as AFFFs. [The Working Group noted that AFFFs were designed to meet firefighting performance criteria; formulations of PFAS have changed over time and by manufacturer (Leeson et al., 2021).] These foams were developed in the 1960s to extinguish liquid fuel fires by efficiently suppressing flammable liquid vapour, suffocating the fire hazard, and preventing re-ignition (Rosenfeld et al., 2023). AFFF containing PFOS was manufactured in the USA from the late 1960s until 2002; however, other fluorotelomer-based AFFF manufactured from the 1970s until 2016 contained precursors of PFOA. Although newer formulations of class B foams exist, the legacy products have been used during fire response, training, and equipment maintenance activities by the military, airport and municipal fire departments, and oil and gas production and refining industries worldwide (Prevedouros et al., 2006; ITRC, 2022b).

PFAS that are known to convert into PFOA and PFOS, frequently referred to as "precursors", are used in a variety of settings (see Section 1.4(d)). Although a detailed description of these uses and functions is beyond the scope of the present monograph, examples include the semiconductor and electronics industry; personal care products, coatings for medical devices, apparel, pharmaceutical equipment; and the pesticide sulfluramid (Löfstedt Gilljam et al., 2016; Glüge et al., 2020; ITRC, 2022a).

1.3 Detection and quantification

General considerations

(a) Analytical method terminology

Analytical methods used for PFAS consist of targeted, non-targeted, and total fluorine analysis approaches. Targeted analyses refer to methods for a pre-defined, known list of analytes for which authentic chemical standards exist. Non-targeted analyses are capable of identifying suspect and unknown analytes in a sample, often through mass spectrometry. Analyte identity can then be confirmed using authentic chemical standards, and unknown analytes can be tentatively identified through matching to existing chemical libraries (US EPA, 2023b). Total fluorine methods quantify the fluorine (often organic fluorine) present in a sample, regardless of chemical structure, and thus are unable to differentiate between chemical structures of analytes (Schultes et al., 2019).

Some methods are able to differentiate between linear and branched isomers. [The Working Group noted that some recent studies

of PFOA and PFOS differentiate between linear and branched isomers. For example, linear, secondary-branched, and tertiary-branched isomers of PFOA and PFOS can be resolved by high-resolution differential ion mobility-mass spectrometry (DMS-MS) (Ahmed et al., 2019).] Isomer profiling can be used in the quantitative assessment of manufacturing source (Benskin et al., 2010b).

[The Working Group noted that, in the papers reviewed for PFOA and PFOS exposure, multiple approaches were used to report the lowest concentration of a chemical analyte. These commonly include the limit of detection (LOD), which describes the lowest concentration identifiable by the analytical instrumentation, and the limit of quantification (LOQ), which describes the lowest concentration that can be determined by means of a given analytical procedure with the established accuracy, precision, and uncertainty. The Working Group noted that some studies reported lowest measurable concentrations as LODs, whereas others reported LOQs. This makes comparison between studies more challenging at the lower end of the concentration range studied.]

Liquid chromatography-mass spectrometry (LC-MS), commonly used for the analysis of PFOA and PFOS, is a sophisticated analytical technique that requires the purchase and maintenance of an expensive instrument. [The Working Group noted that, consequently, access to PFOA and PFOS analyses can be challenging for regions or populations with limited resources, such as low- or middle-income countries (LMICs), and may explain the paucity of available data in some regions of the world.]

(b) Potential for cross-contamination

Consideration of numerous potential sources of cross-contamination (also referred to as "background interference") of PFOA and PFOS have been documented in the context of sample collection and analysis (Method 533, US EPA, 2023b; MDEQ, 2018). Potential sources of PFAS cross-contamination in the typical sampling environment include water used for washing or decontamination and materials used within the sampling environment (MDEQ, 2018).

In a laboratory setting, analytical instrumentation (e.g. mass spectrometry) and laboratory equipment or materials often have fluoropolymer (e.g. PTFE) components that may contain PFOA (Method 533, US EPA, 2023b; MDEQ, 2018). [The Working Group noted that cross-contamination issues may affect the concentrations of PFOA and PFOS in samples and blanks alike. This may contribute to the high LOQs reported in some studies.]

1.3.1 Air

Several methods have been reported for the quantification of PFOA and PFOS in indoor and/or outdoor air using both active and passive air-sampling techniques, and some examples are presented in Table 1.4. These methods generally rely on a combination of sampling media to collect both gas and particle-bound PFOA and PFOS. Most reported active air sampling methods apply a filter (glass fibre or quartz) to capture the particle phase, followed by an adsorbent resin to bind the gaseous-phase PFAS. Few active air-sampling methods reported the use of filters only to capture particle-bound PFAS, or sorbent only to capture both gas- and particle-bound PFAS on the same sampling medium. The passive sampling methods use a compact-design sampler containing a sorbent-impregnated, polyurethane foam (PUF) disc to sample PFAS from both the gaseous and particle phases. Therefore, active sampling methods with two independent sampling media can differentiate between gas- and particle-bound PFAS concentrations, whereas passive sampling methods can only provide PFAS concentrations as the sum of concentrations in the two phases. In general, sampling media (filters and PUF discs)

Table 1.4 Selected analytical methods for the measurement of PFOA and PFOS in air

Sample matrix	Sampler type	Sample collection method	Instrument (LOD)[a]	Reference
Air emissions from stationary sources	Active (flow rate not specified – minimum sample of 3 m^3)	Gas- and particle-bound PFAS collected on a sampling train of GFF or QFF, a packed column of adsorbent material	HPLC-MS/MS (PFOA, 0.35 ng/m^3; PFOS, 0.43 ng/m^3)	EPA-OTM-45 US EPA (2021)
Indoor and outdoor air	Active (flow rate of 6.4 m^3/h)	Gas- and particle-bound analytes collected using GFFs (particle phase) and glass columns with a PUF–XAD-2–PUF sandwich (gaseous phase)	HPLC-TOF/MS (1 pg/m^3)	Barber et al. (2007)
Outdoor air	Active (flow rate of 1.1 m^3/h)	Particle-bound analytes collected using GFF	HPLC-TOF/MS (PFOA, 0.2 pg/m^3; PFOS, 0.4 pg/m^3)	Jahnke et al. (2007)
Outdoor air, $PM_{2.5}$	Active (flow rate of 30 m^3/h)	$PM_{2.5}$-bound analytes collected on QFF	HPLC-MS/MS (0.14 pg/m^3)	Beser et al. (2011)
Indoor and outdoor air	Passive	Gas- and particle-bound analytes collected on sorbent (XAD-4)-impregnated PUF disc samplers	HPLC-MS/MS (PFOA, 0.47 pg/m^3; PFOS, 0.02 pg/m^3)	Shoeib et al. (2010, 2011)
Indoor air and personal breathing zone	Active (flow rate of 0.12 m^3/h)	ISOLUTE ENV+ sorbent (hydroxylated polystyrene–divinylbenzene copolymer) cartridge	HPLC-MS/MS (PFOA, 73 pg/g extract; PFOS, 38 pg/g extract)	Nilsson et al. (2013b)
Outdoor air, $PM_{2.5}$	Active (flow rate of 30 m^3/h)	$PM_{2.5}$-bound analytes collected on QFF	HPLC-HRMS (PFOA, 0.18 pg/mL extract; PFOS, 0.11 pg/mL extract)	Kourtchev et al. (2022)

GFF, glass-fibre filters; h, hour(s); HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; ISOLUTE ENV+, commercial solid-phase extraction column; LOD, limit of detection; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NR. not reported; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; $PM_{2.5}$, particulate matter with diameter < 2.5 µm; PUF, polyurethane foam; QFF, quartz fibre filters; SIP, sorbent-impregnated polyurethane; TOF, time-of-flight; XAD, commercial resin.

[a] Using electrospray ionization in negative ion mode.

are extracted with an organic solvent (mostly methanol), followed by clean-up using filtration, centrifugation, or solid-phase extraction (SPE). Instrumental analysis is usually carried out using LC-MS with an electrospray ionization (ESI) source, operated in negative ion mode. [The Working Group noted that the LC-MS methods reported for the analysis of PFOA and PFOS in air had low instrumental LODs and were appropriate for trace level detection of these chemicals in air samples.]

EPA-OTM-45 is a standardized method that can be used to measure air emissions of PFOA and PFOS from stationary emission sources. In this method, a sampling train of glass fibre or quartz filter is applied, followed by a packed column of adsorbent material to collect both gaseous-phase and particulate-bound target analytes. The samples are then extracted with methanol/5% ammonium hydroxide, cleaned-up and concentrated using SPE and quantified using LC-MS/MS. The method detection limits (MDLs) for PFOA and PFOS were 0.43 ng/m^3 and 0.35 ng/m^3, respectively (US EPA, 2021).

[The Working Group noted that most of these methods have been developed for environmental measurements, and there has been no validated method using personal samplers developed for occupational exposure measurements.]

1.3.2 Water

Several methods have been developed to measure PFOA and PFOS concentrations in water. Some selected methods are summarized in Table 1.5.

The US EPA Methods 537.1 (published in 2009) and 533 (published in 2019) describe methods to analyse PFOA and PFOS in drinking-water (US EPA, 2019; Shoemaker and Tettenhorst, 2020). Water samples are fortified with surrogate standards and passed through a solid-phase sorbent cartridge to extract the PFAS and surrogates. The extract is concentrated, and isotopically labelled performance standards are added. Extracts are analysed by LC-MS/MS. LODs were reported as 0.53 and 1.1 ng/L for PFOA and PFOS, respectively. Interlaboratory comparisons have reported coefficients of variation (CVs) between laboratories of 23% for PFOA and 33–40% for PFOS isomers (van der Veen et al., 2023). An earlier interlaboratory comparison reported substantially higher CVs: 118% for PFOA and 95% for PFOS in water samples (van Leeuwen et al., 2006).

The US EPA has also validated SW-846 Method 8327 using external standard calibration and LC-MS/MS for the analysis of PFOA and PFOS (and other PFAS) in surface water, groundwater, and wastewater effluent (US EPA, 2023c).

In 2023, a draft version was published of US EPA Method 1633, which had already been finalized for the aqueous matrices wastewater, surface water, and groundwater (US EPA 2023d).

Some examples of low detection limits reported for PFOA and PFOS detected via various methods were: PFOA, 0.3 ng/L in demineralized water and 0.5 ng/L in natural spring water (Janda et al., 2019); 0.10 ng/L (Song et al., 2023); PFOA, 0.1 ng/L, and PFOS, 0.5 ng/L (Chen et al., 2016); and PFOA, 0.01 ng/L, and PFOS, 0.01 ng/L (Zheng et al., 2023). [The Working Group noted that detection limits have changed as the methodology for sample processing and detection has improved over time. Differences in LODs might also be explained by the use of different methods to derive these LODs.]

1.3.3 Soil, sediment, consumer products, and foods

Several analytical methods for the quantification of PFOA and PFOS in soil, sediment, dust, and consumer products have been reported. Because of the large variability in sample matrices, the analytical methods involved various extraction techniques, including solvent extraction, ultrasonic extraction, ion-pair

Table 1.5 Selected analytical methods for the measurement of PFOA and PFOS in water

Sample matrix	Sample preparation	Instrument	LOD	Reference
Drinking-water	Adsorb on polystyrene divinylbenzene; elute with methanol; reconstitute in water/methanol with ^{13}C-PFOA internal standards	HPLC-MS/MS	PFOA, 0.53 ng/L; PFOS, 1.1 ng/L	Shoemaker and Tettenhorst (2020) US EPA Method 537.1
Drinking-water	Adsorb on polystyrene divinylbenzene; elute with methanol containing ammonium hydroxide; reconstitute in water/methanol with ^{13}C-PFOA internal standards	HPLC-MS/MS	PFOA, 3.4 ng/L; PFOS, 4.4 ng/L	US EPA (2019) US EPA Method 533
Reagent water, surfacewater, groundwater, and wastewater effluent	Uses US EPA Method 3512 – dilute and filter; does not use SPE or carbon clean-up steps, which is a significant difference from the other US EPA methods	LC-MS/MS	PFOA, 10 ng/L; PFOS, 10 ng/L (LOQ)	US EPA (2023c) US EPA Method 8327
Drinking-water, ground water and surface water (fresh water and sea water)	No pretreatment; adsorb on WAX SPE cartridges, elute with methanol, evaporate with nitrogen gas	HPLC-MS/MS	PFOA, 10 ng/L; PFOS, 2.0 ng/L (LOQ)	ISO (2009) ISO Method 25101
Wastewater, surface water, groundwater, landfill leachate	Glass fibre filtration of total suspended solids; aqueous samples with ≤ 50 mg of suspended solids must not be filtered; aqueous sample: spiking with isotopically labelled standards, SPE, and carbon clean-up	HPLC-MS/MS	PFOA, 0.54 ng/L; PFOS, 0.63 ng/L	US EPA (2023d) US EPA Method 1633 (draft version as of November 2023, finalized for the aqueous matrices: wastewater, surface water, and groundwater)[a]
Non-filtered waters, e.g. drinking-water, natural water (fresh water and sea water) and wastewater	Adsorb on high-purity mixed-mode WAX sorbent; elute with methanol	LC-MS/MS	PFOA, 0.31 ng/L; PFOS, 0.29 ng/L	ISO (2019) ISO Method 21675; Jones and Harden (2022)
Drinking-water	Adsorb on WAX SPE cartridges, elute with 1% ammonium hydroxide in methanol; concentrate to dryness; reconstitute in methanol	LC-MS/MS	PFOA, 0.01 ng/L; PFOS, 0.01 ng/L	Zheng et al. (2023)

HPLC, high-performance liquid chromatography; ISO, International Organization for Standardization; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MS/MS, tandem mass spectrometry PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; SPE, solid-phase extraction; US EPA, United States Environmental Protection Agency; WAX, weak anion exchange.

[a] US EPA recommends the use of Method 1633, and it is currently the only PFAS method that has been validated in multiple laboratories for aqueous matrices that include wastewater, surface water, groundwater, and landfill leachate, as well as for soil, sediment, biosolids, and fish and shellfish tissue.

extraction and dispersive SPE. Sample clean-up methods also varied, from filtration after pH control, to SPE and QuEChERS ("Quick, Easy, Cheap, Effective, Rugged, Safe"). LC-MS-ESI was the method of choice for the analysis of PFOA and PFOS. A summary of these methods is provided in Table 1.6.

The standard test method ASTM D7968 can be used for the determination of PFOA and PFOS in soil samples. The method uses solvent extraction with methanol:water (50:50) under basic conditions, followed by filtration, acidification, and then LC-MS/MS analysis. The MDLs were 6.2 and 18.8 ng/kg for PFOA and PFOS, respectively (ASTM International, 2017).

The United States Food and Drug Administration (US FDA) published a validated method C-010.02 for the analysis of 16 PFAS chemicals, including PFOA and PFOS, in various food items. Target PFAS are extracted from the food samples using acetonitrile and formic acid. After extraction, a modified QuEChERS technique is performed for clean-up, and further SPE is required for clean-up of complex samples. The cleaned extracts are then analysed using LC-MS/MS, with MDLs of 12–24 ng/kg for PFOA and 7–28 ng/kg for PFOS, in the different food items tested (US FDA, 2021b).

1.3.4 Human biospecimens

In early studies on exposed workers, total serum fluorine was used as a surrogate variable for PFOA exposure (e.g. Gilliland and Mandel, 1996). [The Working Group noted that using a total fluorine approach as a surrogate for PFOA is not an accurate quantification method for an individual analyte.] In 2001, LC-MS/MS was used for the first time for the analysis of PFOA and PFOS in biological samples (Hansen et al., 2001). At present, mainly targeted methods are used for the analysis of PFOA and PFOS in whole blood, serum, and plasma. Non-targeted mass spectrometry-based methods, lacking the ability to quantify concentrations, are also used (e.g. Chang et al., 2023). [However, these methods provide semiquantitative intensity levels that allow ranking of participants within a study.] A selection of methods for the analysis of PFOA and PFOS in human biospecimens is shown in Table 1.7. The usual sample preparation step before extraction is protein precipitation (for example, with acetonitrile). An aliquot of the supernatant is analysed using LC-MS/MS. Isotopically labelled internal standards may be used. Typical instrumental LODs are < 0.1 ng/mL for PFOA and PFOS, although higher values were reported in earlier publications (e.g. 10 ng/mL for PFOA; Sottani and Minoia, 2002), and lower values in more recent ones (e.g. 0.023 ng/mL for PFOA and 0.033 ng/mL for PFOS; Gao et al., 2018).

A method for determination of PFOA and PFOS (and other PFAS) in human serum, plasma, and whole blood described the use of methanol for protein precipitation and online SPE-LC-MS/MS. LODs for PFOA in serum, plasma, and whole blood were 0.018, 0.009, and 0.045 ng/mL, respectively, whereas the corresponding LODs for PFOS were 0.009 ng/mL for all three matrices (Poothong et al., 2017).

Earlier interlaboratory comparisons indicated quite large CVs, for example, 51% and 20% for PFOA and 24% and 32% for PFOS, in plasma samples (van Leeuwen et al., 2006; Longnecker et al., 2008). More recently, one interlaboratory comparison reported CVs ranging from 9% for PFOA and from 9% to 38% for PFOS isomers (van der Veen et al., 2023). An interlaboratory comparison and training exercise carried out for four rounds, involving 21 laboratories across Europe, included several PFAS (Nübler et al., 2022). For PFOA, the relative standard deviation improved from 12% to 6% from the second to the fourth round, and the relative standard deviation for PFOS was 11–12% in both rounds. [The study by van Leeuwen et al. (2006) was nearly 20 years old and involved the use of different extraction

Table 1.6 Selected analytical methods for the measurement of PFOA and PFOS in soil, sediment, dust, consumer products, and foods

Sample matrix	Sample preparation	Instrument (LOD)[a]	Reference
Soil	Solvent extraction with methanol:water (50:50) under basic conditions (pH ~9–10, adjusted with ~20 µL NH_4OH), followed by filtration, and acidification (pH ~3–4, adjusted with ~50 µL acetic acid)	HPLC-MS/MS (PFOA, 6.2 ng/g; PFOS, 18.8 ng/g)	ASTM International (2017)
Soil, sediment, and sludge (PFOA)	Solvent extraction with acetonitrile/0.2 M NaOH	HPLC-MS/MS (PFOA, 1 ng/g)	Powley et al. (2005)
Soil (PFOA)	Ultrasonic extraction with acetonitrile/water mixture	HPLC-MS/MS (PFOA, 180 fg on column)	Washington et al. (2008)
Soil and biosolids	Ultrasonic extraction with methanol containing 1% NH_4OH	HPLC-MS/MS (0.02–0.5 ng/g)	Sepulvado et al. (2011)
Soil and riverine sediment	Ion pair extraction with 0.5 M TBAS and 0.25 M sodium carbonate buffer (pH 10)	HPLC-MS/MS (soil: PFOA, 0.34 ng/g; PFOS, 0.32 ng/g; sediment: PFOA, 0.30 ng/g)	Lorenzo et al. (2015)
Sediment and sludge	Ultrasonic extraction with methanol and 1% acetic acid	HPLC-MS/MS (sediment: PFOA, 0.01 ng/g; PFOS, 0.1 ng/g; sludge: PFOA, 1.0 ng/g; PFOS, 0.9 ng/g)	Higgins et al. (2005)
Marine sediment	Ultrasonic extraction with methanol	HPLC-MS/MS (PFOA, 0.01 ng/g; PFOS, 0.05 ng/g)	Wang et al. (2018b)
Lake sediment	Solvent extraction with acetonitrile/0.2 M NaOH	HPLC-MS/MS (PFOA, 0.02 ng/g; PFOS, 0.05 ng/g)	Guo et al. (2016)
Marine plastic litter	Ultrasonic extraction with hexane	HPLC-MS/MS (PFOA, 0.03 ng/g; PFOS, 0.01 ng/g)	Gómez et al. (2021)
Sewage sludge	Ion pair extraction with 0.5 M TBAS and 0.25 M sodium carbonate buffer (pH 10)	HPLC-MS/MS (PFOA, 0.6 ng/g; PFOS, 5 ng/g)	Zhang et al. (2010)
Asphalt	Ultrasonic extraction with methanol and 1% NH_4OH	HPLC-MS/MS (PFOA, 0.6 ng/g; PFOS, 0.7 ng/g)	Srivastava et al. (2022)
Indoor dust	Ultrasonic extraction with acetonitrile	HPLC-MS/MS (PFOA, 2.3 ng/g; PFOS, 4.6 ng/g)	Kubwabo et al. (2005)
Indoor dust	Solvent extraction with methanol followed by filtration	Online SPE-HPLC-TOF/MS (PFOA, 0.03 ng/g; PFOS, 0.01 ng/g)	Padilla-Sánchez and Haug (2016)
Home garden produce (e.g. tomato, pepper, apples)	Dispersive SPE using magnesium sulfate and acetonitrile with 1% NH_4OH	HPLC-MS/MS (PFOA, 0.03 ng/g; PFOS, 0.01 ng/g)	Scher et al. (2018)
Food (various items)	Solvent extraction with acetonitrile plus formic acid, followed by QuEChERS clean-up; further SPE clean-up on WAX sorbent cartridges is required for complex food matrices	HPLC-MS/MS (PFOA, 0.012–0.024 ng/g; PFOS, 0.007–0.028 ng/g)	US FDA (2021b) (Validated US FDA method number C-010.02)
Food (various items)	Ultrasonic extraction with acetonitrile plus NaOH, followed by clean-up on WAX sorbent cartridges	NanoLC – Orbitrap MS (PFOA, 0.001–0.3 ng/g; PFOS, 0.001–0.3 ng/g)	Zacs et al. (2023)

Table 1.6 (continued)

Sample matrix	Sample preparation	Instrument (LOD)[a]	Reference
Microwave paper packaging	FUSLE with ethanol	HPLC-QTOF/MS (PFOA, 1.53 ng/g; PFOS, 0.63 ng/g)	Monge Brenes et al. (2019)
Consumer products (papers and textiles)	Solvent extraction with methanol	HPLC-MS/MS (papers: PFOA, 0.040 μg/m^2, PFOS, 0.038 μg/m^2; textiles: PFOA, 0.12 μg/m^2; PFOS, 0.15 μg/m^2)	Robel et al. (2017)
Consumer products (e.g. waterproofing agents, textiles, paints, cookware, waterproofing agents, firefighting foams, electronics)	Ultrasonic extraction with methanol	HPLC-QTOF/MS (NR)	Herzke et al. (2012)
Consumer products (e.g. textiles (outdoor materials), carpets, cleaning and impregnating agents, leather samples, baking and sandwich papers, paper baking forms and ski waxes)	Depending on the matrix procedures using ion pair extraction, acidic–alkaline sequential extraction or SPE with WAX were applied	HPLC-MS/MS (0.1–0.5 ng/g)	Kotthoff et al. (2015)

FUSLE, focused ultrasonic liquid extraction; HPLC, high-performance liquid chromatography; LOD, limit of detection; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NaOH, sodium hydroxide; NH_4OH, ammonium hydroxide; NR, not reported; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; QTOF, quadrupole time-of-flight; QuEChERS, Quick, Easy, Cheap, Effective, Rugged, and Safe; SPE, solid-phase extraction; TBAS, tetrabutylammonium hydrogen sulfate; TOF, time-of-flight; WAX, weak anion exchange.
[a] Using electrospray ionization (ESI) in negative ion mode.

and instrumental techniques, which led to the large variation and high z-scores.]

Reported serum-to-plasma ratios for PFOA and PFOS were approximately 1:1, whereas serum- or plasma-to-whole blood ratios were approximately 2:1 (Ehresman et al., 2007; Poothong et al., 2017). In the past, total PFOS was normally presented, but in more recent publications PFOS isomers have been distinguished, separating linear and the sum of branched forms; LODs in serum have also improved (e.g. Li et al., 2022c) (see Table 1.7). The method reported by Li et al. (2022c) can be applied for the analysis of PFOA and PFOS in urine; the resulting LODs were 0.01 ng/mL for PFOA and 0.01–0.02 ng/mL for PFOS isomers (Li et al., 2022c).

Similar methods are used for breast milk or colostrum. Existing methods for sample preparation and analysis of PFAS concentrations in human breast milk were reviewed by Macheka-Tendenguwo et al. (2018). SPE is more popular, owing to higher recovery, shorter analysis times, simpler procedures, and less use of solvents (e.g. Kärrman et al., 2007; Abdallah et al., 2020) than in other techniques, such as liquid–liquid extraction (LLE). The LOQ for each PFAS in colostrum and breast milk has been reported as 0.01 ng/mL. In two replication sets with in-house controls (n = 6 each), relative standard deviations were 28% and 11.1% for PFOA, and 20.2% and 8.8% for PFOS, respectively (Blomberg et al., 2023).

Table 1.7 Selected analytical methods for the measurement of PFOA and PFOS in human biospecimens

Sample matrix	Sample preparation	Instrument (LOD)	Comments	Reference
Whole blood	Adding of labelled internal standards; solvent extraction with acetonitrile; carbon–acetic acid, filtration; addition of performance standards $^{13}C_8$-PFOA and $^{13}C_8$-PFOS, with 2 mM ammonium acetate	HPLC-MS/MS (PFOA, 0.4–0.7 ng/mL; PFOS, 0.01–0.1 ng/mL)		Hardell et al. (2014)
Plasma and serum	Protein precipitation with acetonitrile; ^{13}C-labelled PFOA internal standards	LC-MS/MS (PFOA LOQ, 0.5 ng/mL)	Validated to meet US FDA guidelines for bioanalytical methods	Flaherty et al. (2005)
Plasma	Labelled internal standards; protein precipitation with acetonitrile; shaking, centrifugation	LC-MS/MS (PFOA, 0.4 ng/mL; PFOS, 0.5 ng/mL)		Li et al. (2018)
Plasma	Protein precipitation with acetonitrile; reconstitution in MeOH; filtration	HPLC-ESI-MS/MS (PFOA LOQ, 0.5 ng/mL; PFOS LOQ, 0.1 ng/mL)		Tsai et al. (2020)
Plasma	Addition of ^{13}C-labelled PFAS compounds; addition of acetonitrile to precipitate proteins; vortex mixing, centrifugation	LC-HRMS (PFOA, 0.01 µg/L; PFOS, 0.43 µg/L)		Goodrich et al. (2022)
Plasma, serum, and whole blood	Protein precipitation with MeOH, mixing, centrifugation	HPLC-MS/MS (PFOS, 0.009 ng/mL; PFOA, 0.009 ng/mL plasma; 0.018 ng/mL serum; 0.045 ng/mL whole blood)	Validated for human plasma, serum, and whole blood	Poothong et al. (2017)
Serum	Sample with internal standard and TBAS solution mixed; MTBE added and shaken; centrifugation; separation ×2; reconstitution in MeOH; vortex mixing; filtration	HPLC-MS/MS (PFOA, 1.0 ng/mL; PFOS, 1.7 ng/mL)		Hansen et al. (2001)
Serum	Ion-pair extraction	HPLC-MS/MS (PFOA, 10 ng/mL)		Sottani and Minoia (2002)
Serum	Proteins precipitated with formic acid; SPE clean-up	HPLC-MS/MS (PFOA, 0.2 ng/mL; PFOS, 0.2 ng/mL)		Kuklenyik et al. (2005)
Serum	Acidification with HCl, addition of hexanoic acid and THF; vortex-shaking, centrifugation	LC/QQQ MS/MS (2–20 pg/mL)		Luque et al. (2012)
Serum	Dilution with ultrapure water and isotope internal standards in MeOH, centrifugation	HPLC-MS/MS (PFOA, 0.023 ng/mL; PFOS, 0.033 ng/mL)		Gao et al. (2018)
Serum	Alkaline digestion followed by two-stage SPE purification using polymeric HLB and graphitized non-porous carbon cartridges	LC-MS/MS (NR)	Fully validated (2002/657/CE decision) and accredited (ISO 17025 standard)	Mancini et al. (2020)

Table 1.7 (continued)

Sample matrix	Sample preparation	Instrument (LOD)	Comments	Reference
Serum	Precipitation using acetonitrile by vigorous shaking; all sample batches include chemical blanks and three quality control samples	LC-MS/MS (PFOA, 0.09 ng/mL; *n*-PFOS, 0.2 ng/mL; 3/4/5m-PFOS, 0.01 ng/mL)		Li et al. (2022c)
Breast milk, serum	Proteins precipitated with formic acid; SPE clean-up	HPLC-MS/MS (PFOA, 0.2 ng/mL milk; 0.1 ng/mL serum; PFOS, 0.3 ng/mL milk; 0.4 ng/mL serum)		Kuklenyik et al. (2004)
Breast milk	LLE; purification by two successive SPE; reconstitution in fluorometholone solution as external standard in MeOH/water	LC-HRMS (PFOA, 0.003 ng/mL; PFOS, 0.002 ng/mL)		Kadar et al. (2011)
Breast milk	LLE with acetonitrile; purification by dispersive SPE using C18 sorbent; shaking and centrifugation; reconstitution in MeOH; filtration	HPLC-MS/MS (PFOA LOQ, 0.006 ng/mL; *n*-PFOS LOQ, 0.005 ng/mL; br-PFOS LOQ, 0.010 ng/mL)		Lankova et al. (2013)
Semen, serum	Samples were spiked with mass-labelled extraction standard, TBAS solution, $NaHCO_3/Na_2CO_3$ buffer solution and MTBE; shaking; extraction ×2 with MTBE; all three extracts combined, evaporated to dryness under nitrogen at 40 °C, and reconstituted with MeOH	HPLC-MS/MS (PFOA LOQ, 0.004–0.010 ng/mL semen; 0.020 ng/mL serum; PFOS LOQ, 0.004–0.010 ng/mL semen; 0.020 ng/mL serum)		Pan et al. (2019)
Urine and serum	For urine, add isotope-labelled internal standard and ammonium acetate buffer including β-glucuronidase, and subsequently formic acid; for serum, isotope-labelled internal standard was added, and formic acid; samples vortexed	SPE-HPLC-MS/MS (PFOA, 0.1 ng/mL; PFOS, 0.1 ng/mL)		Kato et al. (2018)
Urine	Precipitation using acetonitrile by vigorous shaking for 30 min; all sample batches include chemical blanks and three quality control samples	LC-MS/MS (PFOA, 0.01 ng/mL; *n*-PFOS; 0.01 mL; 3/4/5m-PFOS, 0.02 ng/mL)		Li et al. (2022c)
Hair, nail, urine, serum	For hair and nails: soaking in water, washing twice with acetone, air-drying, grinding to powder, extraction by various organic solvents, cleaning by WAX cartridge, elution with 9% NH_4OH in MeOH, concentration to dryness under nitrogen gas and reconstitution in water/MeOH (v/v; 1/1), filtration	HPLC-MS/MS (PFOA, 0.03 ng/g hair; 0.04 ng/g nail; 0.02 ng/mL serum; 1.07 ng/L urine; PFOS, 0.03 ng/g hair; 0.05 ng/g nail; 0.02 ng/mL serum; 2.09 ng/L urine)		Wang et al. (2018a)

Table 1.7 (continued)

Sample matrix	Sample preparation	Instrument (LOD)	Comments	Reference
Dried blood spots	Punch samples desorbed in ultrapure water, sonicated, and extracted into MTBE with labelled internal standards; dried then reconstituted in MeOH	HPLC-ESI-MS/MS (PFOA, 0.4 ng/mL; PFOS, 0.2 ng/mL)	LODs expressed in units of whole blood equivalents	Spliethoff et al. (2008)
Dried blood spots	Punch samples desorbed into MeOH with labelled internal standards; mixed, sonicated, centrifuged	SPE-HPLC-MS/MS (PFOA, 0.0075 ng/mL; PFOS, 0.03 ng/mL)		Poothong et al. (2019)
Placental tissue	Shaking with MeOH and MPFOA for 5 min, freeze-drying, homogenized with acetonitrile, centrifuged	HPLC-MS/MS (PFOA, 0.03 ng/g; PFOS, 0.03 ng/g)	Linearity, selectivity, accuracy (trueness and precision) and sensitivity validated according to US FDA guidelines	Martín et al. (2016)

br-, branched chain; C18, octadecyl alkyl substituent; ESI, electrospray ionization; HCl, hydrochloric acid; HLB, hydrophilic–lipophilic-balanced; HPLC, high-performance liquid chromatography; ISO, International Organization for Standardization; LC-HRMS, liquid chromatography-high-resolution mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LC/QQQ-MS/MS, liquid chromatography/triple quadrupole-tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; LLE, liquid–liquid extraction; MeOH, methanol; min; minute(s); MPFOA, perfluoro-*n*-[1,2,3,4-$^{13}C_4$]octanoic acid; MS/MS, tandem mass spectrometry; MTBE, methyl *tert*-butyl ether; $NaHCO_3$/Na_2CO_3, sodium bicarbonate/sodium carbonate; NH_4OH, ammonium hydroxide; NR, not reported; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; *n*-PFOS, linear perfluorooctanesulfonic acid; 3/4/5m-PFOS, corresponds to the sum of branched isomers 3m-PFOS, 4m-PFOS, and 5m-PFOS; SPE, solid-phase extraction; TBAS, tetra-*n*-butylammonium hydrogen sulfate; THF, tetrahydrofuran; US FDA, United States Food and Drug Administration; v/v, volume per volume; WAX, weak anion exchange.

Thomsen et al. (2010) reported a different method, with internal standards and acetonitrile added, followed by mixing and centrifugation. After the addition of formic acid, the supernatant is analysed by online column-switching LC-MS/MS.

Dried blood spots have been used to assess PFOA and PFOS exposure (Spliethoff et al., 2008). Detection limits as low as 0.0075 ng/mL for PFOA and 0.030 ng/mL for PFOS, estimated for the corresponding serum concentrations, have been reported.

PFOA and PFOS concentrations have also been measured in placental tissue (Martín et al., 2016), hair, and nails (Wang et al., 2018a).

1.4 Occurrence and exposure

Introduction to occurrence and exposure

(a) Life cycle and practices involved in end-of-life and disposal

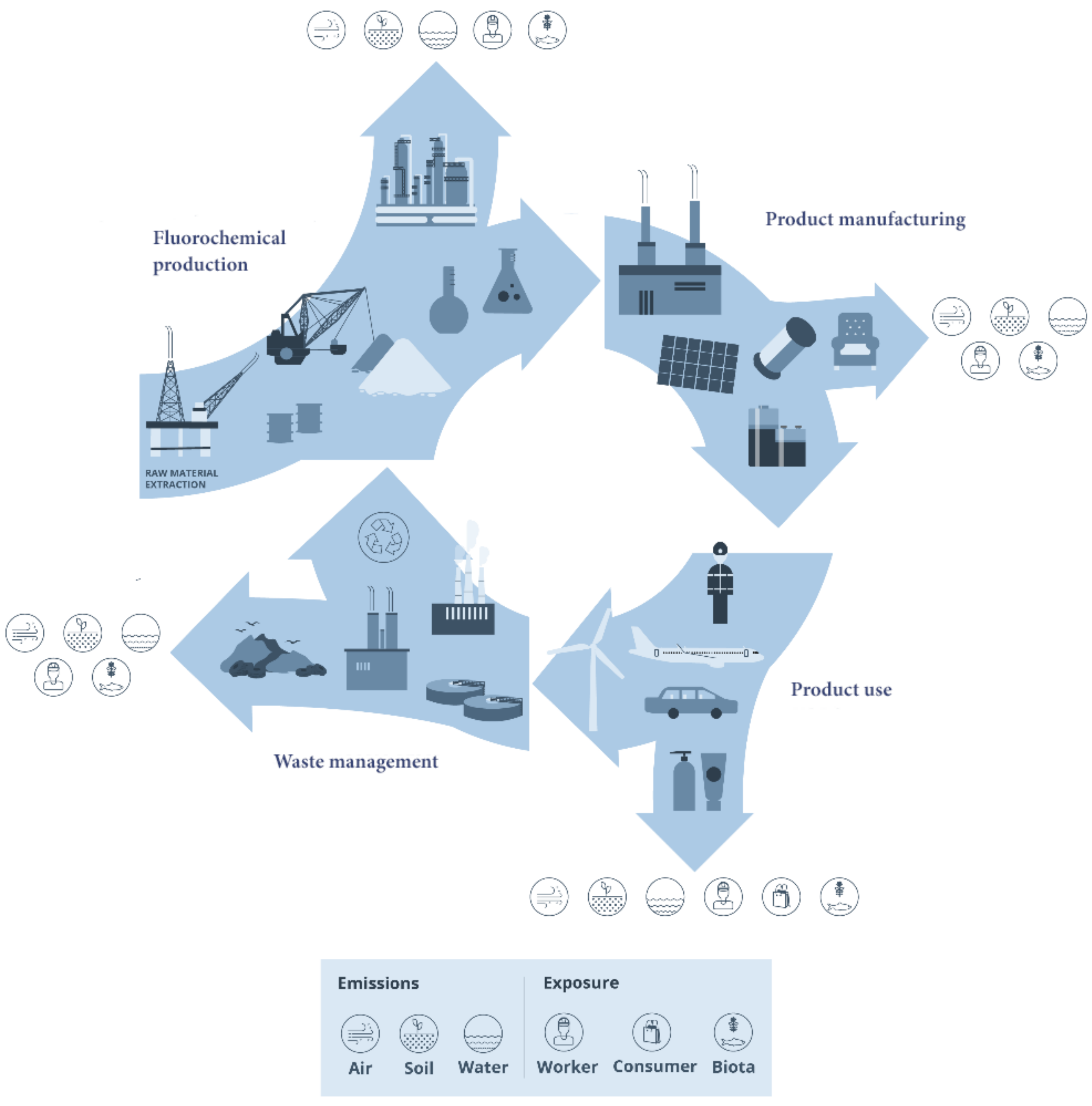
The occurrence of PFOA and PFOS in the environment is influenced by the chemical life cycle, including during fluorochemical production; secondary manufacturing processes (e.g. products containing fluorochemicals or processes using fluorochemicals); product use; and management of waste (industrial waste, products containing PFOA and PFOS, and materials contaminated with PFOA or PFOS) (see Fig. 1.3). The presence of PFOA and PFOS in consumer and industrial products, as well as environmental media subject to remediation, creates avenues for inadvertent, repeated cycles of contamination (Stoiber et al., 2020).

The available approaches to managing large quantities of PFAS wastes include landfilling, incineration, and wastewater treatment (US EPA, 2020). Landfills have been used historically for disposal at perfluorochemical facilities (ATSDR, 2021). The presence of PFAS in leachate from landfills has been documented in several countries, including Australia, China, Germany, and the USA (Stoiber et al., 2020). Incineration of products containing PFOA or PFOS generally requires temperatures of > 800 °C, using a scrubber to remove hydrogen fluoride. Although limited, experimental studies have indicated that incineration can break down PFOA and PFOS (Stoiber et al., 2020; ATSDR, 2021). Liquid wastes are treated with precipitation, decanting, or filtering to separate solids, followed by landfill or incineration of the solids and discharge of the liquids to a wastewater treatment facility (ATSDR, 2021). The US EPA interim guidance also lists underground injection as a possible means of disposal (US EPA, 2020).

In some settings, PFOA- or PFOS-contaminated waste products, including food wastes and sludge from municipal wastewater treatment, have been dispersed over land, for example, by land application of biosolids or composts (Kenny, 2021; ITRC, 2022a). Land application of these products may contribute to the contamination of crops and livestock and the continued cycle of contamination (Stoiber et al., 2020; Kenny, 2021).

[The Working Group acknowledged that in geographical regions with restrictions and phase-out of PFOA and PFOS production and use (e.g. Europe and the USA), trends towards decreases in PFOA and PFOS concentrations in human biospecimens (mainly in serum) have been observed (see Section 1.4.3); however, no clear patterns of declining trends have been observed for abiotic and environmental samples from the same regions. Decreasing concentrations in humans may be influenced by the removal of certain PFAS from consumer products and associated reductions in direct exposure (Land et al., 2018). Persistent levels in the environment may reflect the re-circulation of historically manufactured and released PFOA and PFOS and potentially the breakdown of their precursors.]

Fig. 1.3 Life cycle of PFOA and PFOS



PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.
Adapted from European Environment Agency (2021).

(b) Persistence and mobility

The carbon–fluorine bond is one of the strongest bonds known in nature and makes PFAS extremely resistant to degradation in the natural environment. PFOA and PFOS are among the most environmentally persistent organic chemicals and are, therefore, under the Regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) definition for persistence, classified as "very persistent (vP)" (Cousins et al., 2020).

Unlike other known persistent organic pollutants, PFOA and PFOS are highly mobile in the environment. They are quite soluble in water, and thus can be carried to remote regions through oceanic currents and long-range atmospheric currents. They can also vertically infiltrate sediment layers and move across the water column (ECHA, 2023).

(c) Global and temporal trends

PFOA and PFOS have been detected in environmental media worldwide, including in remote areas like the Arctic, Antarctic, and Mount Everest (Cai et al., 2012; ATSDR, 2021; Miner et al., 2021; Garnett et al., 2022). Estimations of total global annual emissions of PFOA-based products show that emissions steadily increased from 1960 to 2002 and quickly decreased from 2002 to 2012, followed by an increase from 2012 to 2015. The same trend was observed for PFOS-based products (OECD, 2015a). The estimated oceanic transport of PFOA to the Arctic for the period 1951–2004 was greater than the estimated atmospheric transport (Prevedouros et al., 2006). The deposition into soil from the atmosphere and subsequent transport pathways, such as leaching, also contribute to the widespread distribution of these substances in the environment (ATSDR, 2021). Retention by soil is expected to be low (Prevedouros et al., 2006). In the environment, most PFOA and PFOS are estimated to be in ocean water, and smaller amounts are present in freshwater and sediments. The presence of PFOA and PFOS in groundwater is widespread (Johnson et al., 2022).

The presence of PFOA and PFOS in snow and ice core samples indicates their atmospheric deposition from production and/or use (see Section 1.4(c)(i) below). Likewise, sediment cores reflect time trends corresponding to initial production and subsequent changes in patterns of production and use (Section 1.4(c)(ii)).

(i) Snow and ice cores

In a snow core from the Mount Muztagata glacier (western Tibet, China) showed a steady increase in PFOA and PFOS from 1983 to 1999. A more recent (1996–2007) core from Mount Zuoqiupo glacier (south-eastern Tibet) contained lower concentrations of PFOA and PFOS, with no clear trend. Differences in concentrations were attributed to different upwind sources affecting the respective study sites (e.g. sources in Europe or central Asia for Mount Muztagata and sources in India for Mount Zuoqiupu) (Wang et al., 2014).

In glacial ice cores from Svalbard, Norway, representing deposition from 1990 to 2005, higher concentrations of PFOA and PFOS were detected in the layers representing 1997–2000, the period that coincides with the peak production of these compounds (Kwok et al., 2013).

In the eastern Antarctic, a firn core representing the period from 1958 to 2017, showed PFOA levels peaking in 1997–2000. Subsequently there was a short decline, then an increase from 2003 to 2013 with no sign of a decrease, despite recent global restrictions on PFOA production and use (Garnett et al., 2022). [The Working Group noted this may be attributed to increasing production of fluorochemicals in emerging Asian economies, which probably offsets emission reduction in North America and Europe, and may account for the higher concentrations observed in the later years represented in the firn core. PFOS was not detected in any of the studied samples.]

(ii) Sediment cores

In a sediment core containing deposits from the 1950s to 2004, in Tokyo Bay, Japan, concentrations of PFOA increased consistently from 1994 to 2004, which is generally consistent with the PFOA production and usage profile during this period in Japan (Zushi et al., 2010). PFOS concentrations decreased gradually after the early 1990s, whereas concentrations of some PFOS precursors decreased rapidly in the late 1990s. This trend could reflect the shift in PFOS industrial production processes after the phase-out of POSF-based products in 2001 (Zushi et al., 2010). Another study on three sediment cores from Lake Ontario, Canada, (1952–2005) reported a marked increase in PFOA and PFOS concentrations from the mid-1970s to 2005, which is generally in line with PFOA and PFOS production and usage profiles (Yeung et al., 2013). In a sediment core from the Bering Sea, covering almost 70 years of deposition, PFOS concentrations generally showed an upward trend since 1952 and peaked in about 2003, after which concentrations dropped to a lower level until 2015. This largely coincides with the production and usage history of PFOS. Conversely, PFOA concentrations showed a more fluctuating pattern among layers, which was explained by its vertical mobility in pore water (Lin et al., 2020a). [The Working Group noted that although the temporal trends in PFOS concentrations in dated sediment cores reflect PFOS production and usage history, temporal trends in PFOA concentrations can be influenced by its vertical mobility in pore water and thus may not adequately reflect changes in its production and use in certain geographical areas.]

(d) Precursor compounds

In the present monograph, “precursor compounds” refers to PFAS that are known to break down or transform into PFOA or PFOS in the environment or biota, including humans. Precursors include, but are not limited to, fluorotelomer alcohols (FTOH) and polyfluoroalkyl phosphate diesters (diPAP) for PFOA; and perfluorooctane sulfonamides (e.g. *N*-EtFOSA), perfluorooctane sulfonamidoacetic acids (e.g. *N*-EtFOSAA) and perfluorooctane sulfonamidoethanols (e.g. *N*-EtFOSE) for PFOS (Gebbink et al., 2015). While estimates vary by exposure scenario, it has been estimated that a substantial proportion of the body burden of PFOA and PFOS may originate from intake of precursors (Vestergren et al., 2008; Gebbink et al., 2015) (see also Section 4.1). While direct exposure to PFOA and PFOS may decline as a result of regulation or voluntary efforts, production and use of precursors may contribute to ongoing exposure from the breakdown of precursors. Breakdown of precursors has also resulted in PFOA and PFOS contamination in remote areas with no direct sources of pollution (ATSDR, 2021).

1.4.1 Environmental occurrence

(a) Air and dust

The atmospheric environment is not only an important compartment for the transport of PFOA and PFOS, but it is also an exposure pathway for PFOA and PFOS (Liu et al., 2018a). Air is a mixture of particles, gases, and dust. The sources and levels of PFOA and PFOS in outdoor and indoor air differ, and the characteristics of PFOA and PFOS are described here for outdoor air, indoor air, and settled dust separately.

(i) Outdoor air

The sources of PFOA and PFOS in outdoor air include direct emissions from the fluorochemical industry and products containing fluorochemicals (Butt et al., 2010), long-range transport via the gas phase, and degradation of PFAS precursors (McMurdo et al., 2008). Sampling time and location varied among multiple studies; representative concentrations are presented in Table 1.8.

Table 1.8 Occurrence of PFOA and PFOS in outdoor air

Reference	Location and collection date	Characteristics of sampling (number, sites, sampler, time, flow, and duration)	Analytical method (reporting limits)	PFOA		PFOS		Unit	Comments
				Mean (range)	Median (IQR)	Mean (range)	Median (IQR)		
Camoiras González et al. (2021)	15 countries in Africa[a], 2017–2019	118, meteorological station, PAS, 3 mo/sample, 2 yr	LC-MS/MS, (LOQ: PFOA, 13 pg/PUF disc; PFOS, 12 pg/PUF disc)	207 (< 13–1190)	148	185 (< 12–2480)	97.7	pg/PUF disc	Long sampling time. Good reflection of PFAS levels in Africa.
	7 countries in Asia[b], 2017–2019	46, meteorological station, PAS, 3 mo/sample, 2 yr		271 (83.1–965)	183	139 (27.3–634)	101		Long sampling time. Good reflection of PFAS levels in Asia.
	10 countries in Group of Latin America and Caribbean[c], 2017–2019	101, meteorological station, PAS, 3 mo/sample, 2 yr		257 (58.9–655)	233	376 (< 12–2260)	192		Long sampling time. Good reflection of PFAS levels in Group of Latin America and Caribbean countries.
	9 countries in Pacific Islands subregion[d], 2017– 2019	43, meteorological station, PAS, 3 mo/sample, 2 yr		181 (< 13–417)	165	297 (< 12–827)	266		Long sampling time. Good reflection of PFAS levels in Pacific Islands subregion.

Table 1.8 (continued)

Reference	Location and collection date	Characteristics of sampling (number, sites, sampler, time, flow, and duration)	Analytical method (reporting limits)	PFOA		PFOS		Unit	Comments
				Mean (range)	Median (IQR)	Mean (range)	Median (IQR)		
Chaemfa et al. (2010)	UK, July to October 2007	15, background and city centre area, PAS, 2–3 mo/sample	LC-TOF-MS (LOD: PFOA, 27 pg/sample; PFOS, 3.9 pg/sample)	[2657 (< 27–27 000)]	[400]	[53.5 (< 3.9–720)]	[6.5]	pg/sample per day	Long sampling time. Good reflection of PFAS levels in north-western England.
	UK–Norway, June to October 2006	11, background and semi-rural/rural area, PAS, 2–3 mo/sample		[139 (< 27–1200)]	[< 27]	[3.0 (< 3.9–7.7)]	[< 3.9]		Long sampling time. Good reflection of PFAS levels in UK–Norway transect.
	Europe, June to November 2006	23, ranged from background to city centre area, PAS, 2–3 mo/sample		[117 (< 27–540.0)]	[< 27]	[10 (< 3.9–69.0)]	[< 3.9]		Long sampling time. Good reflection of PFAS levels in Europe.
Dreyer et al. (2015)	Geesthacht, Germany, December 2007 to May 2008	11, semirural, AAS, 14–21 days/sample, 2 sample/mo	HPLC-MS/MS, (LOQ: PFOA, 10 pg/sample; PFOS, 10 pg/sample)	0.7 (0.1–4.8)	NR	0.65 (0.2–3.5)	NR	pg/m³	Sampling time in each month was relatively limited. Partially reflects PFAS levels in semirural area.

Table 1.8 (continued)

Reference	Location and collection date	Characteristics of sampling (number, sites, sampler, time, flow, and duration)	Analytical method (reporting limits)	PFOA		PFOS		Unit	Comments
				Mean (range)	Median (IQR)	Mean (range)	Median (IQR)		
Guo et al. (2018)	Shanghai, China, December 2013 to January 2015	18, urban area (reflects long-range transported PFAS from northern or eastern continental China and surrounding seas), AAS, 24 h/sample, 28.3 L/min	HPLC-MS/MS, (LOD: PFOA, 0.35 pg/L; PFOS, 1.30 pg/L)	145.6	101.0 (71.3–230.0)	24.2	24.1 (14.2–29.0)	pg/m³	Sampling time collected in every month was limited. Partially reflects PFAS levels in urban area in winter.
Lin et al. (2020a)	Xiamen, China, December 2016 to September 2018	13, eastern coastal China and commercial/residential area, AAS, 2–3 days/sample, 0–1 sample/mo, 20 L/min	LC-MS/MS (minimum MQL: PFOA, 0.089 pg/m³ ; PFOS, 0.174 pg/m³)	[3.21] (0.211–7.47)	[0.72]	[2.79] (< 0.315–15.7)	[1.50]	pg/m³	Sampling time in each month was relatively limited. Partially reflects PFAS levels in commercial/residential area in Xiamen.
	Delhi, India, December 2017 to May 2018	2, commercial and residential area, AAS, 2–3 days/sample, 0–1 sample/mo, 20 L/min		[0.42] (< 0.367–1.07)	[0.38]	[0.63] (ND to 1.33)	[0.61]		Sampling time in each month was relatively limited. Partially reflects PFAS levels in commercial/residential area in Delhi.

Table 1.8 (continued)

Reference	Location and collection date	Characteristics of sampling (number, sites, sampler, time, flow, and duration)	Analytical method (reporting limits)	PFOA		PFOS		Unit	Comments
				Mean (range)	Median (IQR)	Mean (range)	Median (IQR)		
Lin et al. (2020a) (cont.)	Beijing, China, May 2017 to January 2018	7, rural (surrounded by forest; near some residents), AAS, 2–3 day/sample, 0–1 sample/month, 20 L/min		[0.68] (< 0.182–2.81)	[0.41]	[0.53] (< 0.350–1.16)	[0.41]		Sampling time in each month was relatively limited. Partially reflects PFAS levels in rural area in Beijing.
	Yuxi, China, August 2016 to April 2017	7, rural (fewer residents and low traffic density), AAS, 5 days/sample, 0–1 sample/mo, 20 L/min		[0.12] (< 0.091–0.393)	[0.07]	[0.11] (ND to 0.209)	[0.09]		Sampling time in each month was relatively limited. Partially reflects PFAS levels in rural area in Yuxi.
	Wenchuan, China, May 2017 to October 2017	3, rural (mountain areas), AAS, 2–3 days/sample, 0–1 sample/mo, 20 L/min		[0.70] (0.365–1.22)	[0.66]	[0.57] (ND to 1.37)	[0.47]		Sampling time in each month was relatively limited. Partially reflects PFAS levels in mountain area in Wenchuan.
	Tsukuba, Japan, July to December 2017	5, rural (fewer residents and low traffic density), AAS, 4 days/sample, 0–1 sample/mo, 20 L/min		[0.51] (< 0.124–3.01)	[0.28]	[0.19] (< 0.24–0.709)	[0.14]		Sampling time in each month was relatively limited. Partially reflects PFAS levels in rural Japan.

Table 1.8 (continued)

Reference	Location and collection date	Characteristics of sampling (number, sites, sampler, time, flow, and duration)	Analytical method (reporting limits)	PFOA		PFOS		Unit	Comments
				Mean (range)	Median (IQR)	Mean (range)	Median (IQR)		
Lin et al. (2020a) (cont.)	Jinju, Republic of Korea, April 2017 to January 2018	6, rural (fewer residents and low traffic density), AAS, 3–4 days/sample, 0–1 sample/mo, 20 L/min		[1.47] (0.212–7.84)	[0.65]	[0.39] (ND to 1.16)	[0.38]		Sampling time in each month was relatively limited. Partially reflects PFAS levels in rural Jinjiu.
	Nanjing, China, September 2017 to July 2018	7, urban (industrial area), AAS, 3–5 day/sample, 0–1 sample/mo, 20 L/min		[5.71] (0.695–26.8)	[2.73]	[2.10] (ND to 17.1)	[0.76]		Sampling time in each month was relatively limited. Partially reflects PFAS levels in industrial area in Nanjing.
	Gujarat, India, December 2016 to November 2017	12, urban (western coastal India, residential area), AAS, 2–5 days/sample, 0–1 sample/mo, 20 L/min		[0.28] (ND to 2.06)	[0.17]	[0.37] (ND to 1.81)	[0.35]		Sampling time in each month was relatively limited. Partially reflects PFAS levels in residential area in Gujarat.
Lin et al. (2022)	Karachi, Pakistan, December 2012 to January 2013	18, urban (near industrial area and garbage dumping sites), AAS, 24 h/sample, 16.7 L/min	LC-MS/MS, (MQL: PFOA, 1.0 pg/m³; PFOS, 0.2 pg/m³)	2.01 (0.85–8.70)	1.6	1.69 (0.64–3.17)	1.55	pg/m³	Sampling time was relatively long. Reflects PFAS levels in urban areas.

Table 1.8 (continued)

Reference	Location and collection date	Characteristics of sampling (number, sites, sampler, time, flow, and duration)	Analytical method (reporting limits)	PFOA		PFOS		Unit	Comments
				Mean (range)	Median (IQR)	Mean (range)	Median (IQR)		
Liu et al. (2023)	Pearl River Delta, China, May to July and October to December 2018	186, urban, AAS, 24 h/sample, 100 /min in summer and 1.05 m^3/min in winter	HPLC-MS/MS (LOD: PFOA, 0.0025 ng/mL; PFOS, 0.0003–0.0016 ng/mL)	10.80 (1.02–56.53)	6.05 (3.71–13.04)	45.19 (3.90–378.06)	24.18 (11.94–44.18)	pg/m^3	Sampling time for each sample was limited, but sample sites were representative and sample size was large. Partially reflects PFAS levels in an urban area in the Pearl River Delta.
Seo et al. (2019)	Hyung-san River, Gyeongju and Pohang, Republic of Korea, September 2014	8, urban (near wastewater treatment plants), AAS, 18–24 h/sample, 700 L/min	LC-MS/MS (MDL: PFOA, 0.13 pg/m^3; PFOS, 0.13 pg/m^3)	48.66	43.09	90.52	99.03	pg/m^3	Total sampling time was long. Reflects PFAS levels near wastewater treatment plants in the Republic of Korea.
Wang et al. (2021)	Shandong, China, November 2017	12, urban (fluorochemical industry park), AAS, 20 h/sample, 800 L/min	LC-MS (LOD: PFOA, 0.06 pg/m^3; PFOS, 0.13 pg/m^3; LOQ: PFOA, 0.31 pg/m^3; PFOS, 0.31 pg/m^3	1610 (42.8–9730)	451	1.24 ($<$ 0.31–2.74)	1.01	pg/m^3	Long total sampling time. Reflects PFAS levels at source.

Table 1.8 (continued)

Reference	Location and collection date	Characteristics of sampling (number, sites, sampler, time, flow, and duration)	Analytical method (reporting limits)	PFOA		PFOS		Unit	Comments
				Mean (range)	Median (IQR)	Mean (range)	Median (IQR)		
Yu et al. (2018)	Coastal areas of the Bohai Sea, China, May 2015 to April 2016	48, urban (large emission of PFAS, economic zones), AAS, 48 h/sample, 2 sample/mo, 300 L/min	HPLC-MS/MS (LOD: PFOA, 0.01 pg/m^3; PFOS, 0.02 pg/m^3; LOQ: PFOA, 0.05 pg/m^3; PFOS, 0.05 pg/m^3)	27.0 (0.1–362.9)	[26.2] [(15.0–34.8)]	[1.8] (< 0.05–11.1)	[1.4] [(1.0–2.1)]	pg/m^3	Sampling time in every month was relatively limited, but number of samples was large. Partially reflects PFAS levels in an urban area.
	Coastal areas of the Yellow Sea, China, May 2015 to April 2016	35, urban (large emission of PFAS, economic zones), AAS, 48 h/sample, 2 sample/month, 300 L/min		30.5 (0.6–524.8)	[18.3] [(13.3–46.1)]	[0.6] (< 0.05–8.6)	[0.8] [(0.5–0.9)]		Sampling time in each month was relatively limited, but number of samples was large. Partially reflects PFAS levels in an urban area.
Zhou et al. (2021)	North Carolina, USA, 2018–2019	60, suburban residential areas and on or near university campuses, AAS, 6 days/sample, 3 mo, 10.0 L/min	HPLC-MS/MS (LOD: PFOA, 0.0067 pg/m^3; PFOS, 0.0047 pg/m^3; MDL:PFOA,2.86pg/m^3; PFOS, 0.18 pg/m^3)	(< 0.005–14.06)	NR	(< 0.004–4.75)	NR	pg/m^3	Long sampling time. Good reflection of PFAS levels in North Carolina.

AAS, active air sampler; h, hour(s); HPLC, high-performance liquid chromatography; IQR, interquartile range; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MDL, method detection limit; min, minute(s); mo, month(s); MQL, method quantification limit; MS/MS, tandem mass spectrometry; ND, not detected; NR, not reported; PAS, passive air sampler; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PUF, polyurethane foam; TOF, time-of-flight; UK, United Kingdom; USA, United States of America.

[a] Including Democratic Republic of the Congo, Egypt, Ethiopia, Ghana, Kenya, Mali, Mauritius, Morocco, Nigeria, Senegal, Togo, Tunisia, Uganda, United Republic of Tanzania, Zambia.

[b] Including Cambodia, Indonesia, Lao People's Democratic Republic, Mongolia, Philippines, Thailand, Viet Nam.

[c] Including Antigua and Barbuda, Argentina, Barbados, Brazil, Chile, Colombia, Ecuador, Mexico, Peru, Uruguay.

[d] Including Fiji, Kiribati, Marshall Islands, Niue, Palau, Samoa, Solomon Islands, Tuvalu, Vanuatu.

In general, PFOA and PFOS levels differ according to the surroundings of the sampling sites. Air collected near fluorochemical industrial sites (Yu et al., 2018; Wang et al., 2021), wastewater treatment plants (Seo et al., 2019), and in industrial areas (Lin et al., 2020a) was highly contaminated by PFOA and PFOS, whereas air collected from areas that were remote from exposure sources and had fewer residents and less traffic had lower levels of PFOA and PFOS (Lin et al., 2020a). For example, the average concentration of PFOA in air samples from the fluorochemical industry park in Shangdong, China, was 1610 pg/m^3 (Wang et al., 2021); however, in coastal areas of the Bohai Sea, China, which is more than 100 km from the industry park, the average PFOA concentration was 27.1 pg/m^3 (Yu et al., 2018). This concentration is higher than that in the Pearl River Delta, China, (average 10.8 pg/m^3) (Liu et al., 2023), a coastal area that is farther from fluorochemical industries than is the Bohai Sea.

PFOA and PFOS levels in outdoor air vary widely worldwide, and it is also difficult to compare concentrations when the results are expressed in different units. One study conducted in 2017–2019 used the same method for four regions in LMICs (Camoiras González et al., 2021). The median concentration of PFOA in Africa (148 pg/PUF disc) was similar to that in the Pacific Islands (165 pg/PUF disc), but lower than that in Asia (183 pg/PUF disc) and the Group of Latin America and the Caribbean (GRULAC) (233 pg/PUF disc). The median concentrations of PFOS in Africa (97.7 pg/PUF disc) and Asia (101 pg/PUF disc) were similar, higher levels were observed in GRULAC (192 pg/PUF disc), and the highest levels were found in the Pacific Islands (266 pg/PUF disc) (Camoiras González et al., 2021).

In Asia, PFOA and PFOS levels were mainly reported for samples from China, and some information was available from Pakistan, Japan, India, and the Republic of Korea. Concentrations of PFOA and PFOS in areas remote from exposure sources were similar in China, Japan, India, and the Republic of Korea, and most median concentrations were < 5 pg/m^3 (Lin et al., 2020a, 2022). However, mean PFOA concentrations in highly polluted zones such as industrial areas or areas near fluorochemical industry parks varied from 23.8 pg/m^3 to 1610 pg/m^3, with a maximum of 9730 pg/m^3 (Guo et al., 2018; Seo et al., 2019; Lin et al., 2020a; Wang et al., 2021).

Across Europe and the United Kingdom (UK), PFOA concentrations in outdoor air have been relatively low. According to sampling campaigns conducted in the UK, Norway, and other countries in Europe, more than half of the samples did not contain PFOA and PFOS at concentrations above the detection limits (27 pg/sample per day for PFOA and 3.9 pg/sample per day for PFOS), although in north-west England, the median values for PFOA and PFOS were 400 and 6.5 pg/sample per day, respectively (Chaemfa et al., 2010). Maximum concentrations of PFOA and PFOS in the particle phase measured in Geesthacht, Germany, were both < 5 pg/m^3 (Dreyer et al., 2015).

Available data on PFOA and PFOS levels in outdoor air in other countries or regions including the USA were limited. For example, the production of PFOA and PFOS was phased out in the USA nearly 20 years ago, and in one study in which particulate matter with diameter < 2.5 μm ($PM_{2.5}$) samples were collected from five sites in North Carolina, USA, it was reported that most PFOA and PFOS concentrations were < 1 pg/m^3 (Zhou et al., 2021).

[The Working Group noted that these data suggest that, in the absence of an emission source, levels of PFOA and PFOS in outdoor air are low.]

(ii) Indoor air

The sources of PFOA and PFOS in indoor air include consumer products, building materials, and outdoor air (Winkens et al., 2017; Janousek et al., 2019). The results of previous studies have

suggested that PFOA and PFOS concentrations in indoor air exceed those in outdoor air (Goosey and Harrad, 2012). However, there were only a few studies in which PFOA and PFOS levels in indoor air were reported. The studies were conducted in Canada, the USA, Europe, and China, with samples collected from multiple sites, including bedrooms, homes, offices, cars, living rooms, and a laboratory and hallway (see Table 1.9). The median PFOA concentrations in indoor air collected from bedrooms in Canada (21 pg/m^3) (Shoeib et al., 2011) and eastern Finland (15.2 pg/m^3) (Winkens et al., 2017) were similar to those in living rooms (24 pg/m^3) and offices (18 pg/m^3) in the UK (Goosey and Harrad, 2012), but lower than median values in living rooms (56 pg/m^3), cars (76 pg/m^3), offices (96 pg/m^3), and school classrooms (89 pg/m^3) in Ireland (Harrad et al., 2019). The median PFOS concentrations in bedrooms in Canada (< 0.02 pg/m^3) (Shoeib et al., 2011) and eastern Finland (1.24 pg/m^3) (Winkens et al., 2017), and in living rooms in Ireland (< 0.4 pg/m^3) (Harrad et al., 2019) were similar, and lower than those in living rooms (11 pg/m^3) in the UK (Goosey and Harrad, 2012), and in cars (13 pg/m^3), offices (8.9 pg/m^3), and school classrooms (9.3 pg/m^3) in Ireland (Harrad et al., 2019), and much lower than those in offices in the UK (55 pg/m^3) (Goosey and Harrad, 2012). [These findings suggest that the function of these spaces might influence the concentrations of PFOA and PFOS, but more data are needed to confirm these influences.] In addition, one study at the University of North Carolina, USA, found that the floor waxing process in a laboratory and hallway increased mean PFOS concentrations from < 0.22 pg/m^3 before waxing to 8.88 pg/m^3 during waxing (Zhou et al., 2022).

[The Working Group noted that the available data on PFOA and PFOS levels in indoor air and their determinants were sparse.]

(iii) Settled dust

PFOA and PFOS are widely detected in dust samples because of continuous releases from consumer products (Jian et al., 2017; Zhu et al., 2023). de la Torre et al. (2019) evaluated 65 samples of house dust from three European countries. The median concentrations of PFOA in these dust samples from Belgium, Italy, and Spain were similar (1.54 ng/g, 1.56 ng/g and 1.00 ng/g, respectively), and median concentrations of PFOS in dust samples from these three countries were also low (0.77 ng/g, 0.33 ng/g, and 0.03 ng/g, respectively) (Table 1.10) (de la Torre et al., 2019). A study that collected dust samples from 184 homes in North Carolina, USA, and 49 fire stations in the USA and Canada showed that the median concentration of PFOA in dust samples collected from fire stations (17.6 ng/g) was higher than that from homes (7.9 ng/g) (Table 1.10) (Hall et al., 2020). Likewise, the median concentration of PFOS in fire stations (64.5 ng/g) was much higher than from homes (4.4 ng/g). Another study measured levels in 81 dust samples from homes in Indiana, USA, and found similar median concentrations of PFOA (5.9 ng/g) and PFOS (10 ng/g) (Zheng et al., 2023).

(b) Water

PFOA and PFOS are generally not removed from source water during standard water treatment (Wee and Aris, 2023). They have been detected in surface water, groundwater, wastewater, and in raw and finished drinking-water. Although the global extent of water contamination by PFOA and PFOS has not been completely characterized, PFOA and PFOS have been measured in water sources on all continents (Kurwadkar et al., 2022). Studies on highly contaminated water are discussed in subsection (iv).

Table 1.9 Occurrence of PFOA and PFOS in indoor air

Reference	Location and collection date	Characteristics of sampling (number, sites, sampler, time, flow and duration)	Analytical method (reporting limits)	PFOA concentration (pg/m³)		PFOS concentration (pg/m³)		Comments
				Mean (range)	Median (IQR)	Mean (range)	Median (IQR)	
Goosey and Harrad. (2012)	Birmingham, UK, September 2008 to March 2009	20, living room, PAS, 1.0 m³/day PFOA; 0.8 m³/day PFOS, 28–35 days/sample	HPLC-MS/MS (NR)	52 (< 1.9–440)	24	38 (< 1.0–400)	11	Long sampling time. No. of samples was relatively large. Good reflection of PFAS levels in homes.
		12, offices, PAS, 1.0 m³/day PFOA; 0.8 m³/day PFOS, 28–35 days/sample		58 (< 1.9–200)	18	56 (12–89)	55	Long sampling time. No. of samples was relatively large. Good reflection of PFAS levels in homes.
Harrad et al. (2019)	Dublin, Galway, and Limerick, Ireland, August 2016 to January 2017	34, living room, PAS, 60 days/sample 1.0 m³/day PFOA; 0.8 m³/day PFOS	HPLC-MS/MS (LOD: PFOA, 0.3 pg/m³; PFOS, 0.4 pg/m³)	72 (< 0.3–386)	56	14 (< 0.4–208)	< 0.4	Long sampling time. No. of samples was large. Good reflection of PFAS levels at selected sites.
		31, cars, PAS, 60 days/sample		162 (1.2–790)	76	22 (< 0.4–152)	13	
		34, offices, PAS, 60 days/sample		153 (< 0.3–1210)	96	89 (< 0.4–1290)	8.9	
		28, school classrooms, PAS, 60 days/sample		210 (< 0.3–728)	89	188 (< 0.4–1590)	9.3	
Shoeib et al. (2011)	Vancouver, Canada, 2007–2008	59, bedroom, PAS, 4 wk/sample	LC-MS/MS, MDL, 0.47 pg/m³, 0.02 pg/m³	113 (3.4–2570)	21	(< 0.02, < 0.02)	< 0.02	Long sampling time. No. of samples was large. Good reflection of PFAS levels in bedrooms.
Winkens et al. (2017)	Kuopio, eastern Finland, 2014–2015	57, bedroom, PAS, 21 days/sample	LC-MS/MS (MDL: PFOA, 4.48 pg/m³; PFOS, 0.47 pg/m³)	21.2 (< 4.48–99.8)	15.2	1.33 (< 0.47–5.04)	1.24	Long sampling time. No. of samples was relatively large. Good reflection of PFAS levels in bedrooms.

Table 1.9 (continued)

Reference	Location and collection date	Characteristics of sampling (number, sites, sampler, time, flow and duration)	Analytical method (reporting limits)	PFOA concentration (pg/m³)		PFOS concentration (pg/m³)		Comments
				Mean (range)	Median (IQR)	Mean (range)	Median (IQR)	
Zhou et al. (2022)	University of North Carolina, USA, August to September 2019	3, laboratory and hallway, before floor waxing, AAS, 16/min, 24 h/sample	HPLC-MS/MS (MDL: PFOA, 0.82 pg/m³; PFOS, 0.25 pg/m³)	[12.69]	NR	[< 0.22]	NR	Sampling time and size were limited.
		3, laboratory and hallway, during floor waxing, AAS, 16/min, 18 h/sample		[8.83]	NR	[8.88]	NR	Sampling time and size were limited.
		3, laboratory and hallway, after floor waxing, AAS, 16/min, 24 h/sample		[8.17]	NR	[< 0.22]	NR	Sampling time and size were limited.

AAS, active air sampler; h, hour(s); HPLC, high-performance liquid chromatography; IQR, interquartile range; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MDL, method detection limit; min, minute(s); MS/MS, tandem mass spectrometry; NR, not reported; PAS, passive air sampler; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; UK, United Kingdom; United States of America; wk, week(s).

Table 1.10 Occurrence of PFOA and PFOS in dust

Reference	Location and collection date	Characteristics of sampling (number, sites)	Analytical method (reporting limits)	PFOA concentration (ng/g dust)		PFOS concentration (ng/g dust)		Comments
				Mean (range)	Median (IQR)	Mean (range)	Median (IQR)	
de la Torre et al. (2019)	Belgium, September 2016 to January 2017	Homes (*n* = 22)	HPLC-MS/MS (LOQ: PFOA, 0.11 ng/g; PFOS, 0.04 ng/g)	NR (0.31–24.2)	1.54	NR (< 0.04–6.81)	0.77	Long sampling time. No. of samples was relatively large. Good reflection of PFAS levels in dust.
	Italy, September 2016 to January 2017	Homes (*n* = 22)		NR (0.21–53.0)	1.56	NR (< 0.04–11.9)	0.33	Long sampling time. No. of samples was relatively large. Good reflection of PFAS levels in dust.
	Spain, September 2016 to January 2017	Homes (*n* = 21)		NR (0.42–12.5)	1.00	NR (< 0.04–2.45)	0.03	Long sampling time. No. of samples was relatively large. Good reflection of PFAS levels in dust.
Hall et al. (2020)	Fire stations, USA and Canada, 2015 and 2018	Fire stations (*n* = 49)	HPLC-MS/MS (MDL: PFOA, 1.60 ng/g dust; PFOS, 1.44 ng/g dust)	NR	17.6	NR	64.5	No. of samples was relatively large. Good reflection of PFAS levels in dust.
	North Carolina, USA, 2014–2016	Homes (*n* = 184)	HPLC-MS/MS (MDL: PFOA, 0.26 ng/g dust; PFOS, 0.20 ng/g dust)	NR	7.9	NR	4.4	Long sampling time. No. of samples was relatively large. Good reflection of PFAS levels in dust.
Zheng et al. (2023)	Indiana, USA, August to December 2020	Homes (*n* = 81)	HPLC-MS/MS (MDL: PFOA, 0.01 ng/g dust; PFOS, 0.02 ng/g dust)	(< 0.01–1900)	5.9	(< 0.02–1100)	10	Long sampling time. No. of samples was relatively large. Good reflection of PFAS levels in dust.

HPLC, high-performance liquid chromatography; IQR, interquartile range; LOQ, limit of quantification; MDL, method detection limit; MS/MS, tandem mass spectrometry; NR, not reported; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; UK, United Kingdom; USA, United States of America.

(i) Surveys of surface water

Examples of PFOA and PFOS measurements in surface waters (lakes or rivers) are presented in Table S1.11 (Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636). Mean values of PFOA and PFOS were generally below or in the low nanograms-per-litre range in locations without any reported PFAS pollution source. One example of higher levels reported downstream of an industrial source was in Alabama, USA, where values reported were 598 µg/L for PFOA and 144 µg/L for PFOS (ATSDR, 2021). Reported concentrations in ocean water were generally well below the nanogram-per-litre range (see Table S1.11).

Kurwadkar and colleagues reviewed levels of PFAS substances in surface water, groundwater and wastewater (Kurwadkar et al., 2022). They included information on the Asia–Pacific region, collected under the Second Global Monitoring Report on Persistent Organic Pollutants, which showed that PFOS detection was becoming more frequent. Levels of PFOS ranged from not detected to 47 ng/L in China; from 0.02 to 230 ng/L in Japan; from 0.12 to 33 ng/L in the Republic of Korea; from 0.39 to 42 ng/L in the Philippines; and from not detected to 54 ng/L in Thailand (United Nations Environment Programme, UNEP, as cited in Kurwadkar et al., 2022). Limited data were available for most of South America and Africa.

Muir and Miaz (2021) assembled an extensive summary of PFOA and PFOS measurements and total emissions for rivers across the world. There was a high degree of variability but widespread detectable levels of PFOA, with the highest concentrations identified in Europe in the River Po, Italy (200 ng/L); in Asia, in the Hokkesantanigawa River, Japan (360 ng/L); and in China in numerous rivers (e.g. the Daling River, 233 ng/L). Estimated riverine emissions of PFOA to the sea exceeded 1000 kg/year for many rivers, with estimates for the Yangtze River reaching 10 000 to 40 000 kg/year.

For PFOS, high concentrations were reported for the Llobregat and Besos rivers in Spain (> 250 ng/L) and the Ganges in India (142 ng/L) (Muir and Miaz, 2021). Riverine emissions of PFOS to the sea were estimated to have exceeded 2000 kg/year for the Pearl and Xi Rivers in China, and the Saint Lawrence River in North America. [The Working Group noted that several of these measurements were taken in the early 2000s and may not represent more recent riverine discharges.]

In a meta-analysis of publications on PFAS in wastewater treatment plant effluent streams, some indications of trends over time were presented (Cookson and Detwiler, 2022). Multiple results in China indicated a clear upward trend during 2006–2019 for both PFOA and PFOS. In the data for the USA, a clear downward trend was evident for PFOA over the period 2004–2020, but there was no overall trend for PFOS.

In a systematic review, Land et al. (2018) observed declining trends in PFOA and PFOS levels in Tokyo Bay between 2004 and 2006; in marine and fresh waters on the west coast of the Republic of Korea between 2008 and 2012; and in Bohai Bay on the east coast of China between 2011 and 2013.

Across the USA and across Europe there were many sources of data on PFAS surface-water contamination, from both local government monitoring and research projects, and many of these sources have been assembled into an online searchable resource (Dagorn et al., 2023; Environmental Working Group, 2023; PFAS Project Laboratory, 2023). These maps show the widespread locations where PFOA and PFOS are detectable in the USA and across Europe but do not provide summary exposure data for PFOA and PFOS.

PFOA and PFOS have been detected in many rainwater samples collected from urban and rural areas of Europe, Asia, and North

America. Levels near local emission sources can be very high, for example, PFOA concentrations measured in rainwater near to a fluoropolymer plant in China (median, [615 ng/L]; maximum, 2752 ng/L) (Liu et al., 2017). Dispersion has been very widespread, with detectable concentrations of [0.22 ng/L] for PFOA and [0.006 ng/L] for PFOS reported in Antarctica (Casas et al., 2021). Reported urban rainfall levels tended to be up to about 10 ng/L, and rural levels were generally < 1 ng/L (see Table S1.11).

PFOA and PFOS have been detected in both coastal and sea and ocean waters, with lower concentrations in ocean waters (see Table S1.11). PFOA and PFOS have been found to be substantially concentrated in sea foam and rising mist, which can be blown inland and contaminate surface water (Sha et al., 2022). Muir and Miaz conducted an extensive review of measurements from ocean and coastal waters, lakes, and rivers, and incorporated 29 500 measurements of 87 individual PFAS analytes, including PFOA and PFOS (Muir and Miaz, 2021). During 2015–2019, concentrations in seas were highest for PFOA in the Bohai and Yellow seas (median, 9.0 ng/L) and for PFOS in the Indian Ocean (median, 0.087 ng/L). The lowest concentrations were found in the Mediterranean Sea for PFOA (median, 0.001 ng/L) and in the Arctic Sea for PFOS (0.02 ng/L). Comparison of surveys conducted in 2000–2009, 2010–2014, and 2015–2019 revealed clear upward trends in concentrations of PFOA in the Bohai Sea, Yellow Sea, and East China Sea, and a steep decline in the Mediterranean. For PFOS, upward trends were evident in the Indian Ocean. [The Working Group noted, as did the authors, that deriving medians across studies with different sampling sites, design, and timing of sample collection, as well as different method detection limits (MDLs), introduces considerable uncertainty for assessing contrasts across space and time.]

PFOA and PFOS have been detected in fresh snow at levels that were very low in remote areas such as Antarctica (Xie et al., 2020) and higher in China (Shan et al., 2015), see Table S1.11 (Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636). For PFOA and PFOS concentrations measured in snow and ice core samples, see Section 1.4(c)(i).

(ii) Groundwater

Some examples of PFOA and PFOS concentrations measured in groundwater are presented in Table S1.11 (Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636). The occurrence of PFAS in groundwater from different areas in the world, including Australia, China, India, and islands of Malta has been described in a review (Xu et al., 2021a). PFOA was the dominant PFAS detected in three of the eight locations studied. For instance, in rural areas of eastern China, PFOA concentrations ranged from 7 to 175.2 ng/L, with a mean value of 90.8 ng/L (Chen et al., 2016). Also in China, but in the alluvial–pluvial plain of the Hutuo River, PFOA concentrations ranged from 0 to 1.76 ng/L (mean, 0.63 ng/L) in groundwater (Liu et al., 2019). PFOA was found at concentrations in the range of 0–8.03 ng/L (mean, 1.46 ng/L) in groundwater from valleys in Gozo on the Maltese Islands (Sammut et al., 2019). In the case of PFOS, higher concentrations than those of other PFAS were found only in 13 shallow monitoring bores surrounding legacy landfills in Melbourne, Australia, with a range of 1.3–4800 ng/L and mean value of 413.3 ng/L (Hepburn et al., 2019).

In 2019, 254 samples were collected from five aquifer systems in the eastern USA to evaluate PFAS occurrence in groundwater used as a source of drinking-water. In this study, PFOA and PFOS represent two of the three most frequently detected PFAS in public-supply wells,

with 2.4% ($n = 6$) of the samples containing PFOA plus PFOS at concentrations of > 70 ng/L, and median concentrations detected were 4.6 ng/L and 6.7 ng/L for PFOA and PFOS, respectively (McMahon et al., 2022).

In a study developed in Sweden, a national screening for perfluorinated pollutants in drinking-water was performed. The most abundant individual PFAS in surface and groundwater supplies was PFOS, followed by PFOA (Holmström et al., 2014).

[The Working Group noted that some studies did not report on PFOA and PFOS separately.]

(iii) Drinking-water and drinking-water supplies

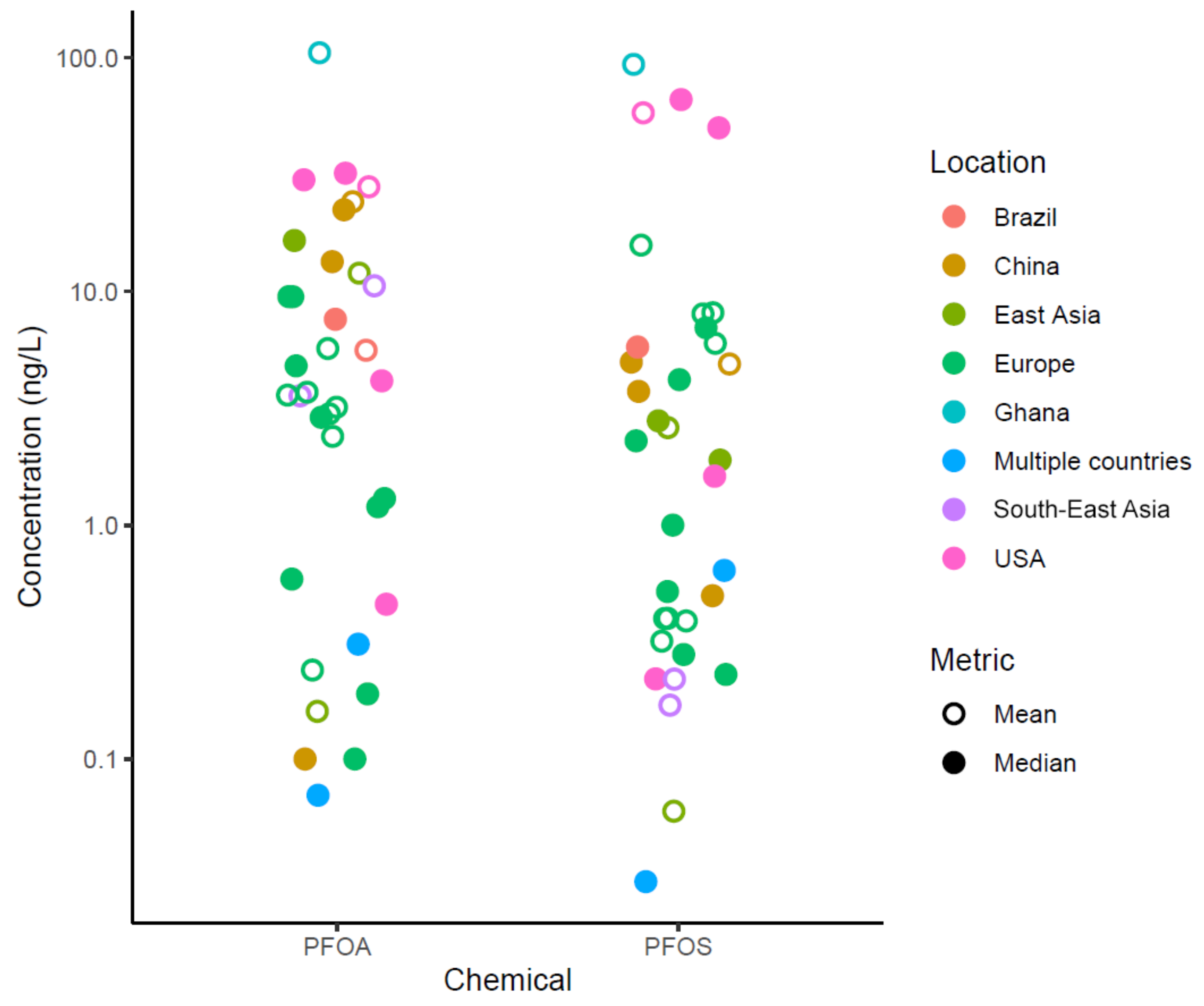
PFOA and PFOS have been measured in drinking-water (e.g. tap water, bottled water) in various locations (Fig. 1.4; Table S1.11, Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636). [The Working Group noted that mean concentrations in drinking-water from sites without any known contamination were usually below 10 ng/L (Fig. 1.4; see also Section 1.4.1(b)(iv) for concentrations measured at sites with reported contamination sources).]

Domingo and Nadal (2019) reviewed the scientific literature on PFAS exposure via drinking-water and highlighted that most information was coming from the EU, USA, and China. They reported on water sampling efforts in Europe (France, Italy, Sweden, Spain, Norway, Belgium, and the Faroe Islands), the Americas (USA, Canada, Brazil), and Asia (China, Japan, Afghanistan, India, and the Republic of Korea), Africa, and Australia. Levels of PFOA and PFOS in drinking-water and drinking-water sources ranged from non-detectable to > 500 ng/L.

There have been few formal efforts to characterize PFOA and PFOS levels in drinking-water sources on a national or international level. Because communities draw drinking-water from both surface and groundwater sources, large-scale efforts focus on water used for drinking-water supplies, regardless of whether this is surface or groundwater. In 2013–2015, the US EPA required 6000 public water systems (PWS) to test for PFOA and PFOS (and four other PFAS) in source water under the Third Unregulated Contaminant Monitoring Rule (UCMR 3) programme (US EPA, 2017b). The prevalence of PFOA at levels above the minimum reporting levels (MRLs) was low (0.09% of samples from 0.3% of PWS were above the MRL of 20 ng/L), as was that of PFOS (0.3% of samples from 0.9% of PWS exceeded the MRL of 40 ng/L) (US EPA, 2017b). Levels of PFOA reported ranged from 20 to 349 ng/L (median, [32 ng/L]); levels of PFOS ranged from 41 to 1800 ng/L (median, [66 ng/L]); the frequency of detection of these chemicals increased over the reporting period (Guelfo and Adamson, 2018). Detectable levels of PFOA and PFOS spanned three orders of magnitude, with PFOS levels being higher than those of PFOA (US EPA, 2017b). On the basis of these detections, it was estimated that more than 6 million people in the USA had drinking-water that exceeded 70 ng/L for the sum of PFOA and PFOS (Hu et al., 2016). Detection of PFOA and PFOS was significantly associated with nearby military firefighting training areas, AFFF-certified airports, and wastewater treatment plants. Detectable PFOA was also associated with major industrial sites that produced or used PFOA and/or PFOS (Hu et al., 2016).

More recently, UCMR 5, being conducted in 2023–2025, is measuring 29 PFAS with lower MRLs (4 ng/L for PFOA and PFOS) and in a larger number of PWS than UCMR 3 (US EPA, 2024). In initial data available up to October 2023 for 10 020 samples from 3072 PWS, PFOA was reported to be above the MRL in 6.1% of samples and 9.5% of PWS, and PFOS was detected at above the MRL in 6.4% of samples and 9.5% of PWS.

Fig. 1.4 Examples of PFOA and PFOS concentrations in drinking-water from sites without known sources of contamination



LOD, limit of detection; LOQ, limit of quantification; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; USA, United States of America.
Selected publications, see Table S1.11 for detail and references (Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636). When available, the median was plotted. When values were below the LOQ or LOD, the LOQ or LOD was plotted instead. Note the logarithmic scale.

(iv) Local major contamination of drinking-water sources

Fluorochemical manufacture and use of fire-fighting foams are associated with PFOA and PFOS contamination of drinking-water around the world. Some examples are described below.

The first such contamination identified was from a facility manufacturing PTFE in Parkersburg, West Virginia, USA, which contaminated surface water and drinking-water supplies in West Virginia and Ohio, with more than 80 000 people supplied with water contaminated with PFOA to varying extents. Levels of PFOA in water supplies measured since the early 2000s ranged from 10 to 100 ng/L in the least-contaminated water district to up to 10 µg/L in the most-contaminated water district (Shin et al., 2011a). Modelling of the water contamination indicated

progressive increases in contamination from the 1950s to 2000, when emissions were curtailed (Shin et al., 2011a). In a series of measurements from 62 private wells used for drinking-water in the same area, the median PFOA concentration was 200 ng/L (range, 6–13 300 ng/L) (Hoffman et al., 2011).

In Veneto, Italy, groundwater used for drinking-water was contaminated by chemical production; PFOA was the main contaminant, together with a mixture of mainly shorter-chain PFAS. In 152 samples collected from the contaminated area in 2013, the PFOA concentration was above the LOQ in 90% of samples, with a median concentration of 319.5 ng/L (maximum, 1475 ng/L), and the PFOS concentration was above the LOQ in 78% of samples, with a median concentration of 18 ng/L (maximum, 117 ng/L) (Pitter et al., 2020).

Firefighting foams and their use at airports and air force bases have resulted in PFAS contamination, particularly PFOS, in drinking-water. In Ronneby, Sweden, about one third of a community of 28 000 people were supplied with contaminated drinking-water in which PFOS was measured at 8000 ng/L and PFOA at 100 ng/L before the waterworks was closed (Xu et al., 2021b).

In Australia, PFOA and PFOS were detected in groundwater near a military base in Williamtown, New South Wales, at concentrations of 1800 ng/L and 5560 ng/L, respectively (Kurwadkar et al., 2022).

(c) Soil

Soil has been highlighted as a global sink for and long-term source of PFOA and PFOS (Brusseau et al., 2020). The estimated half-lives of PFOA and PFOS in soil are at least tens of years, although the true half-lives may be longer, because no significant degradation was noticeable during the experiments that have been conducted (UNEP, 2006, 2017). PFOA and PFOS can reach the soil directly, or via degradation of their precursors, from various input sources including: application of biosolids as fertilizers; the use of PFAS-based firefighting foams; leaching from contaminated asphalt and concrete affected by the extensive use of AFFF in firefighting training centres and airfields; seepage of leachate from landfills; discharge of effluents from wastewater treatment plants; contaminated irrigation water; contaminated discharge from fluorochemical industries; and atmospheric deposition (Costello and Lee, 2020; Abou-Khalil et al., 2022; Panieri et al., 2022; Douglas et al., 2023). Brusseau et al. (2020) comprehensively reviewed the literature on PFAS in soil. Both PFOA and PFOS were ubiquitously distributed globally in soil, with or without nearby point sources. The median for maximum concentrations of PFOA and PFOS reported globally in soil near primary point sources was 8722 and 83 ng/g, respectively, whereas the median for maximum background soil (i.e. no direct input sources) concentrations worldwide was 2.7 and 2.7 ng/g, respectively (Brusseau et al., 2020). The highest reported concentration of PFOA in soil (50 000 ng/g) was measured in soil contaminated with AFFF from a US military site (Brusseau et al., 2020), and the highest PFOA concentration (460 000 ng/g) was measured at firefighting training grounds in Australia (CRCCARE, 2018). Regarding background soil levels, the highest PFOA concentration of 47.5 ng/g was measured in soil from Shanghai, China (Li et al., 2010), and the highest PFOS concentration of 162 ng/g was reported for Alnabru, Norway (NEA, 2017). Table 1.12 provides a summary of selected studies on the occurrence of PFOA and PFOS in soil and lists PFOA and PFOS concentrations from various sites with different sources of contamination, as well as background concentrations from non-contaminated sites.

A systematic review of concentrations of 12 PFAS (including PFOA and PFOS) in 1042 soil samples from 15 countries on 6 continents reported significantly higher Σ_{12}PFAS levels (dominated by PFOA and PFOS) in the northern

Table 1.12 Occurrence of PFOA and PFOS in soil

Location and collection date	No. of samples	PFOA concentrations (ng/g)	PFOS concentrations (ng/g)	Comments	Reference
Antarctica, 2010	3	< MQL	Range, 0.31–0.54 Mean, [0.45]		Llorca et al. (2012a)
Australia, NR	6	Range, 13.6–58.1 Mean, 34.5	Range, 2180–15 300 Mean, 7800	AFFF use at airport sites	Bräunig et al. (2019)
Australia, NR	3	Range, 0.33–0.39 Mean, 0.36	Range, 6.4–7.2 Mean, 6.8	No direct input sources	Bräunig et al. (2019)
Africa					
Uganda, 2015	18	Range, 0.25–0.91	Range, 0.6–3.0	No direct input sources	Dalahmeh et al. (2018)
Asia					
China, 2009	32	Range, < 0.05–34.2 Mean, 2.5	Range, 0.68–189 Mean, 22.6	Soil around a fluorochemical-manufacturing plant	Wang et al. (2010)
China, NR	86	Range, < 0.1–0.9 Mean, 0.2	Range, 0.02–2.4 Mean, 0.3	No direct input sources	Pan et al. (2011)
Nepal, 2010	14	Range, < 0.1–0.26	Range, < 0.09–0.13	No direct input sources	Tan et al. (2014)
Republic of Korea, 2009	13	Range, < 0.2–3.4 Mean, 2.2	Range, < 0.2–1.7 Mean, 0.8	No direct input sources.	Naile et al. (2013)
Europe					
Germany, 2006	1	650	8600	Soil affected by contaminated industrial waste	Wilhelm et al. (2008)
Norway, 2008	39	Range, < 1.0–141.5 Median, 12.8 GM, 16.0	Range, 24.5–11 923 Median, 641 GM, 516.6	Fire training sites (*n* = 4)	SFT (2008)
Norway, 2016	9	< 0.01	Range, < 0.02–7.06 Median < 0.02	No direct input sources	Skaar et al. (2019)
Sweden, 2011–2012	45	Range, < 0.1–219 Median, 1.4 GM, 2.6	Range, < 0.5–8520 Median, 39 GM, 42.5	AFFF-contaminated soil near a military airport	Filipovic et al. (2015a)
Sweden, NR	31	Range, < 0.02–0.57 Median, < 0.02 Mean, 0.04	Range, < 0.02–1.7 Median, 0.3 Mean, 0.43	No direct input sources	Kikuchi et al. (2018)
North America					
USA, 2010	6	Range, 0.29–0.54 Median, 0.33	Range, 0.93–2.1 Median, 1.4	No direct input sources	Scher et al. (2018)
USA, 2019	2469	Range, 0.07–50 000 Median, 1.4 GM, 2	Range, 0.09–373 000 Median, 18 GM, 22	Sites affected by AFFF use	Brusseau et al. (2020)

Table 1.12 (continued)

Location and collection date	No. of samples	PFOA concentrations (ng/g)	PFOS concentrations (ng/g)	Comments	Reference
South America					
Tierra del Fuego, Argentina, 2010	30	Range, < MQL–1.5 Mean, 0.3	Range, < MQL–5.4 Mean, 1.4	No direct input sources	Llorca et al. (2012a)

AFFF, aqueous film-forming foam; GM, geometric mean; MQL, method quantification limit; NR, not reported; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; USA, United States of America.

hemisphere, which was attributed to greater PFAS emissions, compared with the southern hemisphere (Lv et al., 2023). On a continental scale (from highest to lowest), mean concentrations of PFOA were ranked, North America > Asia > Europe > Africa > Oceania > South America, and mean PFOS concentrations were ranked, North America > Africa > Europe > Asia > Oceania > South America (Lv et al., 2023).

A meta-analysis of PFAS soil-to-groundwater concentration ratios for samples collected from 324 sites where AFFF was used across 56 military installations throughout the USA demonstrated that soil is a significant reservoir for PFAS at these contaminated sites (Hunter Anderson et al., 2019). Moreover, analysis of PFAS depth profiles in the soil indicated significant retention of PFOA and PFOS in the vadose zone over decades, serving as a significant long-term source of PFAS in groundwater (Guo et al., 2020; Lv et al., 2023). In a recent study of temporal trends in PFAS concentrations in soil samples from eastern China, it was reported that PFOA concentrations increased by 86.4% between 2011 and 2021, whereas PFOS concentrations decreased by 28.2% during the same period. The distinct difference between PFOA and PFOS in terms of temporal changes in soil concentrations during the studied decade was attributed to the fact that PFOS was added to the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2009, while PFOA was added later in 2019 (Cheng et al., 2023).

(d) Food

PFOA and PFOS are introduced into foods in various ways, mainly depending on the food origin, but also on packaging (Schaider et al., 2017) and processing (Choi et al., 2018). Plant-based foods may be contaminated via atmospheric deposition or uptake from water and soil, including from use of sewage sludge as fertilizer (Ghisi et al., 2019). A study on crops grown in outdoor lysimeters demonstrated uptake of PFOA and PFOS, including in various edible parts of the crop (Felizeter et al., 2021). Uptakes vary both between and within species and may partly be explained by different plant properties (Costello and Lee, 2020). PFOA and PFOS become incorporated into animal-based foods because animals are exposed to these PFAS via water, feed, soil, and air (Death et al., 2021). [The Working Group noted that there was a lack of data on the contributions of different sources of PFOA and PFOS contamination in foods. This may be of more importance for source tracking and reduction than for exposure characterization.]

Concentrations of PFOA and PFOS have been determined in various food products, including food for infants such as formula and baby food, in a range of studies worldwide (Mikolajczyk et al., 2023). Some studies on processed food were available (e.g. Jogsten et al., 2009; Jeong et al., 2019; Genualdi et al., 2022; Vendl et al., 2022), but most of the data were based on the analysis of raw food products. In general, most data were available for fish and seafood, but there have been an increasing number of studies on other food groups during recent years (Domingo and Nadal, 2017; Jian et al., 2017; Pasecnaja et al., 2022). PFOA and PFOS concentrations detailed in selected studies and reports are presented in Table S1.13 and Table S1.14, respectively (see Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636).

Reporting limits varied to a large extent between studies (Pasecnaja et al., 2022). [The Working Group noted that high reporting limits, especially when considering data generated in the early 2000s, have resulted in low detection frequencies, resulting in challenges when comparing studies.] As a result of an increasing focus on the need for more sensitive methods, lower reporting limits have been observed in more recent studies (e.g. Lacina et al., 2011; Vestergren et al., 2012; Sadia et al., 2020). For example, in the study by Vestergren et al. (2012),

in which a particular effort had been made to increase sensitivity, the method limit of quantification (MLQ) ranged between 1.8 and 9.6 pg/g for PFOA, depending on food type, and between 1.5 and 8.0 pg/g for PFOS. In comparison, in the study by Clarke et al. (2010), for example, the LOQ for both PFOA and PFOS was 1000 pg/g. [The Working Group noted that because levels of PFOA and PFOS are low in many food products, improvements in MLQ have an impact on detection frequencies (EU, 2022).]

The studies presented in supplementary Table S1.13 and Table S1.14 were published recently, present data from different regions worldwide, and include information on several food categories. As an example, a study on 266 samples collected during 2018–2019 from 26 countries located in Africa, Asia (excluding China), and Latin America included data on several food groups and had high detection rates (Fiedler et al., 2022). The mean concentrations of PFOA in vegetables, fish and other seafood, beef, chicken, milk, and eggs were 7.58, 12.4, 6.44, 4.61, 0.99, and 8.34 pg/g, respectively. The corresponding mean concentrations of PFOS in the same food groups were 2.45, 124, 37.6, 5.80, 22.1, and 45.6 pg/g, respectively. [The Working Group noted that with the low LOQ in this study, PFOA and PFOS contamination was detected more frequently.]

The mean concentrations in samples from Europe (EFSA Panel on Contaminants in the Food Chain, 2020) and China (Fan et al., 2021) were in general higher than those from Africa, Asia (excluding China), and Latin America (Fiedler et al., 2022), but detection frequencies in studies in the USA were too low to compare, except for fish and seafood (US FDA, 2022a; Young et al., 2022). For example, the mean concentrations of PFOA in eggs were 106 and 150 pg/g in samples from Europe (EFSA Panel on Contaminants in the Food Chain, 2020) and China (Fan et al., 2021), respectively, while the mean concentration in the study on samples from Africa, Asia (excluding China), and Latin America was 8.34 pg/g (Fiedler et al., 2022).

For PFOS, the highest mean concentrations were generally seen in fish and seafood, when compared with other food groups (see Table S1.13 and Table S1.14, Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636). For example, the mean concentration of PFOS in fish and shrimp from China was 2760 pg/g, whereas the mean concentration in meat and meat products was 300 pg/g (Fan et al., 2021). This was in line with data reported in review papers (Domingo and Nadal, 2017; Jian et al., 2017; Pasecnaja et al., 2022). These observations were supported by the results of a study that found PFOS to be very bioaccumulative (bioaccumulation factor, > 5000) and also biomagnifying (trophic magnification factor, > 1) in a freshwater food web in Canada (Munoz et al., 2022). For PFOA, among various countries, the mean concentrations were highest in China across all food groups, with the highest concentrations found in fish and meat (see Table S1.13 and Table S1.14, Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636).

In a study on more than 500 composite samples of locally caught freshwater fish collected across the USA in 2013–2015, the median concentration of total PFAS (of which PFOS constituted 74%) was more than 200 times as high as the levels found in commercially relevant fish analysed by the US FDA in 2019–2022 (Barbo et al., 2023). The median and 90th percentile concentrations of PFOS in locally caught freshwater fish fillets across the USA were 8410 pg/g and 41 400 pg/g, respectively (Barbo et al., 2023), whereas the concentrations reported by the US FDA were generally in the low hundreds of picograms per gram, or less (Barbo et al., 2023).

Elevated concentrations of PFOA and PFOS in food have been reported in areas with known PFAS contamination (e.g. Hölzer et al., 2011; Langberg et al., 2022; Lasters et al., 2022). For instance, elevated concentrations of PFOA and PFOS were measured in hen eggs from private gardens situated within a 10 km radius of a fluorochemical-production plant in Antwerp, Belgium (Lasters et al., 2022). The highest concentrations were observed for PFOS (130–241 000 pg/g), and decreasing concentrations were observed with increasing distance from the plant (Lasters et al., 2022). This was in line with the results of a study showing increasing PFOA and PFOS concentrations in eggs of hens exposed to increasing concentrations in drinking-water (Wilson et al., 2021). In Lyon, France, PFOS concentrations in home-produced eggs near a fluorochemical-production facility were higher than in commercially produced eggs: home-produced, median, [965 pg/g] and range, 105–5240 pg/g; commercially produced, median, [113 pg/g] and range, 34–650 pg/g (Préfète du Rhône, 2023).

Environmental contamination may also result in elevated levels in dairy products. In a study on two dairy farms in the USA with known contamination of groundwater, samples were collected between 2018 and 2021. Milk samples from one of the two farms contained PFOS at elevated concentrations. PFOS concentrations of up to 4.22 ng/g were reported in the milk from this farm, while retail milk and control milk did not contain PFOS at detectable levels (US FDA, 2021a)

Food from wild game species may also contain elevated levels of PFOA and PFOS (Death et al., 2021). The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (EFSA Panel on Contaminants in the Food Chain, 2020) reported that edible offal from game animals contained mean levels of PFOA and PFOS that were more than 10 and 100 times, respectively, as high as in any other food group.

In a study from China that included food samples from two provinces, Hubei (n = 121) and Zhejiang (n = 106), geographical differences were observed between regions and sampling sites (Zhang et al., 2017a). PFOS levels were higher in Hubei than in Zhejiang, particularly for food of animal origin. This was expected because PFOS was produced in Hubei province. The concentrations of PFOA were similar in the two regions.

Temporal trends for abiotic and biological environmental samples, including food items, were evaluated in a systematic review by Land et al. (2018). Both non-significant and decreasing trends were observed for PFOS, depending on region and study. Non-significant trends were predominant for PFOA. The authors concluded that, despite interventions to reduce exposure to PFOA and PFOS, no clear temporal trends have been observed globally, probably because of the high persistence of these compounds in the environment. [There were no available data from the southern hemisphere and Asia.]

(e) Consumer products

PFOA and PFOS are present in numerous consumer products; for example, textiles, outdoor clothing, cleaning products, paints, coatings, carpets, floor coverings, floor polish, leather, cosmetics, printing inks, adhesives, ski wax, and lubricants (Glüge et al., 2020). However, it is often not clear if they were added intentionally, are impurities of other components, or are degradation products (Glüge et al., 2020). Also, PFOA has been used as a processing aid when manufacturing fluoropolymers used, for example, in non-stick cookware. Residues of PFOA may thus be present in these products (Sinclair et al., 2007).

The amount of available data on PFOA and PFOS in consumer products is considerably smaller than that on these compounds in food and drinking-water, and almost all data have been published in the last decade. [The Working Group noted that results are reported in different metrics, e.g. ng/g or μg/m^2, which makes com-

parison challenging.] Below, selected studies on consumer products available on the market in different regions worldwide are presented.

PFOA and PFOS were determined in 115 randomly selected consumer products (textiles, carpets, cleaning and impregnating agents, leather, baking and sandwich papers, paper baking forms, and ski waxes) purchased in Germany in 2010 (Kotthoff et al., 2015). Detection frequencies varied between product categories. For PFOA and PFOS, respectively, the detection frequencies were 100% and 100% for outdoor textiles, 78% and 100% for nanosprays and impregnation sprays, 88% and 100% for ski wax, and 30% and 90% for carpets. PFOA was also found in 100% of the gloves, 100% of textiles for awnings, and 63% of leather products. PFOS was detected in 69% of paper-based food contact materials. PFOA and/or PFOS were detected in < 50% of the remaining products (wood glue and cleaners). For PFOA, the highest median (and maximum) concentrations were observed in ski wax and nano- and impregnation sprays, 15.5 (maximum, 2033.1) and 15.9 (maximum, 28.9) ng/g, respectively (Kotthoff et al., 2015). The highest median (and maximum) levels of PFOS were observed in outdoor textiles and leather with concentrations of 9.5 (maximum, 35.4) and 5.6 (maximum, 5.6) μg/m^2, respectively.

PFOA and PFOS were determined in 25 samples of consumer products available to private consumers in Japan and purchased between 1981 and 2009 (car wash/coating products, sprays for fabrics and textiles, insecticides, rust inhibitors, and paints) (Ye et al., 2015). PFOA was found in one sample of spray for fabrics and textiles (36 ng/g) and one rust inhibitor (11 ng/g), and PFOS was observed in one sample of spray for fabrics and textiles (59 ng/g) (Ye et al., 2015). In a study in Norway, PFOA and PFOS were determined in 45 samples of furniture textile, carpet, clothing, and food contact materials (Vestergren et al., 2015). All samples were imported from China and purchased in Norway in 2012–2013. PFOA was found in 26 samples, at concentrations between 0.005 (carpet) and 0.91 μg/m^2 (curtain). PFOS was detected at 1.7 μg/m^2 in one carpet sample (Vestergren et al., 2015).

Seventeen samples of paper and cardboard food packaging materials purchased from retailers and grocery stores in Egypt in 2013 were analysed for PFAS (Shoeib et al., 2016). PFOA and PFOS were detected in 79% and 58% of the samples, at median concentrations of 2.40 ng/g and 0.29 ng/g, respectively.

In a study in the USA, PFAS were determined in 61 samples of furnishings, apparel, and bedding purchased in 2020 (Rodgers et al., 2022). PFOA and PFOS were detected in seven and one product, respectively. The maximum concentration of PFOA was 22.5 ng/g, and the concentration of PFOS was 2.1 ng/g in the one product in which it was detected (Rodgers et al., 2022). PFAS were determined in 160 textile products purchased in Albany, New York, USA, between 2016 and 2019 (Zhu and Kannan, 2020). PFOA was detected in 20% of the products, at a maximum concentration of 32.7 μg/m^2, whereas PFOS was found in 3.8% of the products, at a maximum concentration of 0.167 μg/m^2 (Zhu and Kannan, 2020). PFOA and PFOS were determined in 32 textile samples purchased in Thailand; mean concentrations were 2.74 μg/m^2 (range, 0.31–14.14 μg/m^2) and 0.18 μg/m^2 (range, 0.02–0.46 μg/m^2), respectively (Supreeyasunthorn et al., 2016).

PFOA was determined in sunscreens and cosmetics, primarily from Japan, for which fluorinated compounds were listed as ingredients. Among these products, 8 of 9 sunscreens and 13 of 15 cosmetics contained PFOA at concentrations above the LOQ; concentrations ranged from 3.7 to 5700 ng/g (Fujii et al., 2013). A wide range of PFAS were determined in 38 cosmetics and personal care products for which organofluorine compounds were listed as ingredients and that were available on the North American market in 2020–22 (Harris et al., 2022). PFOA and PFOS

were found at levels above the LOQ in 65.8% and 26.3% of the samples, respectively. The median (and maximum) concentrations of PFOA and PFOS were 13.6 ng/g (28 600 ng/g) and < LOQ (16.5 ng/g), respectively (Harris et al., 2022). In another study in which PFOA and PFOS concentrations were determined in cosmetics (n = 29, 12 from the USA and 17 from Canada) available on the North American market in 2020, PFOS was detected in only two Canadian samples, at concentrations of 15.5 and 6.6 ng/g (Whitehead et al., 2021). In a study on 43 different cosmetics for which PFAS were listed as ingredients and that were available on the European market in 2020, PFOA was detected in only one foundation/beauty balm cream, at a concentration of 112 ng/g, and PFOS was not detected (Pütz et al., 2022).

[The Working Group noted that there were few data available on the same products at different time points; it was thus not feasible to evaluate potential time trends and the effects of regulations and voluntary phase-outs (see Section 1.5).]

The results of these studies demonstrated that PFOA and PFOS are commonly present in a wide range of consumer products. In several of the studies, PFOA and/or PFOS were most frequently detected in textiles and fabrics at concentrations between the LOD and 35 µg/m^2. Also, in some of the studies on cosmetics, detection frequencies were high, and concentrations detected were up to micrograms per gram product.

1.4.2 Occupational exposure

Populations with occupational exposure are generally recognized as having some of the highest levels of exposure to PFOA and PFOS (Christensen and Calkins, 2023). In occupational settings, the exposure route of greatest importance is typically assumed to be inhalation, but dermal uptake and ingestion of dust may also contribute, depending on the workplace conditions (De Silva et al., 2021).

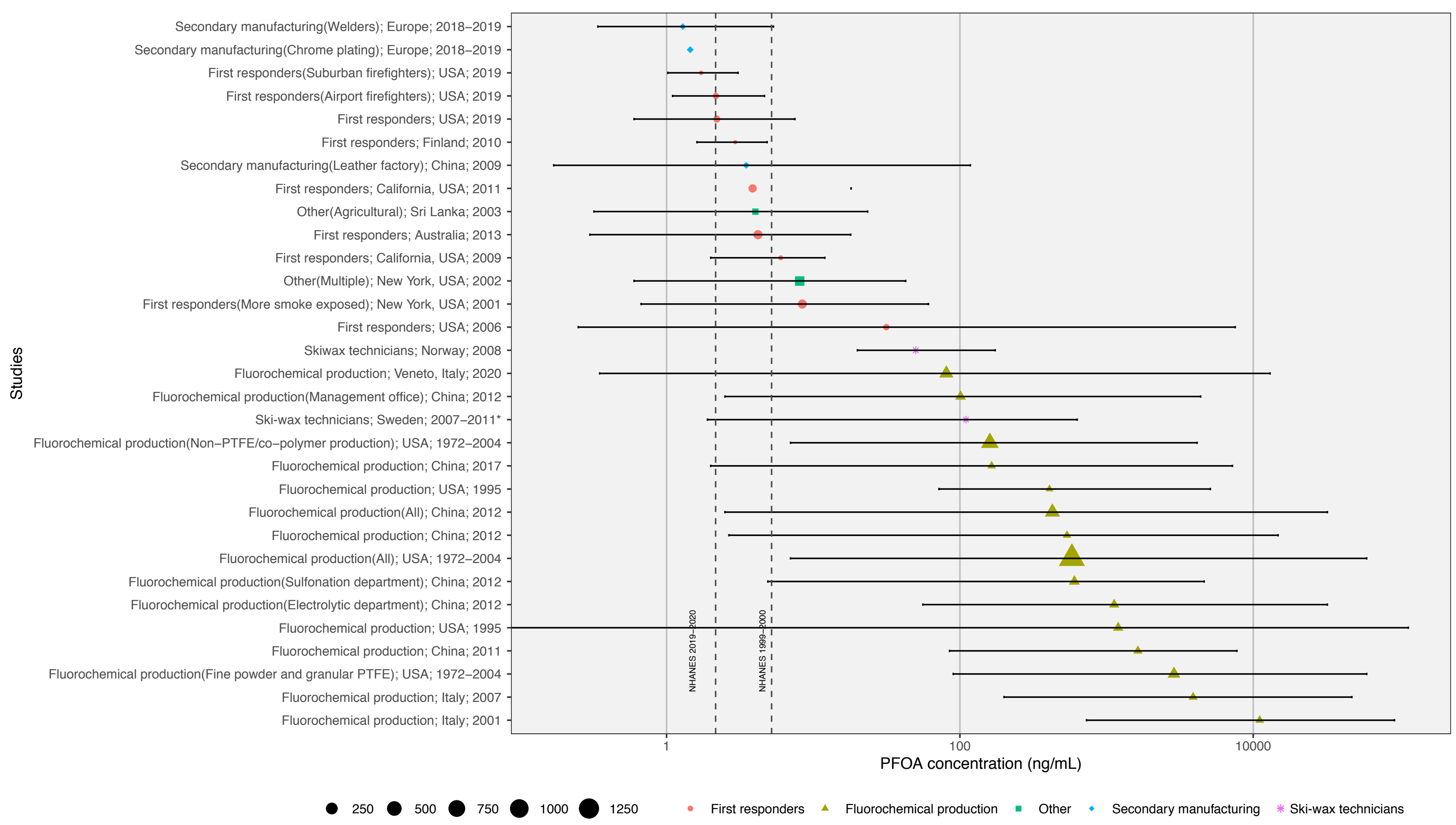
Occupational exposure to PFOA and PFOS may result from fluorochemical-production processes; use of PFAS as a processing aid in other manufacturing settings; use of PFOS as a mist suppressant to reduce exposure to other chemical hazards; contact with products containing PFOA or PFOS, as well as precursor compounds; and contact with contaminated environmental media or waste infrastructure (see Table S1.15, Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636). The information regarding determinants of exposure was limited, but indicated that processing conditions, such as high temperature, low pH, and use of PFAS in dry powder form, are linked to elevated PFAS exposure in fluorochemical-manufacturing settings (Freberg et al., 2010; Kaiser et al., 2010; Christensen and Calkins, 2023). Limited data on bulk and dust monitoring suggested that dust present in speciality textile-manufacturing settings, such as those producing flame-retardant or water-repellent materials, increases the risk of PFAS exposure (Sha et al., 2018; Christensen and Calkins, 2023). For non-manufacturing industries, such as retail and office buildings, factors including the building's age, the presence of carpeting, and the extent of ventilation are strongly linked to PFAS exposure (Langer et al., 2010; Sha et al., 2018; Christensen and Calkins, 2023).

In this section, occupational exposures are first described using biomonitoring data, followed by industrial hygiene samples, and work environment samples.

(a) Biomonitoring data

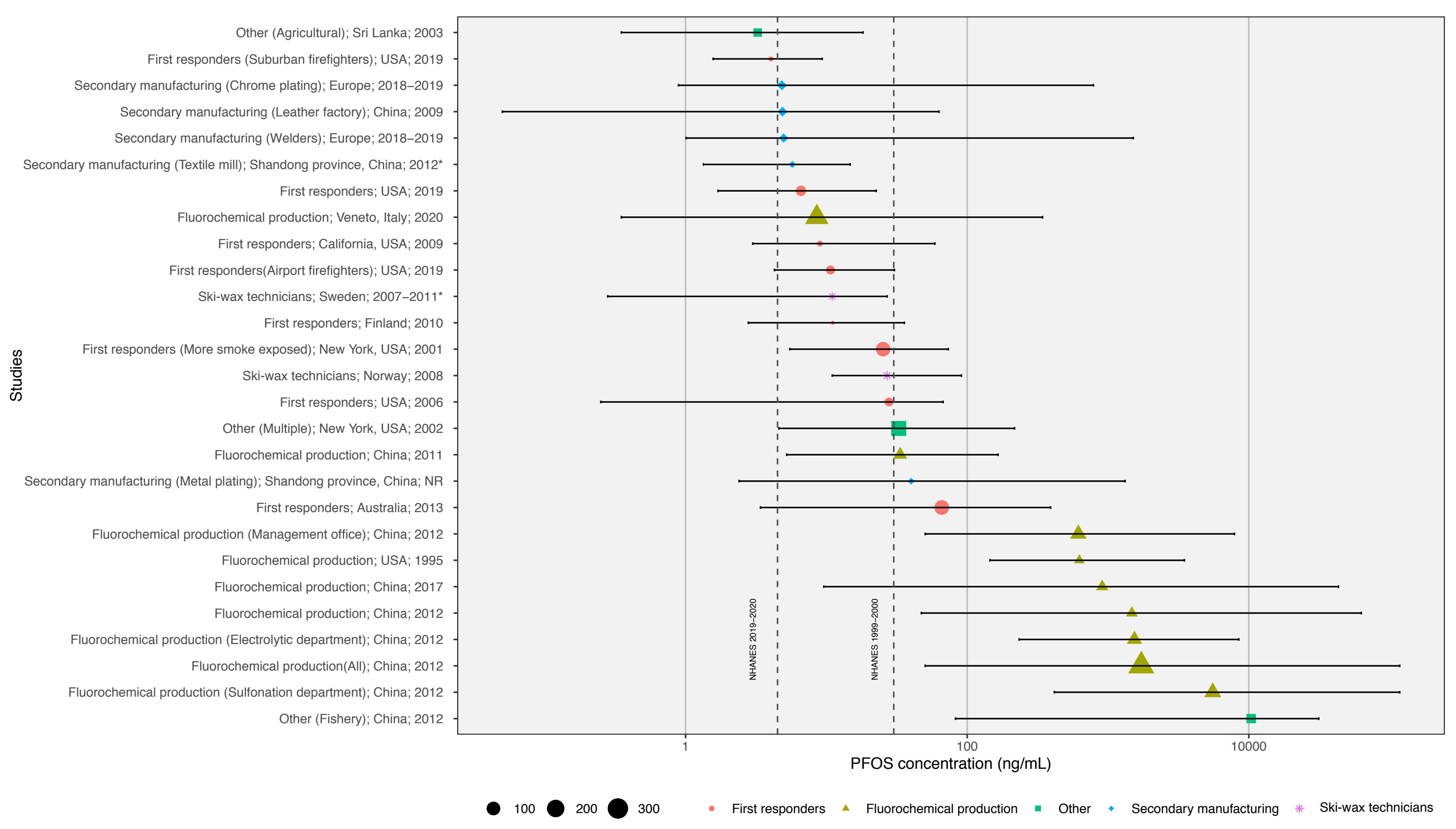
Biomonitoring has been used to assess exposure to PFOA and PFOS in different settings. Fluorochemical-production workers have some of the highest serum PFOA and PFOS concentrations reported in the literature (see Fig. 1.5 and Fig. 1.6; and Table S1.15, Annex 1, Supplementary material for Section 1, Exposure Characterization,

Fig. 1.5 Examples of PFOA concentrations in serum, plasma, and whole blood in occupationally exposed populations



NHANES, National Health and Nutrition Examination Survey; PFOA, perfluorooctanoic acid; PTFE, polytetrafluoroethylene; USA, United States of America. *Whole blood.
Statistics include median, minimum, and maximum concentrations (ng/mL), the most recent year of sample collection is indicated. See Table S1.15 for detail and references (Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636). [The Working Group noted that these values are thought to be representative of the literature on occupational exposure. For studies in which data were reported for multiple subgroups, only selected groups are included.]
Concentrations from the adult general population study NHANES are given for the years 1999–2000 and 2019–2020 for comparison (see Section 1.4.3).

Fig. 1.6 Examples of PFOS concentrations in serum, plasma, or whole blood in occupationally exposed populations



NHANES, National Health and Nutrition Examination Survey; PFOS, perfluorooctanesulfonic acid; USA, United States of America. *Whole blood.
Statistics include median, minimum, and maximum concentrations (ng/mL), the most recent year of sample collection is indicated. See Table S1.15 for detail and references (Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636). [The Working Group noted that these values are thought to be representative of the literature on occupational exposure. For studies in which data were reported for multiple subgroups, only selected groups are included.] Concentrations from the adult general population study NHANES are given for the years 1999–2000 and 2019–2020 for comparison (see Section 1.4.3).

online only, available from: https://publications.iarc.who.int/636). In samples collected in the year 2000 from 25 PFOA-production workers at a site in Italy, Costa et al. (2009) reported a geometric mean concentration of PFOA in serum of 11 700 ng/mL (range, 1540–86 300 ng/mL) (Costa et al., 2009). However, the highest reported PFOA serum concentration (114 100 ng/mL) was in a sample collected in 1995 from a male worker at a US facility that produced APFO, the ammonium salt form of PFOA (Olsen et al., 2000). In a study by Fu et al. (2016) of fluorochemical-production workers from a chemical plant in Hubei province, China, serum samples collected from 101 workers in the sulfonation department contained some of the highest concentrations of PFOS, with a mean of 14 002 ng/mL (range, 416–118 000 ng/mL). PFOA was also reported in this study; however, concentrations were higher for workers in the electrolytic department than in the sulfonation department, peaking at 32 000 ng/mL (Fu et al., 2016).

Using blood samples collected from workers at a fluoropolymer-manufacturing facility in West Virginia, USA, between 1972 and 2004, Woskie et al. (2012) constructed a job-exposure matrix (JEM) to retrospectively assess exposure spanning from 1950 to 2004. This facility used a process to manufacture certain fluoropolymers, such as PTFE, that involved the use of APFO as a surfactant in the polymerization of tetrafluoroethylene (TFE). Serum PFOA concentrations were highest in workers with tasks involving fine powder and granular PTFE (mean, 5470 ng/mL; range, 90–59 400 ng/mL), whereas workers involved in non-PTFE production (no use of APFO or PFOA) had the lowest serum concentrations (mean, 240 ng/mL; range, 7–4140 ng/mL). However even the latter experienced exposure that far exceeded that of the general population. For example, for the period 2000–2004, the geometric mean serum concentration for non-PTFE (no use of APFO or PFOA) production workers was 140 ng/mL, whereas the median serum concentration for the local community in 2005–2006 was 40 ng/mL, and geometric mean concentrations in adults aged > 20 years in the National Health and Nutrition Examination Survey (NHANES) were about 4–5 ng/mL between 1999–2000 and 2003–2004 (Woskie et al., 2012). Trends over time also differed by job category in modelled output, with exposures in fine powder/granular PTFE chemical operators peaking in 1980 (median serum concentration, > 6000 ng/mL), just before the implementation of exposure control measures. However, workers with only intermittent or background exposure to PFOA experienced higher exposure in 2000 (median serum concentration was estimated at nearly 1600 ng/mL), corresponding with peak production (Woskie et al., 2012).

Exposure in first responders (including firefighters), the second most frequently characterized worker population, is typically described as resulting from interactions with products containing PFAS, most notably AFFF, but exposure from turnout gear or the built environment has also been the subject of recent research (Peaslee et al., 2020; Young et al., 2021). Inhalation, dermal contact, and ingestion (e.g. via hand-to-mouth contact) are potential exposure routes because of contact with the product as well as with dust or air at the fire station and response scene (Mazumder et al., 2023; Rosenfeld et al., 2023). The highest serum concentrations of PFOS were reported among 149 firefighters working at AFFF training facilities in Australia; the mean PFOS concentration was 74 ng/mL (range, 3.4–391 ng/mL), compared with a mean concentration of 12 ng/mL for the general population in Australia. In this study, employment before the 2003 phase-out of PFOS-based AFFF at the facilities was positively associated with PFOS concentrations in serum samples collected in 2013. PFOA was not elevated in this population, with mean serum concentrations of 4.6 ng/mL (range, 0.3–18 ng/mL) (Rotander et al., 2015).

Results from other studies of firefighters suggest that there are differences by type of firefighter, firefighting activity, and geography. PFOS concentrations were higher in airport firefighters, (median, 10.69 ng/mL; range, 4.28–30.42 ng/mL) than in suburban firefighters (median, 4.04 ng/mL; range, 1.57–9.34 ng/mL) from the same geographical region; however, no difference was observed for PFOA (Leary et al., 2020). Burgess et al. (2023) compared serum concentrations collected from firefighters in municipal fire departments in three distinctly different regions of the USA and reported concentrations that were elevated above those reported in NHANES for branched PFOS (sm-PFOS, sum of perfluoromethylheptane sulfonate isomers) in all four departments, as well as linear PFOS (*n*-PFOS) and linear PFOA (*n*-PFOA) in two departments (Burgess et al., 2023). In contrast, PFOS levels were similar to those from NHANES for a sample of 101 male municipal firefighters in California, USA (Dobraca et al., 2015), and lower than those from NHANES in 138 volunteer municipal firefighters in New Jersey, USA (Graber et al., 2021).

In plasma from first responders from New York State and National Guard employees who responded to the collapse of the World Trade Center, New York City, USA, in the terrorist attack of 11 September 2001, PFOA concentrations were approximately twofold those of the general population. In this study, Tao et al. (2008a) used samples collected 6 months to 2 years after the collapse to assess exposure to PFAS categorically according to more and less exposure to smoke or dust. They observed higher PFOA concentrations in smoke-exposed individuals than in dust-exposed individuals, with the highest levels occurring in the group that was more highly exposed to smoke (mean, 10.21 ng/mL; range, 0.67–61 ng/mL). Background PFOA concentrations for 2001–2002 in the USA ranged from a median of 4.7 ng/mL for the full population to a mean of 6.98 ng/mL for non-Hispanic White men (see Section 1.4.3 for exposure in the general population). PFOS was detected in all samples, with mean concentrations ranging from 22.9 to 33.9 ng/mL across the study groups; however, concentrations were not elevated above those in the general US population (median, 25.8 ng/mL) or in the general population in two US cities (in Portland, Oregon, the median was 26.0 ng/mL, and in Boston, Massachusetts, the median was 29.5 ng/mL) (Tao et al., 2008a).

There were few available biomonitoring data for other worker populations. In the Human Biomonitoring for Europe (HBM4EU) project, samples collected in 2018–2019 contained median PFOS concentrations of 4.97 ng/mL (maximum, 1513 ng/mL) in welders and 4.83 ng/mL (maximum, 789 ng/mL) in metal plating workers – an exposure that is anticipated to have resulted from the use of PFOS as a mist suppressant in chrome plating baths (Göen et al., 2024). Shi et al. (2016) reported a median serum PFOS concentration in metal plating workers in China of 40 ng/mL (range, 2.4–1323 ng/mL) (Shi et al., 2016). In a study of workers in shoe and leather-related industries (2011) from the same region of China, mean serum concentrations were 6.93 ng/mL (range, 0.17–117.77 ng/mL) and 14.18 ng/mL (range, 0.05–31.66 ng/mL) for PFOA and PFOS, respectively (Zhang et al., 2011). In a separate study of textile factory workers (2009) in another region of China, the mean blood concentration of PFOA was 5.46 ng/mL (range, 2.35–10.93 ng/mL), and that of PFOS was 5.73 ng/mL (range, 1.34–14.75 ng/mL) (Lu et al., 2014). In the same study, mean concentrations measured in barbers, who may be exposed through the use of products containing PFOA and PFOS, were 3.18 ng/mL (range, 0.78–12.18 ng/mL) and 2.56 ng/mL (range, 0.44–7.72 ng/mL) for PFOA and PFOS, respectively (Lu et al., 2014). PFOA concentrations measured in whole blood from ski-wax technicians working with teams competing in World Cup events in Europe between 2007 and 2011

ranged from 1.9 to 630 ng/mL (mean, 130 ng/mL) (Nilsson et al., 2013a). In this population, for which samples were collected at multiple time points across multiple ski seasons, PFOA concentrations increased in technicians with "low" initial concentrations, but decreased or remained at steady-state in technicians with "high" initial concentrations (Nilsson et al., 2010, 2013a). The median PFOS concentration (12.2 ng/mL) was not elevated when compared with exposure in the general population (Nilsson et al., 2010).

In studies of agricultural workers in China and Sri Lanka, as well as retail and office workers in the USA, concentrations of PFOA and PFOS have been reported that are similar to those in the corresponding general population (see Table S1.15, Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636) (Guruge et al., 2005; Fraser et al., 2012; Zhou et al., 2014; Wu et al., 2019; Clarity et al., 2021), with the exception of commercial fishery workers in China, for whom the potential for a large dietary exposure through consumption of employer-provided fish probably contributed to the mean serum PFOS concentration of 11 400 ng/mL (range, 82.6–31 400 ng/mL) (Zhou et al., 2014).

Although biomonitoring data collected over time and by occupation or industry were relatively limited, the available data indicated the potential for exposure to PFOA and PFOS in diverse occupational settings, including primary (e.g. fluorochemical production) and secondary (e.g. metal plating, textile mill) manufacturing, public safety (e.g. firefighters) and services (e.g. ski-wax technicians, barber). The exposure characterization was the most robust for fluorochemical-production workers, followed by first responders. Although the magnitude of exposure in these populations differed substantially, there was evidence for intrapopulation differences in exposure by task or activity, with higher concentrations being measured in workers with tasks or activities involving known contact with materials containing PFOA or PFOS.

[The Working Group noted that blood concentrations across occupational populations differ by orders of magnitude. For example, median concentrations of PFOA and PFOS reported for fluorochemical-production workers are often 100 to 10 000 times as high as those of firefighters, depending on the study. The Working Group also noted that PFOA and PFOS have primarily been measured in worker populations with a known source of exposure (e.g. manufacturing of fluorochemicals, use of AFFF); the absence of data for the many occupations with the potential for PFOA and PFOS exposure is not evidence of the absence of exposure.]

(b) Industrial hygiene samples

Characterization of PFOA and PFOS in the work environment, for example through air samples, is limited by the availability and consistency of methods (see Section 1.3) and comparable data. Although large variability exists in the reported concentrations, measures of the work environment, including samples of workplace air, dust, surfaces, and other work-related materials, frequently contain higher concentrations of PFOA and PFOS in facilities engaged in manufacturing or use of PFAS-laden products (such as fluorochemical production, secondary manufacturing, firefighting, and ski-wax application) than in other occupational environments (such as offices, schools, retail stores, and hotels). In these studies, measurement of PFOA and PFOS is often accompanied by measurement of related or precursor compounds (see Section 1.4), such as fluorotelomer alcohols (e.g. 8:2 FTOH), fluorotelomer sulfonic acids (e.g. 8:2 FTS), sulfonamides (e.g. *N*-EtFOSA), and phosphoric acid diesters (e.g. 8:2 diPAP) (Christensen and Calkins, 2023). Precursor compounds such as these may transform in the environment or the body to PFOA, PFOS, or other PFAS (Kolanczyk et al., 2023). For

additional information on the toxicokinetics of PFOA and PFOS, see Section 4.1.

Occupational exposure at a chemical plant in Hubei province, China, was described by Gao et al. (2015) as indicating higher concentrations of PFOS than PFOA in indoor dust, with geometric mean concentrations of 830 ng/g dust (range, LOD to 658 343 ng/g dust) and 360 ng/g dust (range, 41.3–85 139 ng/g dust), respectively. However, the reverse was observed in total suspended particles, with geometric means of 0.4 ng/m^3 (range, 0.03–78 ng/m^3) and 0.94 ng/m^3 (range, 0.04–1123 ng/m^3) for PFOS and PFOA, respectively (Gao et al., 2015). At a facility producing APFO and PFOA in the USA, Kaiser et al. (2010) reported an 8-hour time-weighted average (TWA) median air concentration of 34 µg/m^3 (range, 4–65 µg/m^3) measured near the process sumps. Concentrations were higher at lower pH and water levels. They also reported that the sublimation rate measured for PFOA (360 µg/hour) was higher than that for APFO (0.302 µg/hour) (Kaiser et al., 2010).

In the studies on ski-wax technicians, personal breathing zone samples collected over three ski seasons spanning 2007 to 2010 contained higher concentrations of 8:2 FTOH (a precursor to PFOA) than PFOA, with concentrations ranging from 0.834 to 997 µg/m^3 and from 0.027 to 14.9 µg/m^3 for 8:2 FTOH and PFOA, respectively. Area aerosol samples did not contain FTOH; however, PFOA concentrations were higher in the inhalable fraction than the respirable fraction, with mean concentrations of 16 µg/m^3 (range, 2.11–52.8 µg/m^3) and 9.91 µg/m^3 (range, 0.62–26.8 µg/m^3), respectively (Nilsson et al., 2013b). The metabolism of FTOH to PFOA in workers' blood was supported by the presence of FTOH degradation products, 5:3 FTCA and 7:3 FTCA, in the blood of workers in this study (Nilsson et al., 2013a). Although concentrations were lower, Freberg et al. (2010) reported a similar relationship between inhalable and respirable fractions, with PFOA concentrations ranging from 5.1 to 35 ng/m^3 and 5.6 to 38 ng/m^3 in inhalable and respirable fractions, respectively. PFOA was detected at higher concentrations in powder wax (median, 2.7 µg/g product; range, 0.29–12 µg/g) than in solid block wax (median, 0.68 µg/g product; range, < LOQ to 3.8 µg/g). PFOS was not detected in any dust samples and was only detected in a few of the powder ski-wax samples (maximum, 0.149 µg/g) (Freberg et al., 2010).

Hall et al. (2020) reported higher concentrations of PFOA and PFOS in dust samples collected from 49 fire stations across the USA and Canada than in samples from 184 homes in North Carolina, with median (and maximum) concentrations in fire stations and homes of 17.6 ng/g dust (maximum, 791 ng/g dust) and 7.9 ng/g dust (2350 ng/g dust) for PFOA, respectively and 64.5 ng/g dust (74 370 ng/g dust) and 4.4 ng/g dust (2810 ng/g dust) for PFOS, respectively (Hall et al., 2020). Within fire stations, concentrations of the PFOS precursor, *N*-ethyl-perfluorooctane sulfonamido acetic acid (*N*-EtFOSAA), in dust were higher in living areas (median, 87.5 ng/g dust; range, 0.748–1800 ng/g dust) than in gear storage (median, 7.84 ng/g dust; range, < MDL to 299 ng/g dust) or the apparatus bay (median, 3.51 ng/g dust; range, < MDL to 159 ng/g dust) (Young et al., 2021). Using silicone wristbands worn while on- or off-shift, Levasseur et al. (2022) reported that PFOS concentrations while on-duty and responding to fires were 2.5 times as high as off-duty exposures; however, PFOA concentrations while on-duty and responding to fires were lower than off-duty exposures (Levasseur et al., 2022). When analysing textiles used in new firefighter personal protective equipment (PPE), Maizel et al. (2023) detected PFOA and PFOS in 7 of 20 textiles tested, with concentrations all < 2 ng/g. However, the highest concentrations were reported for precursor compounds, including 6:2 fluorotelomer methacrylate (mean, 1570 ng/g), 6:2 FTOH (mean, 613 ng/g), and 6:2 fluorotelomer sulfonic acid (mean, 393 ng/g). In

a separate study of PPE worn by firefighters, sets of used and unused turnout gear thermal liners, moisture barriers, and outer shells were analysed. PFOS levels were largely below the LOD, but PFOA was detected at higher concentrations than other PFAS, with the highest concentration measured in a used thermal liner from trousers worn in 2014 (850 ppb) (Peaslee et al., 2020).

Area air and dust collected over 17 days in 2014 from a speciality, water-repellent textile-manufacturing facility in China were analysed for PFOA, PFOS, and numerous precursor compounds. Heydebreck et al. (2016) reported higher concentrations in the gas phase than the particle phase in air samples, with generally lower concentrations in settled dust. Fluorotelomer alcohols (8:2 FTOH and 10:2 FTOH) were the dominant analytes measured in workplace air, with the highest concentrations reported for 8:2 FTOH from heat setting (91.3 μg/m^3) and drying (87.7 μg/m^3) operations in one of the workshops, two processes that occur after the durable water-repellent coating has been applied to the textile. PFOA was the ionic PFAS measured at the highest concentrations in workplace air, with highest concentrations measured during the drying operation in the same workshop (8.48 ng/m^3). PFOS was generally below the method detection limit (5.33 ng/g) (Heydebreck et al., 2016).

Some studies have described exposure in occupational environments where the exposure sources are similar to sources for the general public, such as through direct contact or contact with dust from consumer products (e.g. clothing) or the built environment (e.g. carpets). In these settings, exposures vary across studies of classrooms, offices, and retail stores. In the UK, Goosey and Harrad (2011) reported higher concentrations of PFOS in dust collected from classrooms (mean, 980 ng/g dust; range, 22–3700 ng/g dust) than from offices (mean, 370 ng/g dust; range, 20–1000 ng/g dust). Concentrations of PFOA were variable in classrooms, (mean, 310 ng/g dust; range, 18–1700 ng/g dust), and in offices (mean, 550 ng/g dust; range, < LOD to 6000 ng/g dust) (Goosey and Harrad, 2011). PFOA has been reported to be the predominant compound in samples analysed for perfluoroalkyl carboxylic and sulfonic acids from electronic shops, offices, libraries, and internet cafés (Besis et al., 2019), and to be present at higher concentrations in office than in residential settings. However, this is not the case for PFOS (D'Hollander et al., 2010; Goosey and Harrad, 2011; Fraser et al., 2012). In office settings, Fraser et al. (2012) reported a strong positive association between FTOHs measured in office air and serum PFOA concentrations measured in office workers, with geometric mean air concentration of 8:2 FTOH of 9.92 ng/m^3 (range, 0.28–70.6 ng/m^3).

In the only study of dermal exposure, skin exposure to the pesticide sulfluramid (*N*-EtFOSA), a precursor of PFOS, in pesticide manufacturing workers during an 8-hour shift was measured at six different locations across the body. Exposure was greatest on the hands, with a mean of 89.7 μg/day, followed by the left leg (73.0 μg/day) and arms (72.1 μg/day); however, there was substantial variability (Machado-Neto et al., 1999).

(c) Protection measures to limit exposure

Approaches to reducing occupational exposures are commonly categorized into the hierarchy of controls – an effectiveness-based hierarchy of actions. This framework categorizes elimination and substitution as the most effective risk management measures, with PPE considered the least effective because of reliance on correct and consistent use by individual workers (NIOSH, 2023). Effective control measures have been documented in research studies involving fluorochemical-production facilities and ski waxing. For both industries, the use of local exhaust ventilation near the exposure source was linked to reductions in PFAS exposure. Other measures that led to reductions included

maintaining pH levels above 7 to reduce volatilization potential and wetting PFAS-containing dry powders at fluorochemical-production facilities, and replacing powder wax with block wax at ski-waxing facilities (Christensen and Calkins, 2023). The retrospective assessment by Woskie et al. (2012) demonstrated that, despite increases in production between 1980 and 2000, incorporation of exposure controls such as engineering and PPE resulted in decreasing serum concentrations in workers in the most highly exposed job category (Woskie et al., 2012). [The Working Group noted that the implementation of effective control measures may affect occupational exposures, including those relevant to epidemiological studies. In this case, samples collected after the implementation of effective controls may not be representative of exposures that occurred under prior conditions (and vice versa).]

1.4.3 Exposure of the general population

The general population is exposed via multiple sources to PFOA and PFOS and, given their widespread use, environmental contamination, and long persistence, both compounds are detectable in the blood of virtually all people tested (OECD, 2015b). Serum or plasma are the most common biological matrices used in biomonitoring campaigns. Long-chain PFAS such as PFOA and PFOS are not commonly measured in urine or breast milk samples, because of the lack of sensitivity of most available analytical tools (Worley et al., 2017) (see Section 1.3.4).

Measured levels in whole blood, serum or plasma are useful indicators of exposure since they reflect accumulated intake from all sources and, given the long half-lives of PFOA and PFOS, they are quite stable indicators of body burden (see also Section 4.1). Where people are exposed to a local substantial source of PFOA or PFOS, such as contaminated drinking-water, or occupational exposure, such exposure will be the main source (Pitter et al., 2020). For the general population not living close to a major point source, measured serum levels will reflect diverse exposure sources, including food, water, air, indoor dust, and consumer products. Also, individual serum levels will vary, reflecting not only degree of intake but individual variability in efficiency of uptake, distribution, and excretion (Section 4.1).

(a) Human exposure estimation

The general population is exposed via the diet, drinking-water, household dust, consumer products, and inhalation of contaminated air (Sunderland et al., 2019; De Silva et al., 2021). [The Working Group noted that the data presented in this section mainly draw from studies performed after 2000. Extrapolation to earlier points in time was difficult because of the sparse data available before 2000.] When drinking-water is contaminated by a specific pollution source with high emissions, drinking-water is the main exposure source. For example, in the Mid-Ohio Valley "C8" study population in the USA, the principal PFOA exposure source was drinking-water. In the most highly contaminated water district, the population's PFOA serum levels were 17-fold those in the water district with the lowest contamination, and drinking-water was the main contributor to the total body burden (Steenland et al., 2009).

For general populations without a recognized emission source or not living in a highly contaminated location, several studies have sought to estimate the relative importance of different exposure sources, and these are summarized in Table 1.16 and Table 1.17. Some of the studies have in addition estimated the possible pathways of exposure. There was some variability in the dietary contribution but, in all cases, diet has been estimated to be the most important exposure source. Exposure can derive both from the food being contaminated from uptake during growing or grazing, and from migration from food packaging materials (see Section 1.4.1(d)).

Table 1.16 Estimated relative contribution (%) of various routes of exposure to total PFOA in the general population

Location, sampling time	Relative contribution of exposure route (%)							Comments	Reference
	Oral				Inhalation	Dermal	Via precursors		
	Diet	Dust	Water	Food packaging					
Germany, 2005; Japan, 2004	85	6	1	3			2–8		Vestergren et al. (2008); Vestergren and Cousins (2009)
Norway, 2008	84	5	11		0.13				Haug et al. (2011)
USA, 2003/2004	66	9	24		< 1	< 1			Lorber and Egeghy (2011)
North America, Europe, Republic of Korea, Japan 2007/2008	47	8	12		6		27[a]		Gebbink et al. (2015)
Republic of Korea, 2009	41		37		22		5		Tian et al. (2016)
China, 2013/2014	> 99		< 1						Shan et al. (2016)
Finland, 2005/2006, 2010/2011, 2014/2015	95	< 2.5			< 2.5			Children aged 10 years	Balk et al. (2019)
Ireland, 2016/2017/2018	NR	1	37		62			Adults	Harrad et al. (2019)
	NR	3	74		23			Children	
Norway, 2013/2014	92	4			3	< 1			Poothong et al. (2020)

NR, not reported; PFOA, perfluorooctanoic acid.; USA, United States of America.
[a] Value given for the intermediate exposure scenario; estimated contribution varied according to exposure scenario from 13% to 64%.

In 2020, the European Food Safety Authority made an assessment of exposure of the European population to several PFAS, including PFOA and PFOS, on the basis of data available concerning the presence of these PFAS in different food categories and on consumption data (EFSA Panel on Contaminants in the Food Chain, 2020). For the lower-bound scenario, which was considered the most realistic by the panel, median dietary exposure to PFOA was estimated to range between 0.17 and 0.41 ng/kg body weight (bw) per day for different age categories (Table 1.18). Median dietary exposure to PFOS for the same scenario was estimated to range between 0.36 and 1.34 ng/kg bw per day. Similar average intakes were estimated in the Netherlands: 0.2 ng/kg bw per day for PFOA and 0.3 ng/kg bw per day for PFOS (Noorlander et al., 2011). In both studies and for both compounds, mean dietary exposure was highest for toddlers (defined as children aged 1–3 years).

In a study conducted in the USA in 2020 (Zheng et al., 2020), the occurrence and distribution of PFAS, including PFOA and PFAS, was determined in the childcare environment (dust and nap mats), and children's exposure

Table 1.17 Estimated relative contribution of various routes of exposure to total PFOS in the general population

Location	Relative contribution of exposure route (%)							Comments	Reference
	Oral				Inhalation	Dermal	Via precursors		
	Diet	Dust	Water	Food packaging					
USA, 2003/2004	72	6	22		< 1	< 1			Egeghy and Lorber (2011)
Norway, 2008	96	1	1		2				Haug et al. (2011)
North America, Europe, Republic of Korea, Japan 2007/2008	66	10	7		2		16[a]		Gebbink et al. (2015)
Republic of Korea, 2009	93		4		3				Tian et al. (2016)
China, 2013/2014	100		< 1						Shan et al. (2016)
Finland, 2005/2006, 2010/2011, 2014/2015	95	< 2.5			< 2.5			Children aged 10 years	Balk et al. (2019)
Ireland, 2016/2017/2018	NR	21	30		49			Adults	Harrad et al. (2019)
	NR	55	35		10			Children	
Norway, 2013/2014	75				3				Poothong et al. (2020)

PFOS, perfluorooctanesulfonic acid; USA, United States of America.
[a] Value given for the intermediate exposure scenario; estimated contribution varied with exposure scenario from 11% to 33%.

through dust ingestion and dermal absorption was estimated. The estimated daily intake of PFOA through dust ingestion for toddlers was 0.03 ng/kg bw per day (median value). In the case of dermal absorption, the estimated daily intake was 0.002 ng/kg bw per day (median value). For PFOS, the equivalent values were 0.002 and 0.001 ng/kg bw per day, respectively (Zheng et al., 2020). A modelling exercise for US children and adults considered both direct exposure to PFOS and exposure to precursors. Median adult intake was 4.2 ng/kg bw per day, about half of which was from precursors. This estimate was validated by comparing with intake calculated from a one-compartment pharmacokinetic model with a range of values for the volume of distribution (V_d). With the more plausible V_d values (see Section 4.1), agreement was quite close (Egeghy and Lorber, 2011).

For some individuals, a considerable part of the intake could be from personal care products and cosmetics (Husøy et al., 2023). [The Working Group noted that recent data suggested that dermal uptake is likely to be higher than was previously assumed (see Abraham and Monien, 2022, and Section 4.1).]

(b) Biomonitoring data for the general population (serum and plasma)

Repeated population surveys with the aim of measuring PFOA and PFOS concentrations in serum or plasma, or in archived blood samples,

Table 1.18 Median dietary exposure to PFOA and PFOS for different age groups in the population of Europe

Age group[a]	Dietary exposure (ng/kg bw per day)[b]	
	PFOA	PFOS
Infants	0.19	0.36
Toddlers	0.41	1.34
Other children	0.30	1.02
Adolescents	0.17	0.53
Adults	0.18	0.58
Elderly adults	0.17	0.59

bw, body weight; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.
[a] Age ranges: infants, < 12 months; toddlers, ≥ 12 to < 36 months; other children, ≥ 36 months to < 10 years; adolescents, ≥ 10 to < 18 years; adults: ≥ 18 to < 65 years; elderly, ≥ 65 to < 75 years; very elderly, ≥ 75 years.
[b] Only the lower-bound estimates are presented since these were considered to be more realistic by the European Food Safety Authority Panel.
From EFSA Panel on Contaminants in the Food Chain (2020).

have permitted exposure trends to be observed in several countries (Fig. 1.7). For most studies, estimated total PFOS or PFOA concentrations are presented, but more recent studies present concentrations of isomers, distinguishing linear and different branched isomers, more commonly for PFOS than PFOA (e.g. NHANES, 2023).

In Japan between 1983 and 1999, results for PFOA showed a clear trend, with geometric mean concentrations in men rising from [2.5 to 11] ng/mL, and in women from [1.8 to 8.1] ng/mL, corresponding to a mean annual increase of 0.49 and 0.42 ng/mL, respectively. For PFOS, there was no clear trend, with mean concentrations in the range of approximately [15–23] ng/mL for men and [13 to 19] ng/mL for women (Harada et al., 2007).

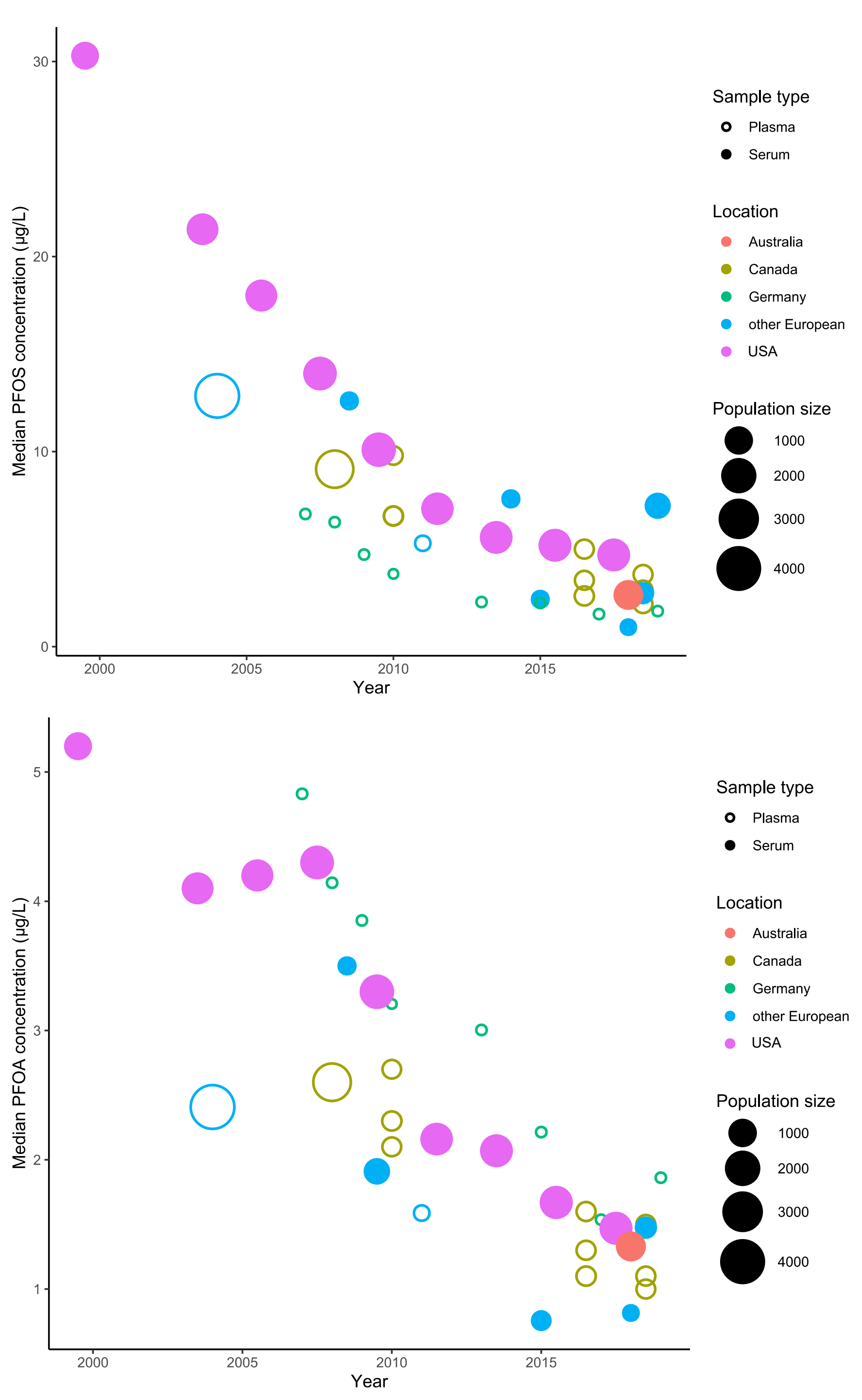
In China, in the region of Shenyang, the results of a study from 2006 showed mean PFOS concentrations of 142 ng/mL (range, 31.7–225 ng/mL) for men and 170 ng/mL (range, 80.4–310 ng/mL) for women (Yeung et al., 2006). Concentrations of PFOS in a previous study (Jin et al., 2003) were 40 ng/mL (range, 5.32–145 ng/mL) for men and 45.5 ng/mL (range, 10.6–142 ng/mL) for women. On this basis, PFOS concentrations measured in the study conducted in 2006 were 3–4 times as high as those reported in the previous study (Yeung et al., 2006).

In the USA, analysis of archived blood samples collected in 1974 (serum) and 1989 (plasma) from volunteer participants in a large community health study indicated an increase in PFOA concentrations from median values of 2.3 µg/L in 1974 to 5.6 µg/L in 1989; for PFOS, the equivalent figures were 29.5 µg/L in 1974 and 34.7 µg/L in 1989 (Olsen et al., 2005).

A unique study reporting PFOA and PFOS concentrations in the same 59 individuals over a long time period in Tromsø, Norway, showed clear trends (Nøst et al., 2014). Samples were collected in five rounds – in 1979, 1986, 1994, 2001, and 2007 – and for both PFOA and PFOS, average concentrations peaked in 2001. Correlations were high between each pair of subsequent rounds for both PFOA and PFOS (Spearman correlation, ρ, in the range 0.6–0.8; all $P < 0.05$), indicating some stability in exposure as determined by single measurements (Nøst et al., 2014).

Trends towards falling concentrations in the last 20–30 years have been shown in several countries (Fig. 1.7). For example, data from the USA derived from NHANES, a large national biomonitoring programme with repeated sampling cycles that has included PFAS in monitoring campaigns

Fig. 1.7 Median PFOA and PFOS concentrations reported in blood samples from the adult general population in several countries



PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; USA, United States of America.
Data aggregated from HBM4EU (2023), CDC (2023), and Government of Canada (2023).

since 1999, have shown this trend very clearly. For PFOA, geometric mean serum concentrations were 5.2 µg/L in 1999–2000, close to 4 µg/L between 2003 and 2008, then declined steadily in subsequent rounds, falling to 1.42 µg/L in 2017–2018. Equivalent trend data were observed for PFOS, with geometric mean serum concentrations of 30.4 µg/L in 1999, falling in each survey, down to 4.3 µg/L in 2017–2018 (Kato et al., 2011; NHANES, 2023). A similar pattern with downward trend was reported for PFOA and PFOS concentrations measured in archived blood spots collected in 1997–2007 from infants in New York, USA (Spliethoff et al., 2008).

A decreasing trend was also observed in Australia (Toms et al., 2014; Eriksson et al., 2017), Japan (Okada et al., 2013), and in several countries in the EU (Fig. 1.7).

In Norway, a steady increasing trend for PFOA concentrations in serum was observed from 1977 (0.58 µg/L) up to the 1990s (5.2 µg/L in 1993), then a decline to 2.7 µg/L in 2006. For PFOS, serum concentrations rose from 3.8 µg/L in 1977 to 33 µg/L during the 1990s, falling to 12 µg/L by 2006 (Haug et al., 2009).

In Germany, data from archived plasma samples from 20 participants (10 men and 10 women) randomly chosen from the monitoring programmes in Münster between 1982 and 2010 were analysed; PFOA concentrations were found to be highest in 1986 (7.4 µg/L) and decreased from 2007 (5.2 µg/L) to 2010 (3.1 µg/L), but in other years there were no clear trends. For PFOS, the pattern was clearer, rising from 15.4 µg/L in 1982 to 28.6 µg/L in 1989, and subsequently falling steadily to 12.7 µg/L in 2005 and 3.8 µg/L in 2010 (Schröter-Kermani et al., 2013).

A similar pattern of decline since 2000 is evident in data assembled from many recent smaller studies across Europe. In the HBM4EU project, data were assembled across 12 European countries, combining 32 different surveys. The surveys were not all directly comparable because of variation in the age and sex composition, but together they provided a picture of falling serum levels over time and exposure ranges between countries at the same points in time (HBM4EU, 2023). In this European project, although early studies were sparse (with only one study including data from 2000), PFOA body burdens were comparable to US NHANES results, with PFOA concentrations in the range of 3 to 6 µg/L up to around 2010, falling to 1 to 2 µg/L in recent years. For PFOS, levels in the EU were somewhat lower than in the USA, being mainly between 6 and 10 µg/L around 2010 for the European data, falling to between 1 and 3 µg/L in recent years (CDC, 2023; HBM4EU, 2023) (Fig. 1.7).

Exposure data for teenagers in this EU project suggested that exposure levels were significantly higher in north and west Europe than in the south and east. Concentrations of PFOA and PFOS were significantly higher in boys than in girls, and significantly higher concentrations were found in teenagers from households with a higher education level. In the same EU project, the consumption of seafood and fish at least twice per week was significantly associated with a 21% (95% CI, 12–31%) increase in PFOS concentrations. The same trend was observed for PFOA but was not statistically significant (Richterová et al., 2023).

PFOA and PFOS levels have been shown to vary by age and sex (Frisbee et al., 2009; Kato et al., 2011; Pitter et al., 2020; NHANES, 2023). Serum levels are consistently higher in males than females, reflecting differences in excretion (with women excreting additionally via menstruation, pregnancy and lactation), and possibly differences in intake and pharmacokinetics (see Li et al., 2022c, and Section 4.1). By age, serum levels measured in cross-sectional surveys showed some differences, with older people having higher serum levels. This may reflect variation in the routes of exposure according to age and biological changes, but the time trends of exposure would also be important, given the long half-lives in people. Infants can have high levels from

maternal and lactational transfer that fall in the post-lactation period (Fromme et al., 2010).With emissions and ambient levels falling, higher levels in older people will in part reflect the fact that they were exposed at earlier time periods when intake was likely to be higher (Nøst et al., 2014). In NHANES data for 1999–2000 and 2003–2004, a modest increasing slope was evident in PFOS levels from age 12 to ≥ 60 years, but no slope was evident for PFOA (Calafat et al., 2007). In the Mid-Ohio C8 population, which had a wider age range, there was a clear increasing trend from age < 10 to ≥ 80 years for PFOS levels in males, but for females the trend was decreasing until age 30–39 years, then rising thereafter (Frisbee et al., 2010). For PFOA, concentrations in females are lower than in males in most age groups, but both males and females show a similar pattern, with a minimum at around age 30 years.

NHANES also provided information on ethnicity: there were some small differences between White and Black people, but PFOS levels were markedly lower for Hispanic people, with smaller differences for PFOA (Calafat et al., 2007).

[The Working Group noted that although there were differences between countries, the overall pattern in general population serum or plasma samples across the world has been a rise in concentrations since the earliest measurements in the 1970s, reaching a peak in the 1990s or close to 2000. Subsequently, trends towards falling serum concentrations have been observed for both PFOA and PFOS. The most notable difference between countries was a higher level of PFOS in earlier samples from the USA compared with other countries.]

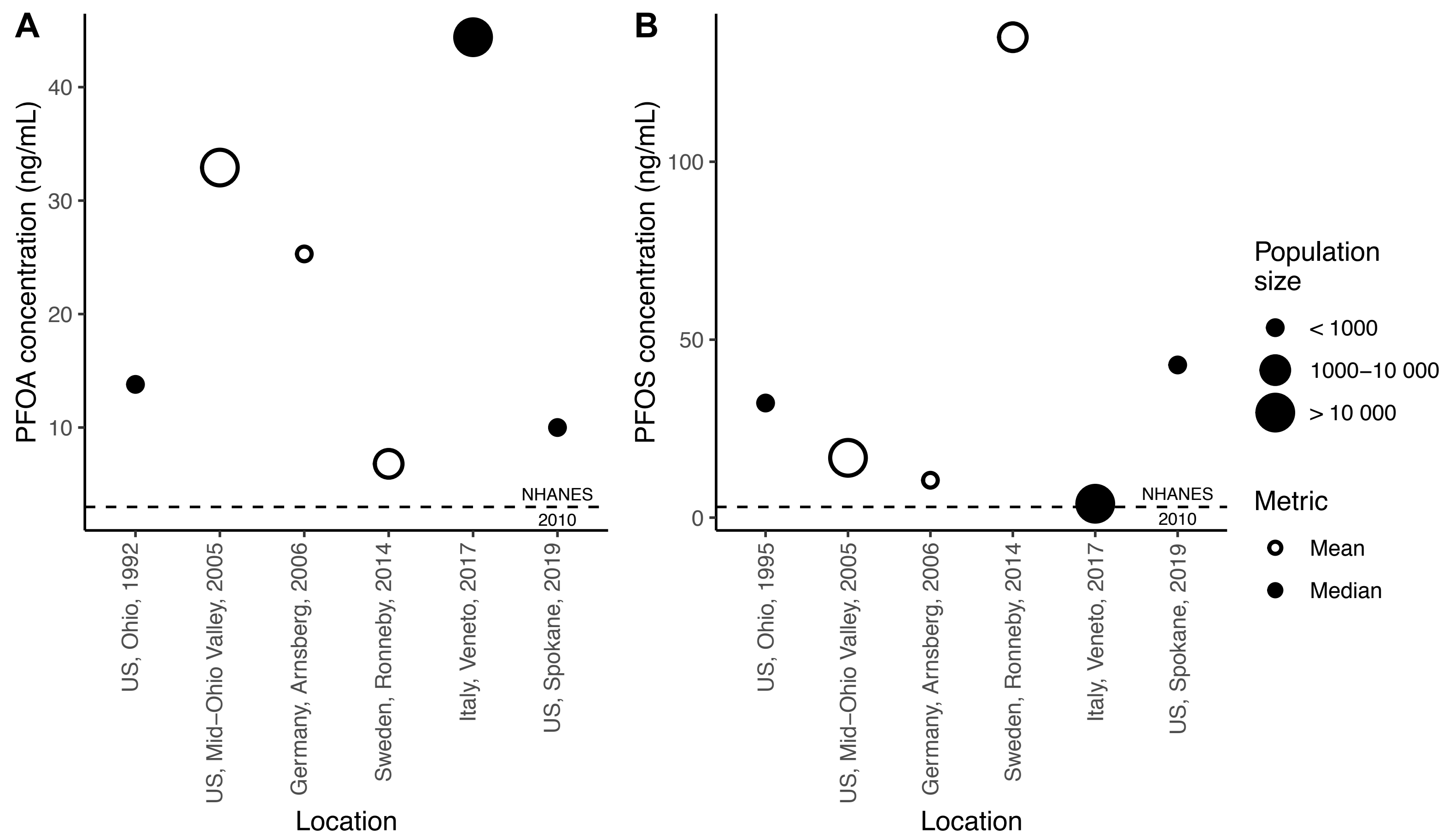
Multiple PFAS with long half-lives and slow rates of excretion, such as PFOA and PFOS, have been monitored in serum samples. Serum concentrations tend to be correlated with each other; for example, logarithmic concentrations showed a significant Pearson correlation coefficient of 0.66 between PFOA and PFOS in the NHANES data (Calafat et al., 2007). Correlation coefficients for circulating PFOA and PFOS levels were similar in several general populations in the cancer studies reviewed in Section 1.6.1, but there was large variability, with values ranging from < 0.15 to > 0.7 (see Table S1.22, Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636). There were also significant correlations with other widespread PFAS with long half-lives, notably perfluorononanoic acid (PFNA) and perfluorohexanesulfonic acid (PFHxS). [The Working Group noted that these correlations may reflect a correlation in exposure or a correlation between different PFAS in individual rates of uptake and excretion.]

(c) *Biomonitoring data for populations living at contaminated sites*

Several large communities have experienced high exposure to PFAS because of environmental contamination, related mainly to the use firefighting foams containing PFAS (e.g. airports, military facilities), certain industrial facilities where PFAS are produced or used and emitted to the environment, and sites related to PFAS-containing waste (Salvatore et al., 2022). This has led to higher blood concentrations of PFOA and PFOS in some of these communities compared with the general population (see Fig. 1.8).

In the USA, high serum concentrations of PFOA were measured in samples collected in 2005–2006 from 69 030 residents living near a PTFE-production facility in West Virginia, USA (the C8 Health Project); the overall geometric mean was 32.9 µg/L, and the arithmetic mean was 82.9 µg/L. Exposures in that community varied substantially across six water districts; the mean serum concentration of PFOA was 16 µg/L in the two water districts with the lowest concentrations of PFOA in water, and 228 µg/L in the water district with the highest concentrations (Frisbee et al., 2009).

Fig. 1.8 PFOA (A) and PFOS (B) concentrations reported in blood samples from the general population in areas reported to be polluted with PFAS



NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; USA, United States of America.
The dotted line shows the respective concentration measured in the NHANES population in 2010 (NHANES, 2023). For the individual study populations, see ATSDR (2023), Pitter et al. (2020), Ingelido et al. (2010), Hölzer et al. (2008), Frisbee et al. (2009), Xu et al. (2021b), and Herrick et al. (2017).

Also in the USA, as early as the 1970s, the Fairchild Air Force Base located in the City of Airway Heights in Spokane County, Washington, used AFFF containing PFAS during firefighter training. Over time, PFAS from the AFFF entered the ground, moved into the groundwater to off-site locations, and affected nearby municipal wells. In samples from 2019, geometric mean PFOA and PFOS serum concentrations of 9.72 µg/L and 42.4 µg/L, respectively, were reported for community residents (ATSDR, 2023).

In China, a lake adjacent to a fluorochemical-production factory was contaminated with PFAS, and serum concentrations of several PFAS were assessed in fishermen and their families who were exposed primarily via eating locally caught fish (Zhou et al., 2014). Among family members of fishery employees, extremely high PFOS levels were found, with a median concentration of linear PFOS of 2720 ng/mL, branched PFOS of 620 ng/mL, and sum of PFOS of 3540 ng/mL. PFOA concentrations were slightly elevated, with a median of 11.7 ng/mL.

At the end of 2013, drinking-water from one of the two municipal waterworks in Ronneby, Blekinge County, Sweden, was found to be contaminated by firefighting foams used at a nearby military airfield. Drinking-water containing high levels of PFOS and PFHxS, and to a lesser extent PFOA, had been distributed to approximately one third of Ronneby households (total population, approximately 30 000) since the mid-1980s (Xu et al., 2021b). Blood samples and demographic data were collected from 3297 Ronneby residents and 226 individuals from a reference group. The population geometric means for serum PFOA and PFOS concentrations were 6.8 and 135 µg/L for all Ronneby residents, i.e. 35 and 4.5 times, respectively, as high as for the reference group (Xu et al., 2021b).

In spring 2013, groundwater of part of the Veneto region in north-eastern Italy was found to be contaminated with mostly PFOA and to a smaller degree with PFOS and other PFAS from a factory that had been manufacturing a variety of PFAS since the 1960s. A population of 140 000 was potentially affected, and a population-based screening programme including measurement of serum PFAS was offered by the regional health service to residents who were exposed to PFAS via contaminated drinking-water (Ingelido et al., 2018). Among 18 122 subjects aged 14–39 years living in the Veneto region, the median concentration of PFOA was elevated, at 44 µg/L (Pitter et al., 2020), whereas the median concentration of PFOS, 3.9 µg/L, was close to levels reported for the Italian general population (Ingelido et al., 2010).

A study of 641 residents of Arnsberg, Germany, in 2006 reported geometric mean PFOA serum concentrations of 22.1, 23.4, and 25.3 µg/L in children, mothers, and men, respectively, because of surface water contamination from upstream agricultural use of soil conditioner mingled with industrial waste (Hölzer et al., 2008). PFOA levels of children and adults living in Arnsberg were 4.5–8.3 times as high as those of the reference population used in the study and living in non-contaminated sites (Hölzer et al., 2008).

(d) Other biological matrices used in biomonitoring

Although serum samples are most commonly used in biomonitoring campaigns, PFOA and PFOS have also been measured in other biological matrices, such as breast milk and urine. [The Working Group noted that these biological matrices could also be used in biomonitoring, particularly in biomonitoring campaigns performed in highly contaminated sites.]

PFOA and PFOS have been detected in breast milk, which is a significant route of exposure to infants through breastfeeding. [The Working Group noted that concentrations in breast milk are much lower than in serum; however, the large volume of breast milk ingested by infants on a

body-weight basis results in considerable exposure.] In a study of 109 paired maternal serum and breast milk samples in a population with high PFAS exposure in Sweden, breast milk concentrations were 0.03 ng/mL for PFOA and 0.130 ng/mL for PFOS (Blomberg et al., 2023). The transfer efficiency or ratio of breast milk to serum concentration was 2.16% for PFOA and 1.02% for PFOS (Blomberg et al., 2023). In a summary of 23 studies, all except 4 reported concentrations in breast milk that were above the LOQ in > 50% of samples; however, LOQs varied between studies (Fromme et al., 2022). Median values above the LOQ for PFOA were 7.2, 26, and 138 ng/L for three studies in Spain (Serrano et al., 2021; Motas Guzmàn et al., 2016; and Beser et al., 2019, respectively), and median values were 139, 121 and 35 ng/L in three studies in China (Awad et al., 2020; Liu et al., 2010, 2011). Median values for PFOA in two studies in the USA were 14 and 36 ng/L (Tao et al., 2008b; Zheng et al., 2021). In a study of a contaminated site in Germany, PFOA could be quantified in all breast milk samples, with a mean value of 199 ng/L (range, 33–854 ng/L) (Fromme et al., 2022). PFOS was observed in only 3 out of 13 samples, at levels of 33 ng/L, 35 ng/L, and 61 ng/L.

PFOA and PFOS can be detected in urine, although concentrations in urine are much lower than in serum. In a study of 104 paired samples in a population with high PFAS exposure in Sweden, the median ratio of urinary to serum level was 0.23% for PFOA and 0.07% for linear PFOS and ranged from 0.02% to 0.07% for branched PFOS. Median urinary concentrations for the three sampling rounds carried out were between 0.017 and 0.025 ng/mL for PFOA and between 0.050 and 0.075 ng/mL for PFOS (Li et al., 2022c). In general population campaigns, values in urine samples are mostly below the LOD. In the NHANES 2013–2014 round, urine levels were above the LOD (0.1 ng/mL) for < 0.1% of the population, even though serum levels were above the LOD for almost 100% of participants (Calafat et al., 2019).

[The Working Group noted that PFOA and PFOS have been measured in other biospecimens, such as nails, hair, and semen, but these have rarely been used to assess exposure for epidemiological studies.]

1.5 Regulations and guidelines

Regulations, guidelines, and guidance for PFOA and PFOS have been established by international, national, and local governing bodies, as well as nongovernmental organizations (e.g. standards, non-profit, and professional organizations). The aim is to reduce human exposure and environmental contamination via approaches covering production, use, and disposal; occupational exposures; food and consumer products; environmental media; and biomonitoring. Unless otherwise stated, numerical standards and guidelines for PFOA and PFOS are generally based on non-cancer effects.

Internationally, PFOS and PFOA and their salts derivatives are recognized as persistent organic pollutants and were included in the Stockholm Convention on 2009 and 2019, respectively. PFOS is listed under Annex B (Restriction) (measures must be taken to restrict production and use), whereas PFOA is listed under Annex A (Elimination) (measures must be taken to eliminate production and use) (UNEP, 2023).

Various regions and countries have also specific regulations in place to prevent the use of PFAS such as PFOA and PFOS (OECD, 2023b). More detailed information on the actions being developed and on regulations in place in each country or region can be found in the supplementary material (Annex 2, Actions and regulations for the elimination of PFAS worldwide, online only, available from: https://publications.iarc.who.int/636).

Table 1.19 Occupational exposure thresholds for PFOA, APFO, and PFOS, by country

Country	PFOA (mg/m^3)		APFO (mg/m^3)		PFOS and its salts (mg/m^3)	
	8-hour	Short-term	8-hour	Short-term	8-hour	Short-term
ACGIH			0.01[b]			
Australia			0.1			
Belgium			0.01[f]			
Canada – Ontario			0.01			
Canada – Quebec			0.01[b]			
Denmark			0.01[b]	0.02[b,c]		
Germany (AGS)					0.01[a,b]	0.08[a,b,c]
Germany (DFG)	0.005[a,b]	0.04[a,b,c]			0.01[a,b]	0.08[a,b,c]
Ireland			0.01			
Japan (JSOH)	0.005[d]					
New Zealand			0.1			
Singapore			0.01			
Spain			0.01[b]			
Sweden					900	1400[c]
Switzerland	0.005[e]	0.04[e]			0.01[e]	0.08[e]

ACGIH, American Conference of Governmental Industrial Hygienists; AGS, Ausschuss für Gefahrstoffe (Hazardous Substances Committee); APFO, ammonium perfluorooctanoate; DFG, Deutsche Forschungsgemeinschaft (German Research Foundation); JSOH, Japan Society for Occupational Health; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.
[a] Inhalable fraction.
[b] Skin.
[c] 15-minute average.
[d] Not applicable to women of child-bearing potential.
[e] Inhalable aerosol.
[f] Skin, mucous membranes, and eyes.
From IFA (2022).

1.5.1 Occupational limits

Occupational exposure limits for air concentrations are available for PFOA, APFO, and PFOS (Table 1.19). Germany and Switzerland have identical 8-hour TWAs and short-term exposure limits (STELs): TWA for inhalable PFOA, 0.005 mg/m^3 (STEL, 0.04 mg/m^3) and TWA for inhalable PFOS, 0.01 mg/m^3 (STEL, 0.08 mg/m^3). Japan also uses the value of 0.05 mg/m^3 as the 8-hour TWA for PFAS; however, Sweden's 8-hour TWA for PFOS is 900 mg/m^3 (and the STEL is 1400 mg/m^3). APFO is assigned an 8-hour TWA of 0.01 mg/m^3 in Belgium, Canada (Ontario and Quebec), Denmark, Ireland, Singapore, and Spain, (IFA, 2022). The same value is adopted by the American Conference of Governmental Industrial Hygienists (ACGIH) (ACGIH, 2023). Australia and New Zealand set their 8-hour TWA at 0.1 mg/m^3, and Denmark has a STEL of 0.02 mg/m^3. Belgium, Denmark, Germany, Quebec, Spain, and the ACGIH all assign a skin notation to their guidance, indicating that dermal protection is needed to prevent skin absorption (IFA, 2022).

1.5.2 Consumer products and food

See Table 1.20.

Numerous countries have set recommended limits for exposure to PFAS in consumer products and food. Food Standards Australia New Zealand (FSANZ) and the National Health and Medical Research Council (NHMRC) of Australia set a tolerable daily intake (TDI) for PFOA of 160 ng/kg bw and a combined intake

Table 1.20 Examples of consumer products in which the presence or use of PFOA and PFOS is restricted

Consumer product	Country or region	Reference
Food packaging	USA, European Union, Japan	US FDA (2022b) OECD (2023a)
Children's products	Some states in the USA	ITRC (2023a) ITRC (2023b)
Carpets, textiles, rugs, and fabric treatments, furniture	European Union, some states in the USA	ITRC (2023a) ITRC (2023b) MNPCA (2023); Maine DEP (2023)
Cookware	Some states in the USA	MNPCA (2023)
Cosmetics and other personal products	Some states in the USA	MNPCA (2023)
Firefighting foams	Canada, European Union, Australia, some states in the USA	ECHA (2023) ECCC (2017) ITRC (2023b)

PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; USA, United States of America.

of PFOS and PFHxS of 20 ng/kg bw (Australian Government, 2017). In the USA, the Agency for Toxic Substances and Disease Registry (ATSDR) developed intermediate-duration oral minimal risk levels of 3 ng/kg per day for PFOA and 2 ng/kg per day for PFOS. The ATSDR minimal risk levels are estimates of the daily intake below which harm to human health is not anticipated to occur and are often used as screening levels for environmental media (e.g. water) (ATSDR, 2021).

In 2008, the European Food Safety Authority`s Panel on Contaminants in the Food Chain (CONTAM) established TDIs of 150 ng/kg bw per day for PFOS and 1500 ng/kg bw per day for PFOA. In 2020, the same agency established a new safety threshold for PFOA, PFOS, and two other PFAS (PFHxS and PFNA), a group tolerable weekly intake (TWI) of 4.4 ng/kg bw per week.

1.5.3 Environmental guidelines

National and local jurisdictions have established regulations and guidelines on acceptable concentrations of PFOA and PFOS in drinking-water and other environmental compartments. [The Working Group noted that these guidelines are evolving on the basis of current science and regulatory processes.] Many of these regulations, particularly those pertaining to drinking-water, have been updated in recent years and are closely tracked by organizations such as the Interstate Technology and Regulatory Council (ITRC). More information on water and soil regulations is available online in tables that are maintained by the ITRC (ITRC, 2023c).

Table 1.21 presents a non-exhaustive list of some regulations for different environmental compartments.

Canada additionally has guidelines for PFOS in surface water, aquatic life, fish tissue, and wildlife diet, as part of the Federal Environmental Quality Guidelines (ECCC, 2023). In the USA, several states have implemented PFAS limits for a variety of environmental media. PFOA and PFOS in drinking-water, surface water, groundwater, and sediment or soil (residential, industrial or commercial, and construction site) are regulated in various combinations in up to 20 states. A few states additionally have testing requirements or allowable concentrations for PFOA and PFOS in biosolids and wastewater. Consumption advisories or limits on concentrations of PFOS and, to a lesser extent, PFOA in fish as well as in shellfish, deer, turkey, beef, and milk exist in numerous states for different consumption patterns and

Table 1.21 Examples of guidelines in place for environmental compartments

Environmental compartment	Country or region	Limit established; year	Reference
Drinking-water	New Zealand	PFOA, 560 ng/L; PFOS, 70 ng/L; 2017	Australian Government (2017)
Drinking-water	Canada	PFOA, 200 ng/L; PFOS, 600 ng/L; 2018	Health Canada (2018a, b)
Drinking-water	European Union	500 ng/L for total PFAS; 100 ng/L for the sum of 20 PFAS, including PFOA and PFOS; 2020	EU (2020)
Drinking-water	Denmark	2 ng/L for the total of PFOA, PFOS, PFNA, and PFHxS; 2021	Danish Environmental Protection Agency (2023)
Drinking-water	UK	10–100 ng/L for PFOS or PFOA; 2021	DWI (2021)
Drinking-water	USA	PFOA, 0.004 ng/L; PFOS, 0.02 ng/L; 2022 (Interim Health Advisory)	Office of the Federal Register (2022)
Recreational water	New Zealand	PFOA, 10 000 ng/L; PFOS, 2000 ng/L; 2017	Australian Government (2017)
Ambient water	Canada (British Columbia)	PFOA, 200 ng/L; PFOS, 600 ng/L; 2020	BC MECCS (2020)
Groundwater	European Union	4.4 ng/L (sum of 24 PFAS, including PFOA and PFOS); 2022	European Commission (2022)
Soil	Canada	PFOS, 0.01 mg/kg dry weight; 2021 PFOA soil screening values are 0.70, 1.05, and 9.94 mg/kg soil, for agricultural/residential, commercial, and industrial land use; 2019	CCME (2021) Health Canada (2019)
Ambient air	USA	PFOS, PFOA, and APFO concentrations ranging from 0.006 to 0.082 μg/m³, 0.007 to 0.07 μg/m³, and 0.024 to 0.05 μg/m³, respectively; varies by regulation	ITRC (2023a, b)

APFO, ammonium perfluorooctanoate; PFAS, per- and polyfluoroalkyl substances; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFHxS, perfluorohexanesulfonic acid; PFOS, perfluorooctanesulfonic acid; UK, United Kingdom; USA, United States of America.

populations (ECOS, 2023; ITRC, 2023a, b). [The Working Group noted that this is a dynamic area and new regulations with lower proposed regulatory thresholds are being established.]

1.5.4 Guidance and biomonitoring reference values

Health-based threshold guidance values for biomonitoring are available in Germany and the USA. In 2016, the Human Biomonitoring Commission (HBM Commission) of the German Environment Agency (UBA) established concentrations below which no adverse health effects are expected to occur (HBM-I values), according to current knowledge and assessment, of 2 ng/mL for PFOA and 5 ng/mL for PFOS in blood serum or plasma (Hölzer et al., 2021). In 2019, concentrations above which there is an increased risk of adverse health effects (HBM-II values) were established of 5 ng/mL and 10 ng/mL for PFOA and PFOS, respectively, in blood plasma in women of childbearing age, and of 10 ng/mL and 20 ng/mL for PFOA and PFOS, respectively, in blood plasma of all other populations (Schümann et al., 2021). In its report of 2022, the US National Academies of Sciences, Engineering, and Medicine (NASEM) identified two threshold values for the sum of seven PFAS in serum or plasma, including PFOA and PFOS, to guide clinical care and exposure reduction efforts: 2 ng/mL and 20 ng/mL. NASEM recommended that clinicians provide the usual standard of care at concentrations of < 2 ng/mL; encourage exposure reduction and screen for certain medical conditions at concentrations of

2 to < 20 ng/mL; and encourage exposure reduction and screen for additional medical conditions at concentrations of ≥ 20 ng/mL (NASEM, 2022).

1.6 Quality of exposure assessment in key epidemiological studies of cancer and mechanistic studies in humans

1.6.1 *Quality of exposure assessment in key cancer epidemiology studies*

(a) *Exposure assessment methods*

The exposure assessment methods employed in 12 case–control studies and 30 cohort studies, including 18 nested case–control studies, were reviewed and are described below by study design. Details on each of the studies are summarized in Table S1.22 (Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636).

These studies employed primarily one of two methods of exposure assessment for PFOA and PFOS: biological measurement of PFOA and PFOS in the blood (whole blood, serum, or plasma) or job (residential history) exposure matrices to estimate historical exposures. The biological matrix for the analysis of PFOA and PFOS was blood in two studies, plasma in five studies, and serum in the other studies.

An overview of chemical analysis methods used for detection and quantification of PFOA and PFOS in human biological samples is presented in Section 1.3.4. In the epidemiological cancer studies in which the exposure assessment was based on biomonitoring, targeted analytical methods were applied in all except four studies that used non-targeted methods, which do not permit quantification of concentrations but rely on semiquantitative determination of intensity level for identified PFAS (Chang et al., 2023; Chen et al., 2023; van Gerwen et al., 2023; Zhang et al., 2023).

As described in Section 1.1, several isomers exist for both PFOA and PFOS. In a few epidemiological cancer studies, isomer-specific determinations were performed that were summed for analysis (Itoh et al., 2021; Li et al., 2022a; Purdue et al., 2023; Rhee et al., 2023b; Winquist et al., 2023), but in most studies only one concentration was reported for PFOA and one for PFOS. The exception was the study by van Gerwen et al. (2023) who considered linear and branched-chain PFOS separately in their non-targeted analysis. [The Working Group noted that when one concentration value was reported for PFOA or PFOS, it was assumed that this represented the sum of branched and linear isomers, even though this was not always specified in the study.] In the studies in which non-targeted methods were used (see Section 1.3.4), compound-specific intensities, not concentrations, were reported (Chang et al., 2023; Chen et al., 2023; van Gerwen et al., 2023; Zhang et al., 2023).

(i) *Case–control studies*

In total, 12 relevant case–control studies were reviewed for the present monograph (Bonefeld-Jørgensen et al., 2011; Vieira et al., 2013; Hardell et al., 2014; Wielsøe et al., 2017; Lin et al., 2020b; Tsai et al., 2020; Itoh et al., 2021; Cao et al., 2022; Li et al., 2022a; Liu et al., 2022a; Velarde et al., 2022; Chen et al., 2023). In all studies except that by Vieira et al. (2013), both PFOA and PFOS were evaluated, and the exposure assessment was based on biomonitoring in the blood (serum, plasma, or whole blood).

Vieira et al. (2013) evaluated incident cancers in residents (according to address at time of cancer diagnosis) in six PFOA-contaminated water districts and 13 counties in Ohio and West Virginia, USA. PFOA concentrations in water, available for each of the six districts, varied by community. Water district information was available for all individuals, and logistic regression

analyses compared individuals in contaminated water districts with those in neighbouring water districts. For residents of Ohio, where approximately one third of the sample population lived, residential addresses were geocoded and then PFOA serum concentrations were assigned on the basis of modelled estimates (Shin et al., 2011a, b), assuming 10 years residence at that address. Exposure was then divided into four categories. However, analysis for residents of West Virginia was limited to residence by water district.

For all other studies based on general populations, blood samples were collected from participants during the same time periods for the cases and controls. For the cases in studies by Bonefeld-Jørgensen et al. (2011) and Cao et al. (2022), the timing of the blood draw relative to when treatment started was not reported, and in the study by Lin et al. (2020b), blood samples were collected 1 week after the identification of the case by pathology. In the study by Chen et al. (2023), blood spot samples were collected at birth, and diagnosis occurred on average 9.3 months after birth for unilateral retinoblastoma and 22 months after birth for bilateral retinoblastoma. For the remaining studies (Hardell et al., 2014; Wielsøe et al., 2017; Tsai et al., 2020; Itoh et al., 2021; Li et al., 2022a; Velarde et al., 2022), blood samples were collected between the time of diagnosis and the start of treatment. Controls were selected from participants in ongoing cross-sectional studies (Bonefeld-Jørgensen et al., 2011; Wielsøe et al., 2017); invited on the basis of selection from population registries (Hardell et al., 2014) or breast cancer screening programmes (Cao et al., 2022; Li et al., 2022a); in connection with medical check-ups (Itoh et al., 2021); through advertisements at the hospital and in the community (Tsai et al., 2020; Liu et al., 2022a); or invited after hospitalization due to other diagnoses or illnesses (Lin et al., 2020b). In studies by Bonefeld-Jørgensen et al. (2011), Hardell et al. (2014), Wielsøe et al. (2017), Itoh et al. (2021) and Chen et al. (2023), cases and controls were matched on age and region of residence, whereas Lin et al. (2020b) matched cases and controls on age and sex. [The Working Group noted that, given the temporal trends in PFOA and PFOS blood levels, it is important that time of blood sample collection is matched or adjusted for.]

In all studies, targeted chemical analyses were performed using LC-MS/MS, except in the study by Chen et al. (2023), in which non-targeted methods were used. Because PFOA and PFOS levels were not quantified using standard targeted methods by Chen et al. (2023), direct comparisons with the levels from other studies were not possible. Li et al. (2022a) performed separate determinations for eight PFOA isomers and nine PFOS isomers, and internal standards of linear PFOA and PFOS isomers were used. In the study by Hardell et al. (2014), only linear isomers of PFOA and PFOS were determined. In the remaining studies, one concentration for PFOA and one for PFOS were reported. In these studies, it was not stated whether only linear isomers were considered or whether other isomers were also included in the reported concentrations.

In addition to PFOA and PFOS, all studies except that by Chen et al. (2023) included at least four of the other most prominent PFAS in human blood (EFSA Panel on Contaminants in the Food Chain, 2020). Bonefeld-Jørgensen et al. (2011), Li et al. (2022a), and Wielsøe et al. (2017) assessed exposure both for single PFAS and for the sum of several PFAS. In the studies by Hardell et al. (2014), Tsai et al. (2020), Lin et al. (2020b), Liu et al. (2022a), Cao et al. (2022), and Chen et al. (2023), only single PFAS were assessed.

Bonefeld-Jørgensen et al. (2011) and Wielsøe et al. (2017) measured other carcinogens, i.e. polychlorinated biphenyls (PCBs), β-hexachlorocyclohexane, cadmium and cotinine (as a biomarker for tobacco smoke), via biomonitoring. Some studies collected information on exposure to other carcinogens, i.e. barbecuing, hair dyeing, smoking, alcohol consumption, use

of estrogen or estrogen-replacement therapy, meat consumption, via questionnaires (see Table S1.22, Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636).

(ii) Cohort studies

This section includes cohort studies designed to study PFOA and PFOS exposure in occupational settings and contaminated communities and case–control studies nested in other general population cohort studies. Eleven cohort studies focusing on cancer incidence or mortality were reviewed by the Working Group. These included eight occupational cohort analyses (Alexander et al., 2003; Alexander and Olsen, 2007; Lundin et al., 2009; Steenland and Woskie, 2012; Consonni et al., 2013; Raleigh et al., 2014; Steenland et al., 2015; Girardi and Merler, 2019), two cohort studies in highly exposed communities (Barry et al., 2013; Li et al., 2022b), and one in the general population in the USA (Wen et al., 2022). Three of the cohort analyses were conducted in the C8 study area (a fluorochemical-production plant in Parkersburg, West Virginia, USA, and the six water districts in Ohio and West Virginia in which water was contaminated by a chemical plant that used APFO in the production of PTFE) and focused on either occupational exposure to PFOA or exposure through residential consumption of drinking-water (Steenland and Woskie, 2012; Barry et al., 2013; Steenland et al., 2015). Additionally, four studies were conducted among fluorochemical-manufacturing workers at a PFOS-production site in Alabama, USA (Alexander and Olsen, 2007) and at an APFO-manufacturing site in Minnesota, USA (Alexander et al., 2003; Lundin et al., 2009; Raleigh et al., 2014). The study by Li et al. (2022b) was based in a general population that was highly exposed to PFAS, but an ecological approach using water districts was followed, rather than measurement of subject-specific PFAS exposure. The majority of these studies that evaluated specific PFAS focused on PFOA. In the general population study (Wen et al., 2022) and the community exposure study (Li et al., 2022b), exposure to both PFOA and PFOS was evaluated.

Occupational cohort studies

Six of the occupational cohort analyses focused on PFOA (Lundin et al., 2009; Steenland and Woskie, 2012; Consonni et al., 2013; Raleigh et al., 2014; Steenland et al., 2015; Girardi and Merler, 2019) and two on PFOS (Alexander et al., 2003; Alexander and Olsen, 2007).

Another cohort analysis on fluoropolymer production was a mortality analysis that did not include estimates for PFOA exposure and is not discussed further in the present monograph (Leonard et al., 2008).

All of these occupational cohort analyses relied on job history to classify potential exposure to PFOA or PFOS. Occupational exposure to PFOS was evaluated in workers in a film and chemical plant in Alabama, USA, (Alexander et al., 2003; Alexander and Olsen, 2007) using an exposure matrix developed by Olsen et al. (2003) that classified workers into three categories on the basis of potential exposure to POSF (a precursor of PFOS): ever high; ever low/never high; or no exposure. No measure of cumulative exposure was included. Serum samples were analysed but were not used to develop an exposure matrix. A variety of perfluorinated amides, alcohols, acrylates, and other fluorochemical polymers were produced at the plant (e.g. PFOA was used as a by-product or emulsifier until 1988) but were not included in the exposure assessment (Olsen et al., 2003).

For PFOA, several different approaches were used in the occupational cohort studies. Two studies used a JEM created using expert opinion (Lundin et al., 2009; Consonni et al., 2013); one used air sampling measurements together with a JEM (Raleigh et al., 2014); and others used biomarkers to enhance JEMs (Steenland and

Woskie, 2012; Steenland et al., 2015; Girardi and Merler, 2019). In the study on workers at the fluorochemical-production plant in Parkersburg, West Virginia (Steenland and Woskie, 2012; Steenland et al., 2015), exposure to PFOA was assessed using a JEM and then linked to serum exposure levels in samples collected between 1979 and 2004 from workers in eight work categories (Woskie et al., 2012). Cumulative exposure was estimated in ppm-years (μg/mL serum-years). Girardi and Merler (2019) used a similar approach to estimate cumulative serum PFOA exposure among workers at a factory in Veneto, Italy. Although PFOS was also produced, at lower volumes, in this factory (an average of [33 tonnes/year] compared with [227 tonnes/year] of PFOA), exposure to PFOS was not estimated. For APFO-manufacturing workers at the Minnesota factory (Lundin et al., 2009), exposure to PFOA was estimated according to three categories: definite; probable; and no occupational exposure, based on job history. Cumulative PFOA exposure was then estimated using weights based on serum levels of workers in different areas of the manufacturing facility; lifetime exposure was estimated based on the product of the weight and the exposure days. For manufacturing workers using APFO (the ammonium salt of PFOA), air samples were collected for combinations of department/job title/work area/equipment/task (Raleigh et al., 2014). Job histories were then linked to the air samples to create a TWA of APFO exposure ($\mu g/m^3$-years), and then all jobs were summed to create an overall summary APFO air-exposure variable.

Consonni et al. (2013) evaluated mortality among workers at a plant involved in TFE synthesis and polymerization. The TFE synthesis and polymerization process uses APFO (the ammonium salt of PFOA) and, as a result, workers were commonly co-exposed to both TFE and APFO (88%, in the study by Consonni et al., 2013). A semiquantitative JEM using arbitrary units was created, and cumulative exposure was estimated.

Studies of communities with contaminated drinking-water

Cancer risk associated with the consumption of PFAS-contaminated drinking-water was evaluated in three communities: the C8 Study in Ohio and West Virginia, in the USA; Ronneby, Sweden; and the Veneto region, in Italy.

The C8 study focused on water districts where drinking-water was contaminated by PFOA, also known as “C8”, from a fluorochemical-production plant (Barry et al., 2013). One cohort study of cancer incidence was conducted in this region. This study, which included both residents and workers, used the exposure assessment metric from the study by Shin et al. (2011a, b) to assign cumulative PFOA exposure to individuals on the basis of residential history, and the exposure metric from Woskie et al. (2012) to assign PFOA exposure related to occupational exposure. Exposure was modelled based on a continuous measure of cumulative PFOA exposure as well as categories of exposure.

The study in Ronneby, Sweden, by Li et al. (2022b) relied on residential history to assign water source into categories: ever high, never high, early high, late high, short high or long high PFAS exposure. Differences in exposure between these categories were supported by measurement of PFAS blood levels in the population, with the highest levels found in the late high group (Li et al., 2022b). Water from this region was contaminated with multiple PFAS, and exposures were particularly high for PFAS related to firefighting foam (PFOS and PFHxS). Exposure assessment in this analysis was not chemical-specific and used residence as a surrogate for exposure. [The Working Group recognized that it was not possible to distinguish PFOS from PFHxS because of the elevated levels of both compounds and the presence of somewhat elevated PFOA levels that correlated with

levels of PFOS and PFHxS, even though PFOA levels were much lower than those of PFOS and PFHxS.]

Another location in the world where there is extensive contamination of water with PFAS is the region of Veneto, Italy, where a factory produced PFOA between 1968 and 2014 (Girardi and Merler, 2019). [No publication has comprehensively described the exposure experience in this community. The Working Group reviewed several papers and reports to characterize PFOA and PFOS exposure in this community and included details in Annex 2, Actions and regulations for the elimination of PFAS worldwide, online only, available from: https://publications.iarc.who.int/636.] Drinking-water contamination was discovered in 2013, and since that time extensive environmental and human biological sampling has been conducted (Ingelido et al., 2018; Pitter et al., 2020; Giglioli et al., 2023). Initially, the highly contaminated area, also known as the "red area", was composed of 21 municipalities, with 126 000 inhabitants. In 2018, nine additional municipalities were added, some of which were only partially supplied by the contaminated waterworks; the updated red area has a size of 595 km² and a total population of approximately 140 000.

General population cohorts including nested case–control studies

Wen et al. (2022) used NHANES exposure data from 1999 to 2014 to evaluate cancer mortality in adults in a general population sample in the USA. The NHANES is a nationally representative sampling of the population, designed to assess the health and nutritional status of adults and children in the USA. This evaluation used serum measurements of PFOA and PFOS, and other PFAS; only one serum measurement was available for each individual. Deaths were identified through linkage to the National Death Index, with a median follow-up of 81 months (range, 46–112 months). Cancer mortality risk was estimated using tertiles of exposure for PFOA and PFOS, but the majority of the analysis focused on the PFAS mixture.

In total there were 18 case–control studies nested within cohorts that used biomonitoring of PFAS in their analyses (Eriksen et al., 2009; Bonefeld-Jørgensen et al., 2014; Ghisari et al., 2017; Hurley et al., 2018; Cohn et al., 2020; Mancini et al., 2020; Shearer et al., 2021; Feng et al., 2022; Frenoy et al., 2022; Goodrich et al., 2022; Chang et al., 2023; Purdue et al., 2023; Rhee et al., 2023a, b; van Gerwen et al., 2023; Winquist et al., 2023; Zhang et al., 2023; Madrigal et al., 2024). Two studies used the E3N (Etude épidémiologique auprès de femmes de la Mutuelle générale de l'Education nationale) prospective cohort of women in the national education system in France (Mancini et al., 2020; Frenoy et al., 2022). Two studies used the Danish National Birth Cohort (Bonefeld-Jørgensen et al., 2014; Ghisari et al., 2017). One study used a cohort of retired Chinese motor-company employees (Feng et al., 2022); another was nested in a cohort of US Air Force Servicemen (Purdue et al., 2023). The others included a cohort of California teachers (Hurley et al., 2018), a Child Health and Development Studies pregnancy cohort in California (Cohn et al., 2020), a population-based national maternity cohort in Finland (Madrigal et al., 2024), and the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study in Finland (Zhang et al., 2023), and the Mount Sinai Bio*Me* medical record-linked biobank in the USA (van Gerwen et al., 2023). Four studies used the US-based Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) Cancer Screening Trial cohort (Shearer et al., 2021; Chang et al., 2023; Rhee et al., 2023a; Zhang et al., 2023), and two used the California- and Hawaii-based Multiethnic Cohort in the USA (Goodrich et al., 2022; Rhee et al., 2023b).

Chang et al. (2023), van Gerwen et al. (2023) and Zhang et al. (2023) used non-targeted analysis. In these studies, the analysis was conducted on quantiles of intensity measures of

the relative levels of PFOA and PFOS found in pre-diagnosis serum samples. Because PFOA and PFOS levels were not quantified using standard targeted methods, direct comparisons with the PFAS levels reported in other studies were not possible. However, the authors (Chang et al., 2023; Zhang et al., 2023) reported strong correlations of between 0.76 and 0.77 between the untargeted analysis and the standard targeted analysis.

In all studies except that by Hurley et al. (2018), blood samples were collected before case ascertainment. Hurley et al. collected samples between 9 months and 8.5 years (average, 35 months) after case diagnosis. In the other studies, the time between sample collection and case ascertainment varied (where this was reported). Zhang et al. (2023) reported that the time between sample collection and cancer diagnosis was 0–18 years (median, 9 years) for the PLCO subcohort analysed. For the study by van Gerwen et al. (2023), sample collection took place 0–1 year before diagnosis (average, 0.08 years) for 65% of cases and an average of 4 years before diagnosis for the remaining 35% of cases. For the study by Goodrich et al. (2022), the median time span between collection of blood sample and diagnosis was 7.2 years (range, 0.9–16.4 years). In the study by Ghisari et al. (2017), cases were diagnosed 11–12 years after initial blood draw, while for Eriksen et al. (2009) cases were diagnosed a median of 7 years after enrolment (and blood draw) (range, 0–12 years). Shearer et al. (2021) reported a mean of 8.8 years (range, 2–18 years) between blood draw and diagnosis, and Chang et al. (2023) reported a median of 5.6 years (range, 2–18 years) between diagnosis and blood draw. In the study by Purdue et al. (2023), the median time between blood collection and diagnosis was 5 years (range, 0–19.8 years). Rhee et al. (2023a) reported a median time between blood collection and diagnosis of 9 years (interquartile range, 5–13 years). Madrigal et al. (2024) reported that cases were diagnosed at least 3 years after delivery (samples were collected during the first trimester of pregnancy).

Both Purdue et al. (2023) and Rhee et al. (2023a) had access to multiple blood samples, which allowed them to evaluate how the rank ordering of exposure might change over time. For a subset of participants in the study by Purdue et al. (2023), a second blood sample was collected. To explore differences related to the time of collection, Purdue et al. (2023) analysed the data separately for participants with two samples and also created a combined variable based on the classification of the median level at each time point. They reported an overall Spearman coefficient of 0.6 for both PFOA and PFOS in repeat samples and an intraclass correlation coefficient (ICC) of 0.5–0.6, with stronger correlation for repeat samples taken after < 4.7 years and weaker correlation for repeat samples taken after > 4.7 years. Rhee et al. (2023a) analysed blood from 60 controls at enrolment, 1 year after enrolment, and 5 years after enrolment to assess long-term intra-individual variability in PFAS concentration. The ICC for three measures was 0.73 for of PFOA and 0.85 for PFOS; these values suggest that measurements of PFOA and PFOS were reliable over time in this study.

Eight of the studies focused on breast cancer (Bonefeld-Jørgensen et al., 2014; Ghisari et al., 2017; Hurley et al., 2018; Cohn et al., 2020; Mancini et al., 2020; Feng et al., 2022; Frenoy et al., 2022; Chang et al., 2023). Some of the studies were in birth cohorts for which blood samples were collected during pregnancy and maternal breast cancers identified subsequently (Bonefeld-Jørgensen et al., 2014; Ghisari et al., 2017). Alternatively, Cohn et al. (2020) used maternal blood collected 1–3 days postpartum to investigate breast cancer in the daughters. Chang et al. (2023), Feng et al. (2022), Frenoy et al. (2022), Mancini et al. (2020), and Hurley et al. (2018) reported on prospective studies of adult general populations. Three studies focused on people in professions related to education

(Hurley et al., 2018; Mancini et al., 2020; Frenoy et al., 2022), and one study was in a cohort in an industrial motor company (Feng et al., 2022), although occupational exposures were not its focus.

Several nested case–control studies were part of general cancer screening or prevention trials, such as the PLCO cohort (Chang et al., 2023; Rhee et al., 2023a; Zhang et al., 2023), the Cancer Prevention Study II Lifelink Cohort (Winquist et al., 2023), or the ATBC Study (Zhang et al., 2023). One study used a hospital-based biobank in the USA (van Gerwen et al., 2023), and another was a population-based national maternity cohort in Finland (Madrigal et al., 2024).

Many studies measured multiple PFAS in their samples; however, much of the outcome analysis focused on potential associations with a limited number of individual PFAS (Eriksen et al., 2009; Ghisari et al., 2017; Hurley et al., 2018; Cohn et al., 2020; Mancini et al., 2020; Shearer et al., 2021; Goodrich et al., 2022). Some authors attempted to sum a variable number of the measured PFOA or PFOS isomers, and use these summed metrics in their analysis (Bonefeld-Jørgensen et al., 2014; Feng et al., 2022), and Frenoy et al. (2022) used principal components analysis and Bayesian kernel machine regression on all the PFAS measurements. van Gerwen et al. (2023) used untargeted analysis to examine intensities of eight detectable PFAS, including linear PFOA and branched and linear PFOS, which were examined individually in their analysis.

(b) Critical review of exposure assessment in key epidemiological studies

Blood is considered a suitable matrix for exposure assessment (Vorkamp et al., 2021), and measured blood concentrations are an objective measure of exposure. In most studies in which blood measurements were used, the analytical methods used were state-of-the-art in 2023, the LOQs for PFOA and PFOS were sufficiently low to ensure high quantification frequencies, and the measurement error in the targeted chemical analyses was low (see Section 1.3.4). In some studies, the quantification method used was non-targeted and thus semiquantitative; therefore, exact concentrations were not available. However, ranking of levels is possible. Several occupational cohort studies that estimated cumulative exposures used older, less specific or precise methods, with higher LODs (Alexander et al., 2003; Steenland and Woskie, 2012). In some studies, estimation of serum levels combined state-of-the-art measurements of community exposures with older data from occupational cohort studies (Barry et al., 2013; Steenland et al., 2015).

The measured concentrations in blood represent combined exposure through all exposure pathways (see Section 1.4.3 on biomonitoring). Since PFOA and PFOS have long elimination half-lives (see Section 4.1), and repeated measures in humans show strong ICCs (Blake et al., 2018; Rhee et al., 2023a), the measured concentrations represent exposure over a relatively long period of time. These factors limit the potential for non-differential exposure misclassification, in general. Using repeated measures data from Rhee et al. (2023a) and Purdue et al. (2023), the Working Group evaluated the potential for exposure misclassification and resulting bias if just one biological sample is used; the results of this analysis demonstrated that using a single sample represented rather well the mean of repeated samples collected a median of 4–5 years apart in two cohort studies of populations with background levels (Spearman correlations of 0.87 and 0.83 for the PLCO and US Air Force Servicemen cohorts, respectively) (see Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). Repeat biomonitoring of PFOA and PFOS in the general population is described in Section 1.4.3.

It is important to be careful when comparing measured concentrations reported in the

various studies, since PFOA and PFOS isomers have been treated differently among studies. This is of particular importance for PFOS since branched isomers may comprise up to 50% of the total concentration of PFOS (Haug et al., 2009); whether or not the branched isomers are included could make a significant difference to participant exposure levels. Results from studies using untargeted methods also present limitations when comparing exposure concentrations with results from other studies.

Most studies relied on a single blood sample to classify lifetime exposure to PFOA and PFOS. In case–control studies, one blood sample was collected near the time of diagnosis. In the cohort and nested case–control studies, the time between blood collection and diagnosis ranged from 0 to 20 years, as described above. Thus, there is a possibility that measured blood levels of PFOA and PFOS do not reflect exposure at crucial windows in cancer development. However, as described above, the results of studies of repeated human serum measurements of PFOA and PFOS have shown strong correlations over time.

(i) Case–control studies

In the study by Vieira et al. (2013), exposure was assigned on the basis of address at the time of cancer diagnosis; this could result in exposure misclassification if individuals changed addresses before cancer diagnosis. However, the authors stated that the median residence time at current address was 17 years, suggesting that this issue was unlikely to be a source of exposure misclassification.

All the other case–control studies used biomonitoring for exposure assessment, and thus generally had the same strengths and limitations. While blood samples provide specific measures of PFOA and PFOS exposure, biological samples are influenced by interindividual variability. For the case–control studies, the fact that blood samples were collected at or near the time of diagnosis means that these biological markers may be influenced by the disease process. If cancer were to alter the absorption, distribution, metabolism, or excretion (ADME) of PFOA and PFOS, then the measured levels in the cases could not be compared with measured levels in the controls, thus resulting in differential exposure misclassification.

A limitation of these studies is that most did not measure other carcinogens in the blood samples, and that only limited information on exposure to other carcinogens was available from the questionnaires. In the studies by Bonefeld-Jørgensen et al. (2011) and Wielsøe et al. (2017), other substances classified by IARC in Group 1, *carcinogenic to humans* (PCBs, β-hexachlorocyclohexane, cadmium, and cotinine as a biomarker of tobacco smoking), were measured. Bonefeld-Jørgensen et al. (2011) reported high correlations between PFAS and other persistent organic pollutants ($r = 0.42–0.55$; $P < 0.05$), although no information on specific compounds was reported. A strength of the exposure assessment in this study was that correlations with biomarkers of co-exposures were assessed.

In summary, for all case–control studies (except Vieira et al., 2013), blood levels were measured and used as the exposure metric. A main strength was that the measured levels represent combined exposure through all exposure pathways. Measurement error was also thought to be low in all studies in which targeted analyses were performed, whereas the untargeted methods applied in other studies might have lower precision. A major weakness of all the case–control studies was that the blood samples for the cases were collected after the participants had been diagnosed. Thus, the measured levels may not reflect exposure at crucial windows in cancer development, and if cancer alters the ADME of PFAS, there could be differential exposure misclassification.

(ii) Cohort studies

In the majority of studies with occupational exposure and in communities with high exposure, PFOA and PFOS exposure was determined by exposure reconstruction, based either on occupational or residential history. Most studies used exposure reconstruction techniques that provided cumulative exposure estimates to rank-order individuals according to PFOA and PFOS exposure. These cumulative exposure estimates allowed for exposure–response analysis, which may strengthen the argument for causality. In several studies, cumulative serum-level estimates were developed using retrospective modelling (Steenland and Woskie, 2012; Barry et al., 2013; Steenland et al., 2015; Girardi and Merler, 2019); these studies have added strength because they included both environmental and biological measurements to support their estimates. In one study, cumulative estimates of air levels of APFO were developed that enabled workers to be ranked according to exposure, because the main source of PFOA was expected to be occupational (Raleigh et al., 2014). In other studies, cumulative categorical estimates were developed based on occupational history information (Alexander et al., 2003; Alexander and Olsen, 2007; Lundin et al., 2009; Consonni et al., 2013). One study relied solely on residence to assign a categorical exposure, although serum levels were said to validate the categories (Li et al., 2022b); this study also lacked specificity for individual PFAS, limiting its utility to the evaluation of the carcinogenicity of PFOA or PFOS individually. Many of these studies focused only on PFOA (Lundin et al., 2009; Steenland and Woskie, 2012; Barry et al., 2013; Steenland et al., 2015; Girardi and Merler, 2019); none presented isomer-specific estimates of exposures. In the study by Girardi and Merler (2019), workers may have been exposed to other PFAS, including PFOS, but these exposures were not evaluated. In one occupational cohort (Consonni et al., 2013) focusing on TFE workers, a very high correlation between cumulative weighted categorical exposures to TFE and cumulative weighted categorical exposures to APFO ($\rho = 0.72$) was reported in exposed workers, therefore, it was difficult to ascertain differences between these exposures. Another study focused on categories of POSF-exposed workers, resulting in estimates only of indirect exposure to its metabolite PFOS; however, serum levels of PFOS were used to validate the exposure estimates. Co-exposure to PFOA was likely but was not assessed (Alexander et al., 2003; Alexander and Olsen, 2007).

In all cohorts, exposure was ascertained before cancer diagnosis or cancer death. Because exposure was assigned before diagnosis and all individuals were evaluated in the same way, the potential for differential exposure misclassification was limited for both cohort studies and the resulting nested case–control studies.

All the nested case–control studies and one cohort analysis (Wen et al., 2022) relied on biomarker measurement of PFAS in serum or plasma samples, although van Gerwen et al. (2023) and Zhang et al. (2023) used an untargeted analysis method. As discussed for the case–control studies, blood is an appropriate matrix for biomonitoring of PFOA and PFOS. The use of non-targeted methods does not allow quantification of PFAS concentrations but does provide appropriate rank ordering of individuals. Most studies evaluated PFOA and PFOS separately. Frenoy et al. (2022) primarily used principal components analysis to characterize exposure to both PFOA and PFOS together with other PFAS and brominated flame retardants, which made individual PFOA or PFOS determinations challenging.

All studies except that by Hurley et al. (2018) used blood samples collected before case ascertainment, although the range of time between blood collection and case ascertainment varied widely. PFOA and PFOS have a relatively long half-life in blood, making them good measures

of long-term exposure. However, single sample exposure measurements may not reflect exposure at crucial windows in cancer disease development. All studies, except those by Purdue et al. (2023) and Rhee et al. (2023a), used a single blood sample to determine exposure status. Purdue et al. (2023) collected samples at two points in time and analysed them both separately and as a combined exposure metric; this may reduce exposure misclassification but also reduced the study power since not all participants had two samples. The results of the ICC analysis by Rhee et al. (2023a) suggested that PFOA and PFOS concentrations in blood samples remain relatively constant over time, suggesting that a single measure may correctly classify individuals. A bias analysis of these samples by the Working Group demonstrated little misclassification error when considering samples collected within an interval of 5–8 years (see Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636).

While all cohort and nested case–control studies accounted for potential co-exposures to some substances classified by IARC as *carcinogenic to humans* (Group 1), mostly by questionnaire, most studies focused solely on PFAS exposure. Many studies quantified additional PFAS in serum samples and presented risk estimates for individual and total PFAS as well. The most common co-exposures to carcinogens were smoking, alcohol consumption, and use of oral contraceptives, although information on occupation type was also collected by Eriksen et al. (2009) and Feng et al. (2022). Madrigal et al. (2024) also measured PCB congeners, organochlorine pesticides, and polybrominated diphenyl ethers (PBDEs) in serum samples. At present, little is known about the correlation between exposure to PFAS and to other substances classified by IARC as carcinogens.

1.6.2 Quality of exposure assessment in key mechanistic studies in exposed humans

(a) Exposure assessment methods

The exposure assessment methods used in the key mechanistic studies in humans are discussed below according to study design. [The Working Group did not review all mechanistic studies in exposed humans but reviewed a representative sample of studies for each type of study design.]

(i) Cross-sectional studies

The Working Group reviewed the exposure assessment methods used in 18 studies with a cross-sectional design (Knox et al., 2011; Fletcher et al., 2013; Watkins et al., 2014; Lin et al., 2016, 2020c; Lopez-Espinosa et al., 2016; Liu et al., 2018b; Pan et al., 2019; Abraham et al., 2020; Aimuzi et al., 2020; Di Nisio et al., 2020; Kvalem et al., 2020; Clarity et al., 2021; Lopez-Espinosa et al., 2021; Omoike et al., 2021; Cheng et al., 2022; Zhang et al., 2022; Wang et al., 2023). The studies were conducted in the USA and several European and several Asian countries. In all these studies, both PFOA and PFOS were evaluated, and the exposure assessment was based on biomonitoring.

In 16 of these studies, PFOA and PFOS concentrations were measured in the serum or plasma fractions of blood. These matrices are considered suitable for exposure assessment of environmental contaminants, including long-chain PFAS such as PFOA and PFOS (Calafat et al., 2019; Vorkamp et al., 2021; NASEM, 2022) and have been used as the exposure metric in most epidemiological studies of PFAS. PFOA and PFOS concentrations were measured in cord blood in one study (Liu et al., 2018b), in semen (as well as in serum) in the study by Pan et al. (2019), and in the placenta in the study by Wang et al. (2023). Relatively few studies have used semen or placenta for the assessment of exposure to PFAS.

Twelve of the 18 studies were of participants from the general population. In 6 of these 12

studies, the study populations included men who visited a fertility clinic (Pan et al., 2019), patients undergoing surgery for benign diseases or an elective reason (Cheng et al., 2022), children (Lin et al., 2016), and pregnant women (Liu et al., 2018b; Aimuzi et al., 2020; Wang et al., 2023). Another 6 of the 12 studies (Knox et al., 2011; Fletcher et al., 2013; Watkins et al., 2014; Lopez-Espinosa et al., 2016, 2021; Di Nisio et al., 2020) were of populations with elevated exposure to PFOA from contaminated drinking-water. However, exposure to PFOS in these populations was not higher than in the general population.

In all studies, PFAS were measured at the same time point as the assessment of the outcome, and in one study (Watkins et al., 2014), they were also measured 4–5 years before assessment of the outcome, but the two measures of PFAS were averaged to give a single exposure measure. In all the studies, PFOA and PFOS were analysed using LC-MS/MS.

Four studies (Fletcher et al., 2013; Di Nisio et al., 2020; Lin et al., 2020c; Cheng et al., 2022) reported only PFOA and PFOS. All the other studies also reported other PFAS. Although Knox et al. (2011) measured levels of other PFAS, they evaluated potential associations with the outcome only for PFOA and PFOS; Xie et al. (2023) reported 17 PFAS and considered the total concentration of the 17 PFAS that were evaluated.

In two studies (Omoike et al., 2021; Zhang et al., 2022), serum cotinine levels were measured as a biomarker for tobacco smoke, and in 11 studies (Knox et al., 2011; Fletcher et al., 2013; Watkins et al., 2014; Lin et al., 2016, 2020c; Pan et al., 2019; Aimuzi et al., 2020; Di Nisio et al., 2020; Lopez-Espinosa et al., 2021; Wang et al., 2023; Xie et al., 2023) information was obtained about either current or overall exposure to tobacco and/or tobacco smoke via questionnaires. In 10 studies (Knox et al., 2011; Watkins et al., 2014; Lin et al., 2016, 2020c; Pan et al., 2019; Aimuzi et al., 2020; Di Nisio et al., 2020; Lopez-Espinosa et al., 2021; Zhang et al., 2022; Xie et al., 2023), information on alcohol consumption was obtained using a questionnaire. Watkins et al. (2014) and Lopez-Espinosa et al. (2021) also obtained information on regular use of anti-inflammatory drugs over time through a questionnaire, Cheng et al. (2022) obtained information on use of hypolipidaemic drugs, and Knox et al. (2011) excluded participants who were taking hormonal medications.

In two studies, biomonitoring data were collected for contaminants other than PFAS. Abraham et al. (2020) measured PFAS in stored blood samples that were collected in the late 1990s and had previously been analysed for 2,3,7,8-substituted polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is classified as *carcinogenic to humans,* Group 1; IARC, 1997); non-dioxin-like-, mono-*ortho*-, and coplanar PCBs (classified as *carcinogenic to humans*, Group 1; IARC, 2015); 4,4′-dichlorodiphenyltrichloroethane (DDT) and its metabolites (classified as *probably carcinogenic to humans*, Group 2A; IARC, 2017); hexachlorobenzene and β-hexachlorocyclohexane (both classified as *possibly carcinogenic to humans*, Group 2B; IARC, 1987, 2001); lead (classified as *probably carcinogenic to humans*, Group 2A; IARC, 1979, 2006), cadmium (classified as *carcinogenic to humans*, Group 1; IARC, 1993, 2012), and mercury. Clarity et al. (2021), in a study of firefighters, measured urinary levels of four brominated flame retardants and metabolites of six organophosphate flame retardants for which there was potential occupational exposure.

(ii) Prospective birth cohort studies

Exposure assessment methods were reviewed for six mechanistic studies with a prospective birth cohort design (Grandjean et al., 2012; Goudarzi et al., 2017; Miura et al., 2018; Manzano-Salgado et al., 2019; Dalsager et al., 2021; Liu et al., 2022b). These studies were conducted in Denmark, the Faroe Islands (Denmark), Spain,

Japan, and the USA. All six studies included mother–child pairs with singleton births from the general population. Both PFOA and PFOS were evaluated, and the exposure assessment was based on blood biomonitoring.

In all six studies, PFOA and PFOS were analysed using LC-MS/MS in maternal blood serum or plasma collected during pregnancy. Grandjean et al. (2012) also measured PFAS in the children at age 5 years. Among the six studies, outcomes were measured in children at time points ranging from birth to age 12 years.

Miura et al. (2018) reported results only for PFOA and PFOS, whereas the other five studies also reported on other PFAS.

In some studies, information was collected on other exposures, including smoking, diet, and other environmental contaminants. Liu et al. (2022b) measured serum cotinine as a biomarker for maternal tobacco smoking. Grandjean et al. (2012), Manzano-Salgado et al. (2019), and Dalsager et al. (2021) collected information on smoking during pregnancy, and Goudarzi et al. (2017) collected information on parental smoking and environmental tobacco smoke when the children were aged 4 years. Manzano-Salgado et al. (2019) also collected information on maternal diet, including fish consumption, with a questionnaire. Miura et al. (2018) did not provide information on exposure to any other agents. Five of the studies did not obtain biomonitoring data for contaminants other than PFAS, whereas Grandjean et al. (2012) measured PCBs in the serum samples; and none of the studies evaluated or measured exposure to agents other than those mentioned above.

(iii) Longitudinal and repeated-measures studies

Exposure assessment methods were reviewed for three studies with a longitudinal or repeated measures design (Kim et al., 2016, 2020; Blake et al., 2018). In all three studies, LC-MS/MS was used to analyse serum levels of PFAS. Kim et al. (2020) measured serum levels of PFOA, PFOS, and 12 other PFAS and assessed outcomes in children from the general population of the Republic of Korea at the same three time points (ages 2, 4, and 6 years). Information on maternal smoking during pregnancy was collected. Blake et al. (2018) measured serum levels of PFOA, PFOS, and six other PFAS in a cohort of adults who were living near a river in the USA that was contaminated with PFOA and who were identified as being at high risk of elevated exposure to PFAS, particularly PFOA. The study group was a subset of residents near a uranium processing site, but this subset was unlikely to have uranium exposure above background. PFAS levels were measured at enrolment in the study and at one or two later time points for each participant, and outcomes were assessed at the same and/or different time point(s) as the collection of samples for measurement of serum levels of PFAS. In the first serum measurement, PFOA and PFOS were detected in all samples. No information on smoking or alcohol consumption was collected. Kim et al. (2016) measured levels of PFOA, PFOS, and 13 other PFAS in the serum of older adults (aged > 60 years) from the general population of the Republic of Korea who participated in a clinical trial on the effect of vitamin C on the outcomes. Serum levels of PFAS were measured at enrolment and at two additional time points over a 10-week period. Exposure to tobacco smoke (using urinary cotinine as a surrogate) and exposure to air pollutants (PM_{10}, ozone, and nitrogen oxide) were evaluated.

(iv) Study on pathology samples

Exposure assessment was reviewed for a study on PFAS levels in glioma and non-glioma brain tissue in patients (aged 2–77 years) with glioma, in China (Xie et al., 2023). The study included paired glioma and non-glioma brain tissue for 18 patients, as well as glioma or non-glioma brain tissue that did not come from the same patients, making a total of 137 glioma and 40 non-glioma

brain tissue samples. PFOA, PFOS, and 15 other PFAS were analysed using LC-MS/MS in these brain tissue samples to evaluate the potential association between PFAS levels and glioma pathological grade, as well as related biomarkers.

The MRL for PFOA and PFOS in brain tissue was 0.05 ng/g (wet weight). PFOA and PFOS were detected at concentrations above the RL in 69% and 82%, respectively, of the glioma tissue samples, and in 33% and 65%, respectively, of the non-glioma tissue samples. The areas of the brain that were sampled for the non-glioma tissue samples were not provided, and a study by Di Nisio et al. (2022) showed that PFAS levels vary widely in different parts of the brain. This study did not report on brain tissue concentrations of contaminants other than PFAS.

(b) Critical review of exposure assessment in key mechanistic studies in exposed humans

(i) Cross-sectional studies

Exposure assessment in all the cross-sectional studies was based on biomonitoring data, and the studies shared many strengths and limitations. In all of these studies, the analytical methods used were state-of-art at the time when the studies were conducted, and the LODs or LOQs for PFOA and PFOS, when provided, were sufficiently low to ensure detection or quantification of PFOA and PFOS (when present) in all or most samples.

In cross-sectional studies in general, it is not possible to determine the temporal relationship between exposure and outcome. Relying solely on measurements made at a certain point in time makes it difficult to comprehensively assess the impact of long-term exposure on health. For cross-sectional studies in general, a single measurement may not accurately reflect long-term exposure levels, because the concentration of chemicals in the human body may fluctuate with changes in the environment and lifestyle habits over time. However, measured serum or plasma concentrations of PFOA and PFOS are objective measures that integrate exposure from various sources and pathways, including contributions from metabolism of precursors to PFOA or PFOS (Section 4.1), and measurement error in the chemical analysis is low. Because PFOA and PFOS have long elimination half-lives (several years; see Section 4.1), the concentrations measured at a single time point represent past exposure over a relatively long period of time (see Section 1.4.3). For these reasons, measurement of serum or plasma PFOA and PFOS concentrations at the same time as the outcome appraisal is considered to be an acceptable method of exposure assessment for the outcomes considered in these studies, and this is also true for measurement of PFOA and PFOS in cord blood (Liu et al., 2018b). In 16 of the 18 cross-sectional studies, it was reported that PFOA and PFOS were detected at levels above the LOD or LOQ in all or almost all samples; Lin et al. (2020c) and Di Nisio et al. (2020) did not provide this information. These factors limit the potential for non-differential exposure misclassification, in general.

All studies except one collected blood samples once and assessed the outcome at the same time point (or during the same period, Zhang et al., 2022) as the serum or plasma PFAS levels. In the study by Watkins et al. (2014), serum levels of PFAS were measured at two time points – several years before and at the same time that the outcome was assessed – and the analysis was based on the mean of the two serum PFAS values.

A potential limitation of cross-sectional studies is that exposures to other agents that were not measured or evaluated may be correlated with PFOA and PFOS exposure and may also have an impact on the outcome (e.g. act as confounders or effect-modifiers). As one example, exposure to dioxins can result in immune system suppression (WHO, 2016). Different outcome(s) were evaluated in each study, and substances that are potential confounders would probably

differ according to the outcome. Thirteen studies assessed exposure to tobacco smoke with serum cotinine measurements or questionnaires, and 10 studies assessed exposure to alcohol with questionnaires. Two studies (Watkins et al., 2014 and Lopez-Espinosa et al., 2021) obtained information on regular use of anti-inflammatory drugs; one study (Cheng et al., 2022) obtained information on use of hypolipidaemic drugs; and one study (Knox et al., 2011) excluded participants who were taking hormonal medications.

Several studies measured exposures to contaminants other than PFAS. Abraham et al. (2020) measured several other POPs and heavy metals in plasma, and Grandjean et al. (2012) measured PCBs; in both studies, these other contaminants were evaluated as potential confounders of associations with PFAS. Additionally, Clarity et al. (2021) measured 10 flame retardants or their metabolites in the urine. However, exposures to other agents that may have an impact on the outcomes were not evaluated in the cross-sectional studies. This consideration may be particularly applicable in the study by Clarity et al. (2021) on firefighters and office workers. In this study, associations between the outcome and PFOA and PFOS were stronger in firefighters, who are exposed to many other contaminants in addition to PFAS (see Table S1.23, Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/636) compared with office workers.

In one of the studies, Abraham et al. (2020) evaluated potential associations between serum levels of PFAS and antibody response to vaccination in children aged 1 year, including breast-fed and formula-fed children. In this study, samples were collected between 1997 and 1999, which corresponds with the period of highest PFOA and PFOS levels in the general population (see Section 1.4.3).

(ii) Prospective birth cohort studies

In the six prospective birth cohort studies, maternal serum or plasma PFAS level measured during pregnancy was used as an indicator of prenatal PFAS exposure for the children, in whom the outcomes were assessed at birth and/or at later time points. In one study (Grandjean et al., 2012), PFAS levels were also assessed in the children at age 5 years. The analytical methods used were state-of-art, and the LODs or LOQs for PFOA and PFOS, when provided, were sufficiently low to ensure detection or quantification of PFOA and PFOS in all or most samples. Because PFOA and PFOS have long elimination half-lives (several years; see Section 4.1), the concentrations measured in serum or plasma represent maternal exposure over a relatively long period of time.

Blood serum or plasma concentrations are an objective measure of exposure; the concentrations represent the combined exposure through all exposure pathways over a period of time and include contributions from the metabolism of precursors to PFOA or PFOS (see Section 1.4(d) or Section 4.1); and the measurement error in the chemical analyses is low. These factors limit the potential for non-differential exposure misclassification, in general. Five of the six studies (Goudarzi et al., 2017; Miura et al., 2018; Manzano-Salgado et al., 2019; Dalsager et al., 2021; Liu et al., 2022b) reported low LODs or LOQs for PFOA and PFOS, and the sixth study (Grandjean et al., 2012) did not provide information on the values of the LODs or LOQs. [The Working Group noted that even though not explicitly reported, data reported on tertiles of measured concentrations suggested that detection frequencies for PFOA and PFOS were high.] In the study by Manzano-Salgado et al. (2019), PFOA and PFOS were detected at concentrations above the LOD or LOQ in all or almost all samples, whereas Grandjean et al. (2012), Miura

et al. (2018), and Liu et al. (2022b) did not provide this information.

Factors such as plasma volume expansion and changes in glomerular filtration rate that occur during pregnancy may result in decreased PFAS concentrations in serum or plasma, and this effect may be greater when PFAS is measured later in pregnancy (reviewed in US EPA SAB, 2022). Maternal PFAS concentration was measured in the first trimester of pregnancy in the studies by Manzano-Salgado et al. (2019) and Dalsager et al. (2021), in the second or third trimester of pregnancy by Miura et al. (2018), in the first, second, or third trimester by Liu et al. (2022b), and in the third trimester by Grandjean et al. (2012) and Goudarzi et al. (2017). [The Working Group noted that although serum PFAS concentrations may decrease during pregnancy, this is unlikely to result in substantial exposure misclassification in studies in which blood PFAS concentrations are measured at the same time point in pregnancy in all participants. There is a higher risk of exposure misclassification in studies when serum PFAS concentrations are not measured during the same time period (e.g. trimester) in all participants.]

In two of the studies (Miura et al., 2018; Liu et al., 2022b), exposure and outcome were assessed in the same cord blood samples at birth, limiting the potential for non-differential exposure misclassification related to PFAS exposures other than from maternal fetal transfer. However, potential associations between the outcome and maternal PFAS concentrations were evaluated by Manzano-Salgado et al. (2019) at ages 1.5, 4, and 7 years, and by Dalsager et al. (2021) and Goudarzi et al. (2017) at up to age 4 years. The potential association between the outcome and maternal PFAS concentrations was evaluated by Liu et al. (2022b) at age 7 or 12 years as well as at birth, and by Grandjean et al. (2012) at ages 5 and 7 years. However, the potential impact of PFAS exposures that occurred postnatally was not considered, except by Grandjean et al. (2012), who also assessed the association between serum PFAS concentration at age 5 years with the outcome at age 7 years. [The Working Group noted that prenatal exposures are an important time window of exposure for epigenetic changes.] Health outcomes assessed in these children may be associated with postnatal PFAS exposure instead of or in addition to prenatal exposure. Breastfeeding has an impact on postnatal exposure, with the magnitude of the impact being dependent on breastfeeding duration, as well as exposure through drinking-water, diet, consumer products, and other sources. Although there may be some relationship between exposure to the mother (and associated prenatal exposure) and postnatal exposure (e.g. if the mother and child both drink the same contaminated drinking-water), maternal/prenatal and postnatal exposure are not necessarily strongly correlated. For example, Grandjean et al. (2012) reported weak correlations (Pearson coefficients of 0.19 for PFOA and 0.27 for PFOS) for maternal PFAS concentrations at week 32 of pregnancy and postnatal PFAS concentrations at age 5 years.

In these studies, exposures to other agents that were not measured in the mothers or children (see Section 1.6.2(a) above) may be correlated with PFAS exposure and may also have an impact on the outcome as confounders or effect-modifiers.

(iii) Longitudinal and repeated-measures studies

Longitudinal or repeated measures were used in three studies. The strengths of these studies include that repeated measurements provide information on the variability of biomarkers over time. Other strengths include that, in all three studies, the analytical methods used were state-of-the-art, and the LODs or LOQs for PFOA and PFOS were sufficiently low to ensure detection or quantification of PFOA and PFOS in all or almost all samples. Because PFOA and PFOS have long elimination half-lives (several years;

see Section 4.1), the concentrations measured in serum represent exposure over a relatively long period of time. Blood serum concentrations are an objective measure of exposure, the concentrations represent the combined exposure through all exposure pathways over a period of time, and the measurement error in the chemical analyses is low. These factors limit the potential for non-differential exposure misclassification, in general.

(iv) Study on pathology samples

In this study with a case–control design, Xie et al. (2023) measured concentrations of PFOA, PFOS, and 17 other PFAS in samples of glioma and non-glioma brain tissue. Although the analytical method (LC-MS/MS) was state-of-the-art, the percentage of samples in which PFOA and PFOS were detected at levels above the RL was 69% and 82%, respectively, of the glioma tissue samples, and 33% and 65%, respectively, of the non-glioma tissue samples, compared with other studies in which PFAS were detected in all or almost all samples in serum or plasma, or other matrices. [The Working Group noted that the low number of samples and low detection frequencies limited the informativeness of this study.]

In this study, paired glioma and non-glioma samples were available from only 18 patients, and the remainder of the total of 137 glioma and 40 non-glioma brain tissue samples did not come from the same patients. Additionally, the areas of the brain that were sampled for the non-glioma tissue samples were not reported. The comparisons of PFAS concentrations in glioma versus non-glioma tissue samples in this study were highly uncertain because, as previously stated, the specific part(s) of the brain that were sampled and compared were not known, and PFAS levels vary widely in different parts of the brain (Di Nisio et al., 2022). Also, comparison of PFAS levels in tumour and non-tumour brain tissues from different individuals is challenging to interpret because PFAS exposures vary widely among individuals. Finally, it is possible that PFAS accumulate more in tumour tissue than in non-tumour tissue in the brain, resulting in reverse causation.

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2. CANCER IN HUMANS

Since the previous evaluation of perfluorooctanoic acid (PFOA) by the *IARC Monographs* programme in 2014 (Volume 110; IARC, 2016), new epidemiological studies have investigated the occurrence of cancer in relation to exposure to PFOA and to perfluorooctanesulfonic acid (PFOS). A comprehensive search was conducted to identify the studies reporting cancer outcomes (defined as incidence or mortality) that were considered in the present evaluation, including studies of cohorts with occupational and high environmental exposure to PFOA or PFOS; prospective nested case–control or case–cohort studies in populations with background levels of exposure; case–control studies evaluating exposure to PFOA and/or perfluoroalkyl and polyfluoroalkyl substance(s) (PFAS), assessed after a cancer diagnosis; and an ecological study in a population with high contrast (determined through measured serum concentrations) between exposure to PFOA and relatively low exposures to other PFAS. The search identified several cohorts that were each reported in multiple publications and included the continuation of follow-up for cancer occurrence over time; in these instances, detailed reviews were conducted only for the most recent or most informative studies in a given cohort.

The Working Group excluded one ecological study of mortality conducted in the Veneto region of Italy, an area with a high level of PFOA contamination, because that study had notable limitations (Mastrantonio et al., 2018). The crude exposure assessment used (contaminated versus uncontaminated area) was based on drinking-water measurements without biological measurements in the population. Although serum concentrations were later assessed in a younger population (aged 15–39 years) in this region (Pitter et al., 2020), they were not available for the older population pertinent to the outcome investigated in the study (mortality). Human biomonitoring subsequent to the publication of Mastrantonio et al. (2018) detected substantial exposure in some areas previously classified as unexposed; this would have biased estimates towards the null value. In addition, many of the risk ratios reported for men and women combined fell outside of the range of the sex-specific risk ratios reported in the study, making it difficult to interpret the findings.

In total, 36 studies were reviewed in detail: 21 cohort studies (also comprising prospective nested case–control or case–cohort studies), some describing different cancer sites in several publications; 11 case–control studies; and 4 meta-analyses. In addition, the Working Group conducted an ecological analysis of orchiectomy rates as a surrogate for testicular cancer in residents of a contaminated area of northern Italy (see Section 2.3, and Annex 3, Supplementary analyses used in reviewing evidence on cancer in

humans, available from: https://publications.iarc.who.int/636). Section 2.1 summarizes the cohort studies, nested case–control and nested case–cohort studies, and two case–control studies on multiple cancer sites (reported in Vieira et al., 2013). Results for specific cancer sites are summarized in Sections 2.2 to 2.7, with findings from cohort and nested case–control or case–cohort studies described first, followed by findings obtained using other study designs. Studies of breast cancer were further sorted by design, with separate subsections for cohort-based studies and case–control studies or meta-analyses. The Working Group also conducted a meta-analysis of studies on kidney cancer, as well as a methodological simulation study to evaluate the representativeness of serum PFOA measurements from a single time point as a surrogate for longer-term measurements (over a period of 5–8 years); this is summarized in Annex 3 (Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). Finally, a synthesis of the evidence relating to cancer in humans is presented in Section 2.8.

2.1 Cohort descriptions

See Table 2.1.

2.1.1 PFOA-production workers (Cottage Grove, Minnesota, USA)

Raleigh et al. (2014) updated data on cancer incidence and mortality in a previously investigated (Gilliland and Mandel, 1993; Lundin et al., 2009) cohort of workers exposed to PFOA at a facility manufacturing ammonium perfluorooctanoate (APFO, the ammonium salt of PFOA) (the Cottage Grove plant) in Minneapolis, Minnesota, USA. The cohort included 4668 workers (men, 79%) who were employed for ≥ 1 year between 1947 (when production of APFO was initiated) and 2002 (when production was terminated). A reference population was also followed, this being a cohort of 4359 workers (men, 88%) who were employed for ≥ 1 year before 1999 at a tape and abrasive production facility (the Saint Paul plant) where there was no production of APFO and that was located in the same suburban area and managed by the same company as the APFO-manufacturing facility.

Workers at the Cottage Grove plant were exposed by inhalation of PFOA vapour and ammonium salt particulates during regular production, through cleaning of equipment, changing filters, quality control checks, and maintenance, and through bystander exposure. For all cohort members, individual exposure by inhalation to APFO (in mg/m^3 of air), as a daily time-weighted average (TWA), was estimated from work history records (period, department, job title), industrial hygiene monitoring data (205 personal samples and 659 area samples collected in 1977–2000, from all processes and tasks in APFO-production areas in the chemical division of the Cottage Grove plant), information from former and current workers and from industrial hygiene professionals, and APFO-production levels. Daily TWAs for jobs in APFO production ranged from 1×10^{-4} to 4.0×10^{-1} g/m^3 [0.1 μg/m^3 to 400 μg/m^3]. Exposures for non-APFO production jobs in the chemical division and the non-chemical division ranged, according to expert judgement, from 1×10^{-8} to 3×10^{-5} mg/m^3 [1×10^{-5} and 3×10^{-2} μg/m^3] and from 1×10^{-8} to 1×10^{-6} mg/m^3 [1×10^{-5} and 1×10^{-3} μg/m^3], respectively. To account for ubiquitous background exposure, all workers (including the reference population) were assigned an exposure that was one order of magnitude lower than that for the workers in the Cottage Grove non-chemical division. The final cumulative exposure metric was quartiles of μg/m^3-years. Medical surveillance of 148 workers employed in the Cottage Grove chemical division in 2000 found a geometric mean serum concentration of PFOA of 815 ng/mL (2538, 979, and

Table 2.1 Description of cohort studies (including nested case–control studies) on exposure to PFOA or PFOS and cancer

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Lundin et al. (2009) MN, USA Enrolment, 1947–1997/ follow-up, 1947–2002 (mortality) Cohort	3993 employees; Cottage Grove (MN) PFOA cohort; workers employed at an APFO-production plant for ≥ 365 days before 31 December 1997; most recent follow-up for some cancer sites (see those listed here), later follow-up by Raleigh et al. (2014) Exposure assessment method: based on job history; jobs classified as definite, probable, and no or minimal occupational APFO exposure	Large intestine, rectum, oesophagus, stomach Thyroid CNS Lymphatic and haematopoietic Lymphosarcoma-reticulosarcoma Hodgkin lymphoma Leukaemia	See Table S2.5[a] See Table 2.4 See Table S2.6[a] See Table S2.6[a] See Table S2.6[a] See Table S2.6[a] See Table S2.6[a]	*Exposure assessment critique*: Key strengths were the industrial hygiene review of jobs; weighting of jobs based on serum measurements; assignment of exposure weights based on blood monitoring (authors indicated that other weights were considered but felt that these weights allowed better differentiation between probable and definite exposures over time). Key limitations were the crude exposure assessment by job classification, and lack of job-specific data on PFOA serum levels (but serum PFOA levels for work areas were collected in 2000). *Other strengths*: Occupational cohort with relatively high exposures; analyses presented based on both job classification and cumulative exposure estimates. *Other limitations*: Small occupational cohort with limited number of deaths; potential healthy-worker effect due to external comparison of rates from general population; limited information on covariates. Smoking data were collected but not included in the final models.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Raleigh et al. (2014) MN, USA Enrolment, 1947–2002/ follow-up, 1947–2008 (mortality), 1988–2008 (incidence) Cohort	9027 employees (4668 exposed workers, 4359 reference workers); Cottage Grove (MN) PFOA cohort latest update (previous: Gilliland and Mandel, 1993, and Lundin et al., 2009); workers employed for ≥ 1 yr in 1947–2002 at an APFO facility (Cottage Grove; *n* = 4668); reference workers without any exposure to APFO employed at a tape and abrasives production facility located in the same suburban geographical area and managed by the same company (Saint Paul; *n* = 4359) Exposure assessment method: exposure matrix used production-process air measurements and expert judgement in applying production volume data and proximity to production areas to assign department and job exposures historically; exposure matrix and job history were used to calculate cumulative exposure (μg/m^3-years)	Kidney Urinary bladder Prostate Breast Liver Pancreas All cancers combined	See Table 2.2 See Table 2.2 See Table 2.3 See Table 2.4 See Table S2.5[a] See Table S2.5[a] See Table S2.7[a]	*Exposure assessment critique*: Key strengths were that the only PFAS exposure in plant was to APFO (PFOA); cumulative co-exposure to TFE was estimated to be minimal Key limitations were that development of cumulative exposure metric was based on APFO air concentrations and not internal dose; reference population in APFO unexposed same plant was assumed to have exposures of the general population, but the method of determination was unclear (1 × 10^{-7} to 1 × 10−9 mg/m^3). *Other strengths*: A reference population sharing similar socioeconomic characteristics as the exposed population and a long follow-up period. *Other limitations*: Lacking data on employees who left MN or WI. Lacking data on cancer-incidence before start of follow-up up to 40 yr after first exposure. No information on health behaviours (potential confounding). Small numbers of cancers of kidney, pancreas, liver, testis. No accounting for alcohol or smoking.
Alexander et al. (2003) Decatur (AL), USA Enrolment, 1961–1997/ follow-up, 1961–1998 (mortality) Cohort	2083 employees; Decatur (AL) PFOS cohort; production workers (men, 83%) who worked ≥ 365 days in a plant producing speciality films and fluorochemicals, a main one being POSF; most recent follow-up of all cancers except bladder, which is described in a later study by Alexander and Olsen (2007) Exposure assessment method: expert judgement; workers were categorized as ever in a "high" exposure job, ever in a "low" exposure job but not a "high" exposure job, only in jobs without POSF exposure, or ≥ 1 yr in a "high" exposure job	Breast Liver and bile ducts, large intestine, oesophagus, digestive organs and peritoneum Lymphatic and haematopoietic Melanoma Respiratory system Bronchus, trachea, lung All cancers combined	See Table 2.4 See Table S2.5[a] See Table S2.6[a] See Table S2.6[a] See Table S2.6[a] See Table S2.6[a] See Table S2.7[a]	*Exposure assessment critique*: A key strength was the range of workplace exposure durations and levels (a large exposure contrast). Key limitations were that exposure assessment did not use any measure of cumulative exposure, but simply categorized each worker in 1 of 3 ever/never/only job classifications, which could produce exposure misclassification; many likely co-exposures to potential carcinogens or other fluorochemicals, including PFOA (however, PFOA concentrations were probably low). *Other limitations*: Occupational cohort with few cancer deaths (overall, 39; high exposure group, 18), limited to mortality, lack of data on smoking and alcohol, mostly male (83%).

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Alexander and Olsen (2007) Decatur (AL), USA Enrolment, 1961–1997/ follow-up, 1970–2002 (mortality and incidence) Cohort	1588; Decatur (AL) PFOS cohort; production workers in the Alexander et al. (2003) cohort; living cohort members completed a questionnaire (response rate, 73.9%) to identify incident bladder cancer cases; bladder cancer decedents were identified using underlying cause of death from death certificates; analyses excluded 495 living cohort members who did not return the questionnaire Based on the exposure assessment described in Alexander et al. (2003), cumulative exposure was calculated weighing the exposure categories of nonexposed, low exposed and high exposed with a factor of 1, 3, and 10, respectively, for each year in that job.	Urinary bladder	See Table 2.2	*Exposure assessment critique*: Key strengths were the range of workplace exposure durations and levels; cumulative exposure was estimated using a weighted approach of exposure categories. Key limitations were that the crude weighted approach to calculate cumulative exposure could produce exposure misclassification; many likely co-exposures to potential carcinogens or other fluorochemicals (however, PFOA concentrations were probably low). *Other strengths*: use of incidence data with 74% participation rate in survey; attempt to validate self-reported cancer for survey respondents. *Other limitations*: occupational cohort with only 11 cases of bladder cancer, 2 in the highest category of exposure. Bladder cancer incidence identified by survey of cohort (6 cases) and death certificates (5 deaths) no cancer registry matching, only partial data on smoking, no ability to validate 5 cases of bladder cancer identified by death certificate, mostly male (83%).
Leonard et al. (2008) Parkersburg (WV), USA Enrolment, 1948–2002/follow-up, 1948–2002 (mortality) Cohort	6027 workers; Parkersburg (WV), polymer-production PFOA cohort; most recent follow-up for some cancer sites (see those listed here), later follow-up by Steenland and Woskie (2012); workers (men, 81%) at a polymer-manufacturing facility for ≥ 1 day in 1948–2002 Exposure assessment method: no quantitative exposure assessment; workers in a polymer-production facility were identified using the company's administrative records; ~30% worked in processes using APFO; all participants had detectable levels of serum PFOA	Large intestine, rectum, oesophagus, stomach Thyroid Melanoma	See Table S2.5[a] See Table 2.4 See Table S2.6[a]	*Strengths*: Occupational cohort with relatively high exposures. Complete cohort ascertainment and follow-up. Local reference groups increase comparability with respect to socioeconomic factors and health behaviours. *Limitations*: No assessment of exposure to specific chemicals (the company used a wide variety of chemicals including PFOA). Small numbers.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Steenland and Woskie (2012) Parkersburg (WV), USA Enrolment, 1948–2002/follow-up, 1952–2008 (mortality) Cohort	5791 workers; Parkersburg (WV), polymer-production PFOA cohort; workers (men, 81%) at a polymer-manufacturing facility who had potential exposure to fluoropolymers and sufficiently detailed work histories Exposure assessment method: JEM was based on a total of eight job category/job group combinations; jobs were classified on the basis of PFOA exposure potential and the JEM was improved through the use of blood samples to assign serum PFOA levels over time	Kidney Urinary bladder Testis Prostate Breast Liver and gallbladder Pancreas NHL Leukaemia Lung Mesothelioma All cancers combined	See Table 2.2 See Table 2.2 See Table 2.3 See Table 2.3 See Table 2.4 See Table S2.5[a] See Table S2.5[a] See Table S2.6[a] See Table S2.6[a] See Table S2.6[a] See Table S2.6[a] See Table S2.7[a]	*Exposure assessment critique*: Key strengths were that the JEM incorporated changes in exposure over time; because serum levels were used to construct the JEM, residential exposure to PFOA in drinking-water was included in estimates; any exposure misclassification is likely to be non-differential. A key limitation was the lack of description of other exposures. *Other strengths*: Ability to evaluate associations with PFOA in a population exposed to levels much higher than in the general population. *Other limitations*: Limited ability to evaluate mortality for some cancers due to small numbers of deaths, particularly for cancers among women (given the small number of female workers in the study) and cancers that are relatively rare and/or less likely to be fatal.
Steenland et al. (2015) Parkersburg (WV), USA Enrolment, 1948–2002/follow-up, 1951 to interview date in 2008–2011 (incidence) Cohort	3713 employees; a subset of the Parkersburg (WV) polymer-production PFOA cohort in Steenland and Woskie (2012); polymer-production workers (men, 80%) who responded (self or next-of-kin) to a questionnaire about health outcomes and for whom measured or estimated occupational and residential exposure estimates were available Exposure assessment method: cumulative PFOA serum concentrations estimated on the basis of JEMs and residential history; historical PFOA serum levels were modelled via a JEM based on > 2000 serum measurements (Woskie et al., 2012); non-occupational exposure from drinking-water (address-based) was also estimated;	Urinary bladder Prostate Colon and rectum Melanoma	See Table 2.2 See Table 2.3 See Table S2.5[a] See Table S2.6[a]	*Exposure assessment critique*: Key strengths were good characterization of exposure in both occupational and residential settings; minimal potential for non-differential exposure misclassification. A key limitation was that loss to follow-up of 40% of workers could lead to differential exposure misclassification if related to PFOA exposure. *Other strengths*: Ability to evaluate associations between PFOA and cancer incidence in a population exposed to levels much higher than in the general population. Use of medical records to confirm self-reported cancer diagnoses likely reduced non-differential outcome misclassification.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Steenland et al. (2015) Parkersburg (WV), USA Enrolment, 1948–2002/follow-up, 1951 to interview date in 2008–2011 (incidence) Cohort (cont.)	yearly serum estimates from the occupational exposure model were used for the years when people worked at the plant if these were higher than residential estimates, or if they were lower, the residential (community) estimates were used			*Other limitations*: Possibility of selection bias as the investigation included only 62% of the target population; relatively small numbers of validated cancer cases and inability to evaluate less-common malignancies. Possible under-ascertainment of cases due to medical record confirmation.
Eriksen et al. (2009) Denmark Enrolment, 1 December 1993 to 31 May 1997/follow-up, 1 December 1993 to 1 July 2006 (incidence) Case–cohort	Case–cohort within the Diet, Cancer, and Health cohort, which included men and women aged 50–65 yr without cancer at enrolment. Cases: urinary bladder, 332; prostate, 713; liver, 67; pancreas, 128; incident cases identified through cancer registry linkage Comparison cohort: 772 (680 men, 92 women); subcohort of participants randomly selected without cancer at the end of follow-up Exposure assessment method: quantitative plasma measurements; analytical method was state-of-the-art; a single sample collected at enrolment (1993–1997) was analysed for PFOA and PFOS	Urinary bladder Prostate Liver Pancreas	See Table 2.2 See Table 2.3 See Table S2.5[a] See Table S2.5[a]	*Exposure assessment critique*: Key strengths were that plasma levels measured at baseline represent the combined exposure through all exposure pathways; measurement error low. Key limitations were that single samples collected at time of enrolment may not reflect exposure at crucial windows in cancer development; measured only PFOA and PFOS and no information on exposure to other PFAS. *Other strengths*: Large cohort with numerous incident cancers (n = 1240) followed 0–12 yr after baseline enrolment; control of confounders; internal comparison; low loss to follow-up. *Other limitations*: Low exposure contrast in a population with background exposure levels. Analyses only considered PFOA and PFOS separately.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Barry et al. (2013) Mid-Ohio Valley (OH and WV), USA Enrolment, August 2005 to August 2006/ follow-up, 1952 to 2011 (incidence) Cohort	32 254 (28 541 community members and 3713 workers); C8 Science Panel Study; included people enrolled in the C8 Health Project who lived, worked, or attended school for ≥ 1 yr between 1950 and 3 December 2004 in a district with contaminated water in the vicinity of a chemical plant using PFOA in manufacturing processes (Parkersburg, WV, polymer-production facility), as well as a subset of those from the original Parkersburg (WV) polymer-production PFOA occupational cohort who worked at the plant between 1948 and 2002 Exposure assessment method: residential and occupational exposure estimates were combined to estimate cumulative PFOA serum concentrations; historical PFOA serum levels were modelled via a JEM based on > 2000 serum measurements (Woskie et al., 2012); non-occupational exposure from drinking-water (address-based) was also estimated	Kidney	See Table 2.2	*Exposure assessment critique*: Key strengths were that exposure assessment was done the same way for all participants; estimates accounted for both residential and occupational exposure to PFOA. A key limitation was that serum PFOA levels were available only in 2005–2006. *Other strengths*: Wide range of PFOA exposure levels; authors presented both no lag and 10-yr lag models; availability of detailed information on potential confounding factors; relatively high participation rates; and validation of cancer diagnoses through medical chart review and state registries. *Other limitations*: possibility of selection bias, particularly for cancers with a high rate of fatality; and relatively few validated cases for prospective analyses (after C8 Health Project enrolment). Potential limitation of a survivor cohort but unlikely to be biased unless those with higher exposure had lower post-diagnosis survival rates than those with lower exposure (Barry et al., 2015). *Other comments*: 62% of the polymer production plant cohort (Steenland and Woskie, 2012) is included in the study population (including workers who did and did not participate in the C8 Health Project).
		Urinary bladder	See Table 2.2	
		Testis	See Table 2.3	
		Prostate	See Table 2.3	
		Breast	See Table 2.4	
		Liver, pancreas, colon and rectum, oesophagus, stomach	See Table S2.5[a]	
		Thyroid	See Table 2.4	
		Brain	See Table S2.6[a]	
		Leukaemia, lymphoma	See Table S2.6[a]	
		Lung	See Table S2.6[a]	
		Melanoma	See Table S2.6[a]	

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Consonni et al. (2013) USA, UK, Italy, Germany, Netherlands Enrolment, 1950–2002/follow-up, 1950–2008 Cohort	5879 male workers (APFO-exposed, 4205); The pooled international TFE cohort included male workers who were ever employed or employed for a minimum of 6 or 12 mo at one or more of six TFE production sites in North America and Europe between 1950 and 2002; the principal occupational exposures were TFE and APFO (facilitates production of TFE) Exposure assessment method: a JEM provided yearly semiquantitative estimates (in arbitrary units) of TFE and APFO exposure for relevant job titles at each production site, from the start of TFE production to 2002 (Sleeuwenhoek and Cherrie, 2012)	Kidney and other organs of the urinary tract	See Table 2.2	*Exposure assessment critique*: A key strength was the availability of job history for all participants. Key limitations were that only expert judgement was used to determine exposure levels; no measured exposures; high correlations between exposure to TFE monomer (IARC Group 2A) and PFOA, which precludes evaluation of effects of the individual compounds. *Other strengths*: The cohort included all TFE production sites worldwide during the entire period of production and had almost complete enrolment and follow-up. *Other limitations*: low statistical power to detect risk of rare cancers.
		Urinary bladder	See Table 2.2	
		Testis	See Table 2.3	
		Prostate	See Table 2.3	
		Liver and intrahepatic bile duct, pancreas, colon, rectum, oesophagus, stomach	See Table S2.5[a]	
		Brain	See Table S2.6[a]	
		Lymphatic and haematopoietic, NHL, multiple myeloma, leukaemia	See Table S2.6[a]	
		Lung	See Table S2.6[a]	
		All cancers combined	See Table S2.7[a]	
Ghisari et al. (2017) Denmark Enrolment, 1996–2002/follow-up, through 2010 Nested case–control	Nested within the Danish National Birth Cohort of ~100 000 pregnant women: nulliparous women at the time of blood draw during pregnancy were followed for breast cancer. Cases: 178 cases of breast cancer in nulliparous women at the time of blood draw during pregnancy Controls: 233; frequency-matched on age Exposure assessment method: quantitative serum measurement; analytical method was state-of-the-art; a single sample collected at enrolment (1996–2002) was analysed for PFAS at ascertainment for cases and controls	Breast (premenopausal)	See Table 2.4	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; measurement error low. Key limitations were that single samples at time of enrolment may not reflect exposure at crucial windows in cancer development; focused analysis on only 4 PFAS separately even though others had 98.8–100% samples detectable (PFHpS, PFNA) and others had 50–89% detectable (PFHpA, PFDA, PFDoA, PFUnA, PFTrA) (Bonefeld-Jørgensen et al., 2014). Did not sum PFAS in any way.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Ghisari et al. (2017) Denmark Enrolment, 1996–2002/follow-up, through 2010 Nested case–control (cont.)				*Other strengths*: Blood samples collected before breast cancer diagnosis; exposure during pregnancy may be an important exposure window for breast cancer; large sample of premenopausal cases; consideration of relevant SNPs. *Other limitations*: Focused on premenopausal breast cancer, did not consider postmenopausal breast cancer; no information on tumour characteristics; small exposure contrast. *Other comments*: Earlier follow-up by Bonefeld-Jørgensen et al. (2014). A few dozen breast cancer cases from that study were excluded here due to concern about status due to a coding error.
Hurley et al. (2018) CA, USA Enrolment, 1995–1996/follow-up, 1 January 2006 to 1 August 2014 (incidence) Nested case–control	Nested within the California Teachers Study; 133 479 female public-school teachers and other professionals were followed annually for cancer incidence Cases: 902 cases with a diagnosis of invasive breast cancer at age < 80 yr, no prior history of breast cancer, who provided a blood specimen, answered the questionnaire, and were continuous residents of CA; participation rate, 65% Controls: 858 women drawn from a probability sample of at-risk cohort members, frequency-matched on age, race/ethnicity, and residence; participation rate, 55% Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; a single sample was collected after diagnosis of invasive breast cancer (average, 35 mo) and analysed for PFAS	Breast	See Table 2.4	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; measurement error low. Key limitations were that blood samples were collected on average 35 mo (range, 9 mo to 8.5 yr) after diagnosis, which may not reflect exposure at crucial windows in cancer development; if breast cancer alters ADME of PFAS, there could be possible differential exposure misclassification; did not account for mixtures of PFAS and did not use all PFAS measurement data available. *Other strengths*: Case ascertainment with statewide cancer registry linkage and pathology confirmation; considered several established breast cancer risk factors as confounders/modifiers; evaluated associations by combined ER and PR status and menopausal status; large number of cancer-registry identified cases and controls.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Girardi and Merler (2019) Vicenza province, Veneto Region, Italy Enrolment, 1960–2008/follow-up, 1970–2018 (mortality) Cohort	462 PFAS workers; 1383 railroad workers (comparison cohort); workers in a perfluorocarbon-production facility in Trissino manufacturing PFOA, PFOS, other perfluorinated compounds, and other chemicals; comparison populations included the regional general population and workers in a local railroad industry who were not exposed to chemicals; for both occupational cohorts, the workers included were men employed for ≥ 6 mo Exposure assessment method: cumulative serum levels were estimated for each worker's history, 1970–2008; serum data collected in 2000–2013 were used to model historical exposures in three job categories by incorporating fixed effects for variables related to subject of measurement as well as historical data on PFOA production.	Liver and intrahepatic bile ducts (ICD-9, 155) Colon Oesophagus, stomach Lymphatic and haematopoietic, NHL Lung All cancers combined	See Table S2.5[a] See Table S2.5[a] See Table S2.5[a] See Table S2.6[a] See Table S2.6[a] See Table S2.7[a]	*Exposure assessment critique*: Key strengths were that historical exposures were modelled using serum measurements and estimation of cumulative exposures to PFOA (ng/mL-years); the study evaluated whether workers' home drinking-water was in contaminated area (Red Zone), but unclear how this information was used; APFO exposures were accounted for in PFOA measurements as it dissociates to PFOA in the body. Key limitations were that few samples were available to model serum levels in job categories 2 and 3; other PFAS exposures in plant were not accounted for (including PFOS and perfluorobutylsulfonyl fluoride); other potential carcinogenic co-exposures within factory were not accounted for, nor were alcohol or smoking use assessed. *Other strengths*: High exposure contrast; internal comparisons with non-exposed workers. *Other limitations*: Included only men; small occupational cohort with few deaths (n = 107); few cancer deaths for liver and lympho-haematopoietic (7 each) (the 2 causes with positive trends with exposure); no data on some causes of death of interest (e.g. bladder, prostate).

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Mancini et al. (2020a) France Enrolment, 1990/ follow-up, through 2013 (incidence) Nested case–control	Nested within the E3N cohort of 98 995 women born in 1925–1950 and covered by the French National Education System insurance; participants were invited to complete follow-up questionnaires (including dietary) every 2–3 yr and donate blood between 1994 and 1999 Cases: 194 incident cases of post-menopausal breast cancer diagnosed among women with serum (≥ 3 aliquots) collected before diagnosis, a completed dietary questionnaire in 1993, and randomly selected from 240 eligible cases of breast cancer Controls: 194; density-sampled at time of case occurrence and matched on age within 2 yr, menopausal status at blood collection, BMI at blood collection, and year of blood collection Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; a single sample collected before diagnosis of breast cancer was analysed for total PFOA and PFOS, not for isomers of PFOA or PFOS	Breast (post-menopausal)	See Table 2.4	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; measurement error low. Key limitations were that single samples before diagnosis may not reflect exposure at crucial windows in cancer development. *Other strengths*: Blood samples collected before diagnosis with a long follow-up period; extensive adjustment for plausible confounders; inclusion of hormone receptor subtype information; low loss to follow-up. *Other limitations*: Limited statistical power, particularly when exploring differences by subtype; low exposure contrast in general population sample; did not include premenopausal breast cancer cases. *Other comments*: Frenoy et al. (2022) conducted additional exposure–response analyses.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Shearer et al. (2021) USA Recruitment: 1993–2001, Follow-up (from blood drawn): median 8.8 yr (incidence) Nested case–control	Nested within the PLCO cohort, which comprises ~150 000 adults aged 55–74 yr from study centres in 10 cities; about half (assigned to the screening arm) provided a blood sample at baseline and were followed for incident cancer Cases: 324; source of cancer diagnosis not reported. Controls: 324; density-sampled on calendar time and individually matched on age categories, sex, race and ethnicity, study centre, and year of blood draw Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; blood samples collected at enrolment into PLCO study; samples from cases and controls were analysed at the same time for PFAS in serum	Kidney (RCC)	See Table 2.2	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; measurement error low. A key limitation was that single measurement of serum levels may not have reflected cumulative or long-term exposure, although only minor misclassification of long-term exposure over a period of 5–8 yr was seen, based on a simulation study (see Annex 3 in the present monograph). *Other strengths*: Large number of kidney cancer cases (n = 324); an average of 8 yr of follow-up following baseline serum measurement of a variety of PFAS; good data on confounders; internal comparisons with control over kidney function; adjustment for exposure to other PFAS. *Other limitations*: Low exposure contrast in a population with background levels. *Other comments*: PFAS concentrations were missing for two (excluded) cases.
Chang et al. (2023) USA Enrolment, 1993–2001/ follow-up, through November 2013 (incidence) Nested case–control	Nested within the PLCO cohort (see Shearer et al., 2021) Cases: 621; all incident cases of invasive breast cancer diagnosed among postmenopausal women who were not using MHT at baseline (unless their cancers were hormone receptor-negative) Controls: 621; controls were selected using incidence-density sampling; all were postmenopausal, still alive and cancer-free at the time of case diagnosis; matching on age at baseline, date of blood draw, and baseline MHT use	Breast (post-menopausal)	See Table 2.4	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; exposure was assessed before outcome; any misclassification is likely to be non-differential. Key limitations were that single measurement of serum levels may not capture relevant window of exposure for cancer development, especially among the cases diagnosed close to sample collection (but the authors conducted analyses stratified by time since blood draw, which addresses this concern); also, only minor misclassification of long-term exposure over a period of 5–8 yr, based on a simulation study (Annex 3); exposure assessment relied upon relative quantification of PFOA and PFOS (but relative measures have correlated well with targeted absolute concentration measurements).

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Chang et al. (2023) USA Enrolment, 1993–2001/ follow-up, through November 2013 (incidence) Nested case–control (cont.)	Exposure assessment method: PFOA and PFOS serum levels were measured using a non-targeted method (unclear whether both branched and linear isomers were included); in the same participants, untargeted serum levels were correlated with levels measured using a standard, targeted method (Spearman correlation coefficient was 0.76 and 0.77 for total PFOS and total PFOA, respectively)			*Other strengths*: Large number of breast cancer cases (n = 621); an average of 8 yr of follow-up after serum measurements on a variety of PFAS at baseline; good data on confounders, internal comparisons; adjustment for exposure to other PFAS; stratified analyses by hormone status of cancer. *Other limitations*: Low exposure contrast in a population with background levels; limited power to consider hormone receptor negative tumours, no premenopausal cases. *Other comments*: PFAS concentrations were missing for two (excluded) cases.
Rhee et al. (2023a) USA Recruitment, 1993–2001, follow-up (from blood draw), median, 9 yr (incidence) Nested case–control	Nested within the PLCO cohort (see Shearer et al., 2021) Cases: 750 cases of aggressive prostate cancer (defined as stage III or IV, Gleason score ≥ 8, or Gleason score 7 and death from prostate cancer), diagnosed > 300 days after blood collection Controls: 750; alive and cancer-free at time of case diagnosis, and individually matched to cases on age at baseline, race/ethnicity, study centre, calendar and study year of blood collection, and prior freeze–thaw cycle Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; a single sample was collected at time of enrolment into PLCO; time between blood draw and diagnosis was 9 yr (median IQR, 5.13 yr)	Prostate (aggressive/advanced)	See Table 2.3	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; measurement error low; PFAS were measured in blood samples collected before diagnosis; analysis of blood samples collected 0,1, and 5 yr after enrolment showed a high degree of reproducibility with ICCs of > 0.7 for PFOA and PFOS. *Other strengths*: large case control study with 750 cases and matched controls; data on a broad range of confounders; smoking was controlled for in the analysis; other exposures are unlikely to be correlated with PFAS in this general population sample; mutual adjustment for other PFAS under study. *Other limitations*: general population with low exposure contrast; large number of comparisons.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Cohn et al. (2020) Oakland (CA), USA Enrolment, at birth between 1959–1967/ follow-up, birth to March 2013 (incidence) Nested case–control	Nested within the CHDS pregnancy cohort, which includes 19 044 live births from pregnant members of the Kaiser Foundation Health Plan who received obstetric care between 1959 and 1967 and who provided blood specimens during pregnancy and at birth; > 99% of eligible women enrolled, and 74% of cases had a blood sample and complete information on potential confounders and effect modifiers Cases: 102 incident cases of invasive or non-invasive breast cancer diagnosed by age 52 yr, with a maternal perinatal blood sample and complete information on potential confounders and effect modifiers Controls: 310; 3 per case, density-sampled on case age and matched on birth year and trimester of maternal blood draw Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; maternal blood samples were collected before offspring birth; blood samples from cases and controls were retrieved ~50 yr later for PFAS analysis	Breast	See Table 2.4	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; measurement error low; perinatal exposure may be relevant for later breast cancer; misclassification of exposure is unlikely because cases were matched to controls for year of enrolment in the study (thus, changes in concentration over time were addressed). Key limitations were that PFAS exposure was not measured directly in study participants; only one maternal blood sample (during pregnancy or after labour) was used, while PFAS levels may vary during pregnancy; no information on exposures during the individual's lifetime. *Other strengths*: long follow-up; cases were likely to have been accurately determined via the California cancer registry. *Other limitations*: Only cases diagnosed before age 52 yr were included; risk of incomplete or biased case ascertainment; small sample size and limited statistical power; no information concerning tumour hormone-receptor status; adjustment only on potential maternal confounders and no variable collected at the daughter's individual level; lack of information on migration out of state.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Li et al. (2022a) Ronneby, southern Sweden Enrolment,1985–201/ follow-up, 1985–2016 (incidence) Cohort	60 507; the Ronneby Register cohort includes all individuals who ever lived in the Ronneby municipality in 1985–2013; one third of the households received PFAS-contaminated drinking-water from a waterworks situated near a military airfield where PFAS-containing firefighting foam was used in 1985–2013 (individuals considered to have "ever-high" exposure, 15 811); subsets with long-term exposure (≥ 11 yr) in the latest part of the follow-up period (2005–2013) were considered to be more highly exposed Exposure assessment method: residential location (water source) used to categorize participants into groups of potential exposure based on time period or duration of residency, or a residence in a neighbouring reference municipality; serum levels collected in 2014–2015 for residents and the neighbouring municipality were used to validate categories	Kidney	See Table 2.2	*Exposure assessment critique*: Key strengths were that categories reflect serum levels, which include exposure through all pathways; attempted to incorporate length of exposure period in analysis. Key limitations were the potential for misclassification of exposures, since there was no information on individual water consumption patterns or use of bottled water or filtration at home; no cumulative years of exposure used, except two categories of short and long high exposure; no accounting for potential co-exposures; lack of historical information on area-level PFAS drinking-water contamination, particularly during earlier years of the study period. *Other strengths*: Large study population; strong exposure contrast; unbiased inclusion; complete follow-up; long follow-up for part of the population; reference group from same municipality. *Other limitations*: Mixed exposure profile without possibility to single out effects due to specific compounds; little information on potential confounders. *Other comments*: PFAS exposure mainly PFOS, PFHxS, together comprising > 90% of total PFAS in water, and PFOA (water and blood samples).
		Urinary bladder	See Table 2.2	
		Testis	See Table 2.3	
		Prostate	See Table 2.3	
		Breast	See Table 2.4	
		Liver, bile duct or gall bladder, pancreas, colon, rectum, oesophagus, stomach	See Table S2.5[a]	
		Thyroid	See Table 2.4	
		Brain	See Table S2.6[a]	
		NHL, multiple myeloma, chronic lymphocytic leukaemia, chronic myelogenous leukaemia	See Table S2.6[a]	
		Melanoma	See Table S2.6[a]	
		Trachea and lung	See Table S2.6[a]	
		All cancers combined	See Table S2.7[a]	

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Feng et al. (2022) Shiyan, China Enrolment, September 2008 to June 2010 and April to October 2013/ follow-up, 2008–2018 (incidence) Case–cohort	Case–cohort within the Dongfeng-Tongji cohort, which included 18 387 female retirees of automotive companies, without cancer at enrolment, with sufficient blood samples Cases: 226; the total number of diagnoses of incident breast cancer included 13 diagnoses in the comparison cohort Comparison cohort: 990 (including 13 cases); women randomly selected according to age strata The 13 cases included in the comparison cohort of 990 women served as controls until they received a cancer diagnosis Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; a single serum sample collected before diagnosis of breast cancer was analysed for six PFAS (including PFOA and PFOS), but not for isomers of PFOA or PFOS	Breast	See Table 2.4	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; measurement error low; availability of prediagnostic serum samples (mean, 9.6 yr before diagnosis); measurement of several PFAS compounds. A key limitation was that single samples before diagnosis may not reflect exposure at crucial windows in cancer development. *Other strengths*: cases identified by reviewing medical records or death certificates; information on potential confounding variables collected through face-to-face interview and physical examination; high baseline participation. *Other limitations*: study population limited to retired workers; no information concerning tumour hormone-receptor status; no information on the likely completeness of diagnoses; cases identified by death certificate only (number not identified) would have an unknown diagnosis date; low exposure contrast. *Other comments*: ~10% lost to follow-up; does not mention how age-stratification was used to select comparison cohort; 90% of cases were postmenopausal; examined individual PFAS (6) and summed categories of PFCAs and PFSAs as well as sum of all PFAS.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Wen et al. (2022) USA Enrolment, 1999–2014/ follow-up, 1999–2015 (mortality) Cohort	11 747 from the NHANES cohort, a nationally representative cross-sectional survey of adults (aged ≥ 18 yr) followed for mortality through 2015 Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; a single serum sample collected before death was analysed for 12 PFAS, but not for isomers of PFOA or PFOS	All cancers combined	See Table S2.7[a]	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; considered mixtures of PFAS; measurement error low. Key limitations were that single samples before death may not reflect exposure at crucial windows in cancer development; unclear timing of blood sample relative to diagnosis/treatment. *Other strengths*: NDI linkage, nationally representative of the USA; relatively good control for potential confounders; adjustment for other PFAS. *Other limitations*: Short follow-up time (median, 81 mo); heterogenous outcome, representative of incidence only in the case of high fatality of cancers; use of volunteer-based population that may be healthier than the general population. *Other comments*: Analysed PFOA and PFOS separately as well as total PFAS, total PFAS excluding PFOA and total PFAS excluding PFOS to address mixture issues.
Goodrich et al. (2022) CA and HI, USA Enrolment, 1993–1996/ follow-up, from mid-1990s for > 20 yr Nested case–control	Nested within the MEC cohort, which is a community sample of 215 251 men and women aged 45–75 yr enrolled during 1993–1996 in HI and CA (primarily Los Angeles county) when responding to a 26-page postal questionnaire on mainly diet, demographic, and health issues Cases: 50 incident cases of non-viral HCC Controls: 50 individuals from the MEC, matched on age, sex, race/ethnicity, and study area	Liver (HCC)	See Table S2.5[a]	*Exposure assessment critique*: Key strengths were use of prediagnostic plasma PFAS measurements; plasma levels represent the combined exposure through all exposure pathways; measurement error low. A key limitation was that single samples before diagnosis (at recruitment) may not reflect exposure at relevant windows in HCC development.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Goodrich et al. (2022) CA and HI, USA Enrolment, 1993–1996/ follow-up, from mid-1990s for > 20 yr Nested case–control (cont.)	Exposure assessment method: quantitative plasma measurements; analytical method state-of-the-art; a single plasma sample was collected at recruitment, before HCC diagnosis, and analysed for 6 PFAS including PFOA and PFOS, but not for isomers of PFOA or PFOS			*Other strengths*: Exposure and outcome were ascertained independently and with high accuracy, with a median 7.2 yr between blood sample and diagnosis; BMI and diabetes status considered as potential confounders. *Other limitations*: Insufficient information on attrition, completeness of follow-up, statistical analysis; low exposure contrast (general population sample); did not account for mixture of PFAS in analysis of exposures.
Rhee et al. (2023b) CA and HI, USA Enrolment, 1993–1996; Follow-up through 2018 Nested case–control	Nested within the MEC cohort; see Goodrich et al. (2022) Cases: 428; all RCC cases identified as of 2018 in the MEC study with available pre-diagnostic serum sample; incident cases identified through linkage with the SEER HI registry and the CA state cancer registry Controls: 428 controls who were MEC participants alive at the time of the matched case diagnosis and matched 1:1 to cases on sex, race/ethnicity, study centre, age and date at serum collection, time of serum collection, and fasting status Exposure assessment method: quantitative plasma measurements; analytical method was state-of-the-art and included isomers of PFOA and PFOS; single plasma sample collected before or after (21%) RCC diagnosis; all were analysed for 11 PFAS, including PFOA and PFOS; separate analysis of linear and branched isomers of PFOS and PFOA was performed but only the summed results were reported.	Kidney (RCC)	See Table 2.2	*Exposure assessment critique*: Key strengths were the availability of pre-diagnostic sample for most participants, plasma levels represent the combined exposure through all exposure pathways; measurement error low. Key limitations were that single samples may not reflect exposure at crucial windows in RCC disease development; if RCC development alters ADME of PFAS, there could be possible differential exposure misclassification for samples collected after diagnosis (21%). *Other strengths*: Large sample size; consideration of multiple PFAS adjustment; stratification by race/ethnicity. *Other limitations*: Some stratified analyses by race/ethnicity had low statistical power. *Other comments*: pre-diagnostic sample collected between 1994–2006; in 1994, samples were collected only among participants selected to be included in case–control studies, then between 2001–2006, samples were taken from all survivors in the MEC cohort.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Purdue et al. (2023) USA Enrolment, 1988–2017/ follow-up, through 2018 Nested case–control	Nested within a cohort of active-duty US Air Force servicemen with ≥ 1 serum sample stored in the Department of Defence Serum Repository between 1988 and 2017; further eligibility criteria were no prior history of cancer 1990–2018 and age ≤ 39 yr Cases: 530 overall (187 with two samples); TGCTs diagnosed in the Department of Defence Cancer Registry Controls: 530 overall (187 with two samples); one control per case density-sampled with replacement among eligible US Air Force servicemen on active duty and cancer-free as of the case diagnosis date and matched on date of birth, race/ethnicity (seven groups), year entering military service, year of baseline serum sample collection, and year of second sample collection (if applicable) Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; stored serum samples were analysed for PFAS; in analyses of men with two samples, categories based on above or below the median at each time were evaluated	Testis	See Table 2.3	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; low potential for exposure misclassification; measurements of several PFAS compounds including PFOS and PFOA isomers; 2 repeated prediagnostic samples several years apart in a subset of the population; other military related PFAS exposures were considered (drinking-water). Key limitations were that for most participants only one serum measurement was available; no information on other exposures; no cumulative exposure metric and in particular inability to examine specific exposure windows in early life. *Other strengths*: Nested design; well characterized source population; large number of cases; serum samples obtained 0–19 yr before diagnosis; reasonable exposure contrast, analyses adjusted for other PFAS compounds, information on a range of covariates. *Other limitations*: Loss to follow-up (men leaving the military) and completeness of case ascertainment not quantified; large percentage (29%) excluded due to missing serum specimens; residual confounding by prenatal PFAS concentrations is an unresolved issue of potential importance; data on strong determinants of TGCT are lacking.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Madrigal et al. (2024) Finland Enrolment, 1986–2010/ follow-up, through 2016 Nested case–control	Nested in the Finnish Maternity Cohort, a national registry of women who donated serum during the first trimester of pregnancy (> 90% of pregnancies in Finland between 1983 and 2016) Cases: 400 cases were randomly selected from those diagnosed among women who donated serum for their first pregnancy and had a live, full-term birth delivered between 1987 and 2010, and who had no prior diagnosis of cancer at enrolment Controls: 400 controls individually matched on year of delivery (4–5 yr increments) and age at first birth (3 yr increments) Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; a single serum sample was collected ≥ 3 yr before diagnosis	Thyroid (papillary)	See Table 2.4	*Exposure assessment critique*: Key strengths were that prediagnostic serum sample levels were measured, which represent the combined exposure through all exposure pathways; all samples analysed in the same manner; measurement error low; selected Group 1 carcinogens were measured in the blood samples. A key limitation was that single sample collected during pregnancy may not reflect exposure at crucial windows in cancer development, although only minor misclassification of long-term exposure over a period of 5–8 yr, based on a simulation study (Annex 3). *Other strengths*: Analyses controlled for 19 PFAS as well as several PCBs, organochlorine pesticides, and PBDEs; the age of the cohort during follow-up included peak years of thyroid cancer incidence. *Other limitations*: Low-level exposure with small exposure contrast; controls were not matched on the exact year of delivery, but on increments of 4–5 yr, which might affect comparison of PFAS levels because of temporal trends in levels of PFAS; data on pre-pregnancy body mass index, a thyroid cancer risk factor, was largely missing; no information was available on medical or environmental exposure radiation.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Zhang et al. (2023) ATBC cohort: Finland, PLCO: USA ATBC: Enrolment, 1985–1988/follow-up, through 2011; PLCO: Enrolment, 1993–2001/follow-up, through 2010 Nested case–control	Two nested case–control studies, one within the ATBC study, the other within PLCO study (for PLCO see Shearer et al., 2021) ATBC is a randomized trial in White male smokers (aged 50–69 yr at recruitment) to evaluate the chemopreventive effects of alpha-tocopherol and beta-carotene on lung cancer (*n* = 29 246) Cases: 251 from ATBC and 360 from PLCO; cases from the ATBC study were male smokers who participated in a prevention trial who developed pancreatic ductal adenocarcinoma identified in the Finnish Cancer Registry; cases from the PLCO study were men and women ascertained by annual mail-in surveys, cancer registries and/or the NDI Controls: 251 from ATBC, 360 from PLCO; in both cohorts, controls were individually matched on age and date of blood draws, and sex; there was additional matching on race in PLCO only Exposure assessment method: PFOA and PFOS levels were measured in serum using a non-targeted method; a single serum sample was collected before diagnosis	Pancreas (ductal adenocarcinoma)	See Table S2.5[a]	*Exposure assessment critique*: Key strengths were that prediagnostic serum levels represent the combined exposure through all exposure pathways; measurement error low; long follow-up time (median time between blood draw and diagnosis was 9–12 yr). Key limitations were that non-targeted analyses prevented comparison of sample concentrations across studies; single samples may not reflect exposure at crucial windows in cancer, although only minor misclassification of long-term exposure over a period of 5–8 yr, based on a simulation study (Annex 3); exposure assessment relied upon relative quantification of PFOA and PFOS, but relative measures have correlated well with targeted absolute concentration measurements. *Other strengths*: Information on potential confounders collected by trained staff through questionnaires and for height and weight in the ATBC Study; excellent case ascertainment. *Other limitations*: Low-level exposure with small exposure contrast; included only White men who smoke. *Other comments*: ATBC study participants were aged 50–69 yr at baseline and PLCO participants were aged 55–74 yr at baseline; the two nested case–controls were analysed separately.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
van Gerwen et al. (2023) Mount Sinai (NY), USA Enrolment, 2008–2021 Nested case–control	Nested within Bio*Me*, a medical record-linked biobank within the Institute for Personalized Medicine at the Icahn School of Medicine at Mount Sinai, including residents of New York City and the larger metropolitan area Cases: 88 adult patients diagnosed with thyroid cancer according to ICD codes 193 (9th revision) and C73 (10th revision) Controls: 88 healthy (non-cancer) participants, pair-matched on sex, age (± 5 yr), race/ethnicity, BMI, smoking status (ever/never), and calendar year of sample collection Exposure assessment method: PFOA and PFOS levels were measured in plasma using an untargeted analytical method; a single plasma sample was collected, only 35% of these were collected > 1 yr before diagnosis; all samples were analysed for 8 PFAS including PFOA and PFOS; analysed linear and branched isomers of PFOS, but not PFOA separately	Thyroid	See Table 2.4	*Exposure assessment critique*: Key strengths were that plasma levels represent the combined exposure through all exposure pathways; plasma samples were collected ≥ 1 yr before diagnosis for a subset, albeit small, of the cases. Key limitations were the use of untargeted analysis with semiquantitative measurements, so concentrations not known or comparable with other studies (however, participant exposures can be ranked); single samples may not reflect exposure at crucial windows in cancer development, especially since 65% samples were collected < 1 yr before diagnosis; if thyroid cancer development alters ADME of PFAS there could be possible differential exposure misclassification for those samples collected after diagnosis. *Other strengths*: Source population represents a diverse racial/ethnic and socioeconomic population; availability of histologic data for the cases; analyses adjusted for age, sex, race, and body mass index, and sample storage time, and, for some analyses, adjustment of analyses of specific PFAS compounds for other PFAS compounds. *Other limitations*: Small sample size, particularly for cases with plasma collected > 1 yr before diagnosis; short follow-up time; the possibility of detection bias, given cases were identified in a hospital and ambulatory practice setting (however, this was minimized by selection of controls from the same network, and patients and practitioners were unaware of PFAS measurements).

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
van Gerwen et al. (2023) Mount Sinai (NY), USA Enrolment, 2008–2021 Nested case–control (cont.)				*Other comments*: Analyses were conducted for all thyroid cancer cases, cases whose plasma collection was < 1 yr before diagnosis (cross-sectional group) and cases whose plasma collection was ≥ 1 yr before diagnosis (longitudinal group).
Winquist et al. (2023) 20 US states Enrolment 1998–2001; follow-up through 30 June 2015 Case–cohort	Case–cohort design within the CPS-II Lifelink Cohort (*n* =39 371); participants in the CPS-II Nutrition cohort (recruitment, 1992–1993) who were alive and agreed to a blood sample collection between 1998 and 2001 Cases: 3762 incident cases with a first cancer diagnosis of kidney, bladder, breast (females only), prostate (males only), or pancreatic cancer, leukaemia, or lymphoma, detected through self-report or NDI linkage and verified through medical records review or cancer registry Controls: 999; a sex-stratified simple random sample of 499 women and 500 men (~3% of the eligible cohort); stratification sampling was to ensure an adequate number of subcohort participants in sex-specific analyses (for breast and prostate cancers) Exposure assessment method: quantitative plasma measurements; analytical method was state-of-the-art, except no branched isomers of PFOA and PFOS were analysed; a single plasma sample was collected before diagnosis	Kidney (all combined) Kidney (RCC) Urinary bladder Prostate Breast (post-menopausal) Pancreas Haematological malignancies	See Table 2.2 See Table 2.2 See Table 2.2 See Table 2.3 See Table 2.4 See Table S2.5[a] See Table S2.6[a]	*Exposure assessment critique*: Key strengths were that prediagnostic plasma levels represent the combined exposure through all exposure pathways; measurement error low. A key limitation was that single samples may not reflect exposure at crucial windows in cancer development, although only minor misclassification of long-term exposure is expected over a period of 5–8 yr, based on a simulation study (Annex 3). *Other strengths*: Large number of cases. *Other limitations*: Survivor cohort with blood collected from persons mostly over aged 65, thus the study would not include persons who may have had PFOA- or PFOS-related cancer developed earlier in life, resulting in downward bias.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Vieira et al. (2013) OH and WV, USA 1996–2005 (incidence) Case–control	Cases: 25 107 index cancer cases were retrieved from cancer registries covering a community sample with relatively high exposure to PFOA because of contamination of drinking-water from the Parkersburg (WV) polymer-production plant; 18 different cancers were analysed Controls: number varied; for each cancer site evaluated, controls were cases of cancer for all other sites, with the exclusion of four cancers of a priori interest (kidney, testis, pancreas, and liver) that have been associated with PFOA in studies in experimental animals or humans Two case–control studies are described, one including both WV and OH cases (first), the other only OH cases (second)	Kidney (urinary pelvis/ UUT) Urinary bladder Testis Prostate Breast Liver Pancreas Colon and rectum Thyroid Brain Leukaemia, multiple myeloma, NHL, melanoma Lung	See Table 2.2 See Table 2.2 See Table 2.3 See Table 2.3 See Table 2.4 See Table S2.5[a] See Table S2.5[a] See Table S2.5[a] See Table 2.4 See Table S2.6[a] See Table S2.6[a] See Table S2.6[a]	*Exposure assessment critique*: Key strengths were the availability of data on measured serum levels for a large number of residents of contaminated water districts, permitting analyses by approximate levels of exposure in each water district; exposure reconstruction for OH provided detailed exposure assessment; any misclassification is likely to be non-differential; the second case–control study based in OH estimated serum level for individuals based on a model shown to be able to predict well observed levels for 30 000 residents of the six contaminated water districts at one point in time (2005/2006, Spearman correlation 0.71). Key limitations were the assignment of an ecological exposure (by water district) in the first case–control study; the use of estimated individual serum levels in the 2nd case–control study based in OH, and the somewhat arbitrary assumption in that second study that the estimated serum levels 10 yr before case diagnosis were the most relevant, as well as the assumption that cases and controls had remained in the same residence for 10 yr; no other exposures (except smoking) were considered.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology)	Exposure category or level	Comments
Vieira et al. (2013) OH and WV, USA 1996–2005 (incidence) Case–control (cont.)	Exposure assessment method: address at diagnosis was used to assign PFOA exposure; individuals from OH (about one third of the sample) were geocoded whereas individuals from WV were assigned exposure on the basis of geographical unit; water-district PFOA levels were available for all individuals; for individuals from OH, PFOA serum values could be estimated on the basis of exposure models (Shin et al., 2011a, b) A five-level exposure variable was created			*Other strengths*: The large number of incident cancers from cancer registries; the reasonably large number of exposed cases in the contaminated water districts for many specific cancers (although for analyses of rarer cancers by categories of exposure small numbers were sometimes an issue); the large exposure contrasts between water districts as well as within individuals in the second case–control study. *Limitations*: Fairly few potential confounders used in the analyses; the use of controls with cancer was a less marked limitation.

ADME, absorption, distribution, metabolism, and excretion; AL, Alabama; approx., approximately; APFO, ammonium perfluorooctanoate; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention; BMI, body mass index; CA, California; CHDS, Offspring in the Child Health and Development Studies; CI, confidence interval; CNS, central nervous system; CPS-II, Cancer Prevention Study II; E3N, Etude épidémiologique auprès de femmes de la Mutuelle générale de l'Education nationale; HCC, hepatocellular carcinoma; HI, Hawaii; ICD, International Classification of Diseases; IQR, interquartile range; JEM, job-exposure matrix; MEC, Multiethnic Cohort; MHT, menopausal hormone therapy; MN, Minnesota; mo, month(s); NDI, National Death Index; NHANES, National Health and Nutrition Examination Survey; NHL, non-Hodgkin lymphoma; NY, New York; OH, Ohio; PCB, polychlorinated biphenyl; PBDE, polybrominated diphenyl ethers; PFCA, perfluoroalkyl carboxylic acid; PFDA, perfluorodecanoic acid; PFDoA, perfluorododecanoic acid; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptanesulfonic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFSA, perfluorosulfonic acid; PFTrA, perfluorotridecanoic acid; PFUnA, perfluoroundecanoic acid; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; POSF, perfluorooctanesulfonyl fluoride; RCC, renal cell carcinoma; SEER, Surveillance, Epidemiology, and End Results; SMR, standardized mortality ratio; SNP, single-nucleotide polymorphism; TFE, tetrafluoroethylene; TGCT, testicular germ cell tumour; UK, United Kingdom; USA, United States of America; WI, Wisconsin; WV, West Virginia.

[a] Tables S2.5, S2.6, and S2.7 are available in Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636.

282 ng/mL in workers with all work, some work, and no work, respectively, in PFOA production) versus 4.5 ng/mL in blood donors in the same area (Raleigh et al., 2014). The toxicokinetics of PFOA were not considered when modelling the cumulative exposure metric, which was based solely on air concentration and duration of exposure.

Vital status was ascertained by the National Death Index (NDI) until the end of 2008 for 99.3% of the population, and cases of incident cancer were identified by linkage to the Minnesota Cancer Surveillance System and the Wisconsin Cancer Reporting System; reporting of cancer had been mandatory since 1988. The case capture of the cohort was estimated to be about 85%, and higher for highly exposed workers (individual out-of-region migration data were not available).

Standardized mortality ratios (SMRs) for cancers of the prostate, pancreas, urinary bladder, female breast, kidney, and liver were estimated from 1960 through 2008 using the entire Minnesota population as the referent.

The follow-up period for cancer incidence was from 1 January 1988 until the end of 2008. In total, 665 incident cancers of the prostate, kidney, pancreas, bladder, liver, breast, testis, and thyroid were identified. [Estimates for testicular, thyroid, and liver cancer were not provided.] Relations between cancer-specific risk and time-dependent cumulative APFO exposure were estimated by Cox regression, with adjustment for sex, year of birth, and age.

[The Working Group noted several strengths associated with this primarily male cohort, including individual assessment of cumulative air exposure, with some evidence that this exposure metric was correlated with serum concentration; limited and minimal co-exposure to tetrafluoroethylene (TFE); a relatively large number of some incident cancers in the exposed cohort (prostate, bladder, breast); a reference population with socioeconomic characteristics that were similar to those of the exposed population; and a long follow-up period. Limitations were the lack of information on dermal exposure; the fact that this was a survivor cohort (in which follow-up for cancer incidence began many years after the start of follow-up) with the potential for downward healthy-worker survivor bias; the lack of data on workers who left Minnesota or Wisconsin (although the out-migration of the workers with higher exposure was similar to that of the reference workers); and the small numbers of some cancers (kidney, pancreas, liver, testes, and thyroid). Risk estimates were most probably biased towards the null, because measurement error is likely to be non-differential. Finally, a discrepancy was noted between the description of the adjustment for Cox regression models in the text (age, year of birth, and sex) and that in the tables (age and year of birth only).]

2.1.2 PFOS-production workers (Decatur, Alabama, USA)

Alexander et al. (2003) studied a cohort of 3512 production workers who had been exposed to perfluorooctanesulfonyl fluoride (POSF), which is degraded or metabolized to PFOS, at a plant in Decatur, Alabama, USA, that produced speciality films and fluorochemicals in two different facilities. All the cohort members had worked at the plant between 1961 and 1997. The study was limited to those who had worked for ≥ 1 year at the plant (n = 2083; men, 83%). Follow-up for mortality was conducted between 1961 and 1998. Exposure was estimated using serum concentration of PFOS, based on a sample of 232 employees randomly selected for serum sampling in 1998, with 80% participation (chemical plant, n = 126; film plant, n = 60). The authors noted that there was also exposure to PFOA in this subsample, at slightly lower levels; serum PFOA concentrations correlated well with serum PFOS concentrations. PFOA manufacturing at this plant started in 1998; this was the same year that serum samples were

collected, but there may have been incidental exposure previously. Chemical plant workers had high serum concentrations of PFOS (geometric mean, 0.9 ppm [900 μg/L]), whereas film plant workers had lower concentrations (geometric mean, 0.1 ppm [100 μg/L]). On the basis of these measurements, a job-exposure matrix (JEM) was developed for all workers, jobs being classified into three exposure groups. The groups were defined as having: (i) no or minimal workplace exposure to PFOS (e.g. jobs in film plant); (ii) low potential workplace exposure to PFOS (e.g. engineers, quality control technicians, environmental health and safety workers, administrative assistants, managers); or (iii) high potential workplace exposure to PFOS (e.g. cell operators, chemical operators, maintenance workers, mill operators, waste operators, crew supervisors). Cumulative exposure (duration multiplied by intensity of exposure) was estimated after accounting for changing jobs over time, based on assigned weights of 1, 3, and 10, respectively, for jobs in the three exposure groups. The SMRs were calculated by comparing the mortality of all workers with that of residents of Alabama; in further analyses, each worker subgroup was compared with the Alabama population. Of the 2083 workers included, 47% (982) had worked at some time in jobs in which exposure to PFOS was considered high, 14% (289) worked in low-exposure areas, but never held a job in the high-exposure areas, and 812 (39%) were considered to have no or minimal workplace exposure to fluorochemicals. In the cohort, 145 deaths were identified (including 39 deaths from cancer); there were 65 deaths in the high-exposure group, 27 in the low-exposure group, and 53 in the non-exposed group. Deaths were ascertained using the NDI and the US Social Security Death Index. There were 3 deaths from bladder cancer, which was the cause of death that showed the highest excess, compared with the reference population.

Alexander and Olsen (2007) further followed the PFOS cohort studied by Alexander et al. (2003), focusing on bladder cancer. A postal questionnaire sent to all living current and former employees of the facility in 2002 (n = 1895) to identify cases of incident bladder cancer was returned by 1400 cohort members (response rate, 74%). The underlying cause of death (ascertained through the NDI) from death certificates for 188 deceased workers was used to identify bladder cancer decedents. The analysis drew on data from questionnaire respondents and from decedents (1588 cohort members); 11 cases of bladder cancer were included (6 identified in the questionnaire and 5 identified using death certificates). Cumulative exposure was analysed for the same three groups as before (none, low, high). Serum concentrations of PFOS were estimated from a subsample, as described in Alexander et al. (2003). Job categories assigned to no, low, and high exposure had geometric mean serum PFOS concentrations of 0.11–0.29 μg/mL, 0.39–0.89 μg/mL, and 1.30–1.97 μg/mL, respectively (slightly different from the values reported in Alexander et al., 2003). The incidence of bladder cancer was analysed using national rates from 1970 to 2002, adjusted for age, sex, and calendar year. Internal analyses used Poisson regression, adjusting for age and sex.

[The Working Group noted that this cohort, the same in both studies, had large exposure contrast, and that some serum measurements were available, although they were not used in the exposure assessment. Cancer incidence data were published by Alexander and Olsen (2007), who also used internal comparisons. Limitations included the small number of observed cancers. Alexander et al. (2003) limited their study to mortality, reporting a large excess for bladder cancer on the basis of only 3 cases, and analyses only against national rates. Alexander and Olsen (2007) studied only 11 cases of incident bladder cancer (with no bladder cancer excess). Another limitation was co-exposure to PFOA, which was measured at somewhat similar levels to those of PFOS in a sample of employees in 1998

(Alexander and Olsen, 2007), and correlated with PFOS serum concentrations – although PFOA was not produced until 1998 and earlier levels were probably lower. Both studies were limited by sparse data on smoking, but there were fewer concerns about confounding by smoking in the study by Alexander and Olsen (2007) because the authors conducted an internal analysis (which is less subject to confounding) as well as external comparisons. Another limitation was the failure to adjust for other PFAS in the plant, and in general for other workplace exposures in a chemical plant. The Working Group also noted that perfluorooctanesulfonyl fluoride (POSF), the predecessor of PFOS used in this plant, is itself a reactive compound and may be toxic.]

2.1.3 Polymer-production workers in Parkersburg (West Virginia, USA)

The polymer-production plant in Parkersburg, West Virginia, USA, was a facility producing several types of polymer from a wide variety of monomers and other chemicals; it began operating in 1948. Workers in the plant, especially those involved in various activities related to the production of certain polymers or copolymers, were exposed to PFOA. Steenland and Woskie (2012) conducted an updated investigation of mortality in a previously studied cohort of plant workers (Leonard et al., 2008). Briefly, this cohort included 6027 workers who were employed at the plant for ≥ 1 day between 1948 and 2002. The analyses by Steenland and Woskie (2012) focused on 5791 workers (women, 19%) with sufficiently detailed work histories for PFOA exposure estimation and non-missing values for date of birth. Workers were followed for mortality from 1952 through 2008; deaths were identified using the NDI (for 1979 or later), or from death certificate data (from the US Social Security Administration and state death certificates) for earlier years. For the PFOA exposure assessment, the investigators used serum PFOA concentrations determined from 2125 blood samples collected from 1308 workers between 1979 and 2004 to produce regression models estimating serum PFOA levels for eight different combinations of job category and job group over time. Comparisons of modelled PFOA levels with the serum concentrations measured for this study population demonstrated high agreement (Spearman rank correlation coefficient, $\rho = 0.8$) (Woskie et al., 2012). The SMRs for mortality from cancer overall and for specific cancers – liver, pancreas, lung, breast, prostate, testis, kidney, and bladder, and mesothelioma, non-Hodgkin lymphoma (NHL), and leukaemia – were calculated for workers in the cohort (overall and by quartiles of cumulative estimated PFOA exposure) compared with workers at other plants in the Appalachian region and managed by the same company, as well as with reference rates for the general US population. A total of 1084 deaths were recorded during follow-up (with a mean length of follow-up of 30 years). In a subsequent investigation for a subset of 3713 workers, who were all included in both the occupational cohort and the C8 Science Panel study (described in Section 2.1.5) by Steenland et al. (2015), the incidence of selected cancers for which there were 20 or more cases during follow-up (melanoma and cancers of the bladder, colorectum, and prostate) was evaluated for workers aged from 20 years or from the year 1951 (whichever was later) until the period (2008–2011) when interviews were conducted with workers or their next of kin (the latter making up 6% of the interviews included in the analysis). Analyses focused on self-reported cancers or cancers reported by the next of kin that were validated by a review of medical records (355 valid cases). Cox proportional hazards regression analyses were used to estimate the risks of developing specific cancers in relation to quartiles of estimated cumulative PFOA exposure with age, as the underlying

timescale, with adjustment for sex, race, education, body mass index (BMI), and time-varying smoking and alcohol consumption.

[The Working Group noted several strengths of this study, including the detailed historical exposure assessment and the ability to evaluate associations between PFOA and cancer in a population exposed to levels much higher than those in the general population. The estimated average annual serum PFOA concentration in the cohort overall (mean, 350 ng/mL) was nearly two orders of magnitude higher than the measured serum PFOA concentration (geometric mean, 3.9 ng/mL) reported in the US National Health and Nutrition Examination Survey (NHANES) in 2003–2004 (Calafat et al., 2007). The modelled PFOA exposure levels were highly correlated with serum PFOA concentrations in the more than 2000 measurements available for this study population (Spearman correlation, 0.8), indicating a valid model. Exposure to PFOS or other PFAS was not characterized in the cohort but was considered likely to be low, given the nature of the polymer-production work at the plant at the time of the study. Although some processes in the production of fluoropolymers involve TFE (classified by IARC as *probably carcinogenic to humans*, Group 2A; IARC, 2016), the Working Group noted that, owing to its high volatility and explosive potential, processes involving TFE were conducted in a separate area of the plant with limited access and that exposure control measures (e.g. closed systems) were used to prevent emissions of this gas. Therefore, workers' exposure to TFE in the plant was probably minimal during normal operations.

The use of a reference group of workers from other plants in the same region was also a strength of the mortality analysis, in terms of addressing the potential downward bias of the risk estimates because of the healthy-worker effect. However, exposures to other potential carcinogens were not assessed for the reference workers; if such exposures were higher or more prevalent in the reference group, or both, then the resulting risk estimates for some cancers could have been biased towards the null. The Working Group noted that mortality from mesothelioma was elevated in the cohort (on the basis of 6 deaths among the PFOA-exposed workers), suggesting possible exposure to asbestos in this population. However, the potential for confounding by asbestos exposure in analyses for other cancer sites of particular interest with respect to PFOA was considered likely to be low. Other limitations of the mortality analysis included the inability to evaluate some cancers because of the small numbers of deaths, in particular for cancers among women (given the small number of female workers included in the cohort) and for cancers that are less likely to be fatal (e.g. testicular cancer).]

[The Working Group did not consider the overlap between the subset of workers included in both the investigation of cancer incidence by Steenland et al. (2015) and the C8 Science Panel investigation (Barry et al., 2013) to be a major limitation, given that workers comprised a small proportion of the population in the latter study. In addition to the strengths of the PFOA exposure assessment already noted, a strength of the study by Steenland et al. (2015) was the ability to control for established cancer risk factors (e.g. BMI, smoking, and alcohol consumption) that might have confounded the associations between PFOA and specific cancers. The use of medical records to confirm self-reported cancer diagnoses probably reduced non-differential outcome misclassification, thereby potentially improving the ability to detect any true associations with PFOA. However, this approach might also have resulted in the underascertainment of cancer cases. Taken together with the relatively small sample size, this might have further limited the statistical power for analyses of specific cancers, and there were too few cases to evaluate cancers of the kidney and testis. Finally, the Working Group noted that the subset of workers in this analysis were less likely to have died than those

who were not included, and also differed with respect to several demographic characteristics (e.g. those who were included were younger and more likely to be female), raising the possibility of selection bias if the exposure–response patterns differed between the workers who were included and those who were excluded. The potential effect of such selection bias (if present) on the direction and magnitude of the reported risk estimates was unclear.]

2.1.4 Diet, Cancer, and Health Cohort

Eriksen et al. (2009) studied data collected for 57 053 participants enrolled in a prospective Danish cohort between 1993 and 1997 – the Diet, Cancer, and Health Cohort. Approximately 160 000 potential participants were recruited from the general population, with data accessible from a national database, in two counties in Denmark (Aarhus and Copenhagen) (Tjønneland et al., 2007); of these, 57 053 agreed to participate. Participants were Danish citizens aged 50–65 years with no previous cancer diagnosis at enrolment. Plasma concentrations of PFOA and PFOS were measured at baseline using liquid chromatography and tandem mass spectrometry (LC-MS/MS). Virtually all samples had concentrations above the limits of detection, and 50 random samples were measured twice, with good agreement between measurements. Cancer incidence data for the cohort were available from the Danish Cancer Register. Investigators identified 1240 cases of cancer of the prostate, bladder, pancreas, or liver (in 1111 men and 129 women), diagnosed in 1993–2006, with a follow-up period of 0–12 years (median, 7 years) after baseline. Cancers of the prostate, bladder, pancreas, and liver (713, 332, 128, and 67 cases, respectively) were analysed in relation to baseline plasma concentrations of PFOA and PFOS. A subcohort of 680 men and 92 women who were randomly selected and had not had a diagnosis of cancer at the end of the follow-up period were used as controls for the cancer cases, according to a case–cohort design. Median plasma levels (5th and 95th percentiles) of PFOA and PFOS in those not later diagnosed with cancer were 6.6 (3.0–13.0) ng/mL and 34.3 (16.2–61.8) ng/mL, respectively. Information on potential confounders (BMI, smoking, occupation, education, alcohol intake, diet) was collected using a questionnaire and differed according to cancer type. Analyses were conducted by quartile of PFOA or PFOS concentration. Linearity was first evaluated using spline models; where there was no significant deviation from linearity, a linear trend was assessed using a continuous variable for plasma PFOA or PFOS concentration.

[The strengths of this study included the use of a large cohort with numerous incident cancers identified using a reliable cancer registry (n = 1240), the measurement of plasma PFOA and PFOS concentrations at baseline, good control for confounders (e.g. age, sex, BMI, detailed smoking data, diet), internal comparisons, and little loss to follow-up. Limitations were a relatively low exposure contrast in a population with background exposure levels, the characterization of exposure on the basis of a single measurement at enrolment, and a somewhat limited period of follow-up.]

2.1.5 C8 Science Panel study

The C8 Science Panel conducted a cohort study of community residents and workers exposed to PFOA (C8 is a synonym of PFOA) from a fluoropolymer-production plant in the Mid-Ohio Valley on the border of West Virginia and Ohio, USA (Barry et al., 2013). Between the 1950s and the early 2000s, PFOA was released from the plant in air emissions and as liquid and solid waste, contaminating local public water supplies and private wells. A settlement from a class action lawsuit initially funded a large community health study known as the C8 Health Project, which was conducted in 2005–2006

(Frisbee et al., 2009). The C8 Science Panel investigated a cohort of adults aged ≥ 20 years enrolled in the C8 Health Project (Winquist et al., 2013), as well as individuals who were employed in the plant and included in an occupational cohort, as described in Section 2.1.3; analyses were restricted to individuals who had completed at least one subsequent survey between 2008 and 2011 and who had retrospective environmental or occupational PFOA exposure estimates. These surveys solicited detailed information on demographic and health characteristics; an extensive residential history was included. Of the 32 254 individuals included in the analytical cohort, 28 541 were community cohort participants and 3713 had worked at the plant. For community participants, annual estimates of serum PFOA concentrations were calculated using an environmental fate and transport model for each year of life between 1952 and 2011, as described in Shin et al. (2011a, b). For the workers, estimates of occupational PFOA exposure were calculated as described in Section 2.1.3 and combined with environmental exposure estimates. Barry et al. (2013) conducted an investigation of cancer incidence among cohort participants, who were followed up from age 20 years onwards for an average of 33 years. For this analysis, cancers reported by participants in the surveys conducted in 2008–2011 were confirmed by consulting state cancer registries in Ohio and West Virginia or a review of medical records; a total of 2507 validated cancer diagnoses were identified and included in the statistical analyses. Proportional hazard regression models were used to estimate the risk of developing specific cancers at each year of age in relation to estimated serum PFOA concentrations (modelled per 1-unit increase on the natural log scale) both up to the time of diagnosis or censoring (unlagged analysis) and for estimated exposures 10 years in the past (lagged analyses). Exposure–response associations based on quartiles of estimated PFOA concentrations were also reported for kidney, testicular, and thyroid cancers. All models were run with age as the timescale and were adjusted for sex, education, 5-year birth year period, and time-varying smoking and alcohol consumption. Data from this study and a nested case–control study of Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO study) (Shearer et al., 2021) were combined for a pooled analysis of renal cell carcinoma (RCC) (Steenland et al., 2022).

[The Working Group considered this cohort study to be highly informative, in light of several important features and strengths. With 32 254 participants, it is, to the knowledge of the Working Group, the largest cohort to evaluate cancer risk in community members and workers with high exposure to PFOA. Based on measurements of serum PFOA concentration from the C8 Health Project in 2005–2006, exposure levels were found in the investigation of Barry et al. (2013) to be considerably higher in the overall cohort, compared with the general US population (medians of 26.1 ng/mL and 4.0 μg/L [ng/mL], respectively) (Winquist et al., 2013). Among C8 Health Project participants (Frisbee et al., 2009), serum concentrations of perfluorohexanesulfonic acid (PFHxS) and perfluorononanoic acid (PFNA) were more modestly elevated, relative to the general US population (geometric means were 39% and 73% higher, respectively, compared with 2003–3004 US NHANES data), and serum PFOS concentrations were similar to those observed in the general population. A particular strength of the C8 Science Panel study was the detailed characterization of estimated serum PFOA concentrations from 1952 or the participant's year of birth (whichever was later) through 2011; modelled serum PFOA concentrations corresponded well (Spearman correlation, 0.71) with measured serum concentrations for the cohort in 2005–2006 (Winquist et al., 2013). This assessment of PFOA enabled analyses of exposure–response associations with cancer incidence in both unlagged and lagged analyses. The adjustment for established cancer risk

factors (e.g. smoking, alcohol consumption) that might have confounded the associations between PFOA and specific cancers was also a strength of the analyses in this cohort. A strength of the outcome ascertainment was the validation of self-reported cancer cases by linking with Ohio and West Virginia cancer registries and medical chart reviews; this approach could have reduced the potential for attenuation of risk estimates, owing to non-differential disease misclassification. Finally, there were high rates of participation in the target populations of community members in the C8 Health Project and workers in the occupational cohort (81.5% and 72.9%, respectively) (Winquist et al., 2013), reducing the likelihood of selection bias affecting the direction or magnitude of the observed associations. The Working Group also noted several limitations of this study. Direct measurements of serum PFOA concentrations were available only in 2005–2006; this might not have reflected PFOA exposure during etiologically relevant time periods when data on measured concentrations were not available. However, as noted previously, modelled estimates and measurements of serum PFOA concentrations were highly correlated, and any exposure misclassification would be non-differential and more likely to bias risk estimates towards the null. Also, given the design of the study as a survivor cohort, community members and workers who died before the cohort enumeration would not have been included, resulting in the potential underascertainment of cancers with a high rate of fatality in this population. However, given that PFOA exposure was considered to be unlikely to be related to survival time, the effect of this aspect of the study design on the resulting risk estimates was considered likely to be minimal (Barry et al., 2015). Despite the large sample size, the study had relatively limited statistical power to detect associations with some less common cancers, and for prospective analyses of cancer risk (i.e. for cases diagnosed after enrolment in the C8 Health Project). Finally, the Working Group noted that the cancer cases included in this study probably overlapped with those included in the study by Vieira et al. (2013), although the case ascertainment approaches differed for the two studies. The study of Barry et al. (2013) included self-reported cases of cancer that were confirmed either by linking with West Virginia or Ohio cancer registries or by medical record abstraction, including those diagnosed in other states or before the availability of data from state registries. In contrast, the study of Vieira et al. (2013) considered cancer cases from 13 counties in West Virginia and Ohio (including both contaminated water districts and other adjacent areas without water contamination) that were identified from West Virginia and Ohio cancer registries for the years 1996–2005. However, the degree of overlap of the cases included by Barry et al. (2013) and Vieira et al. (2013) was unknown.]

2.1.6 Pooled cohort of international tetrafluoroethylene workers

This pooled cohort of international TFE workers included workers employed at one or more of six TFE synthesis and polymerization sites in North America and Europe (Gendorf, Germany; Dordrecht, the Netherlands; Spinetta Marengo, Italy; Thornton-Cleveleys, UK; Bayonne, New Jersey, USA; Parkersburg, West Virginia, USA) that, at the time of the study, comprised the entire population of workers in TFE manufacture in Europe and the USA (Consonni et al., 2013). TFE is a flammable and explosive gas and is mainly used in closed systems as a monomer in the production of fluorinated polymers, including polytetrafluoroethylene (PTFE), which is widely used in consumer products such as waterproof and breathable membranes for clothes and as coatings on carpets. APFO, the ammonium salt of PFOA, is used as a polymerization aid in PTFE production.

Excluding 778 female workers and 122 male workers with missing data, the cohort included

5879 male workers who, for ≥ 1 day (in three plants), 6 months (in one plant), or 1 year (the other plants), were employed at a TFE-manufacturing facility in 1950–2002. Enrolment of eligible workers was based on company rosters. [Completeness of enrolment of eligible workers was not reported.]

The synthesis and polymerization of TFE entail potential exposure to the TFE monomer and to PFOA, which is released from its ammonium salt (APFO) during production. Individual semiquantitative levels of work-related exposure to TFE and PFOA were estimated using expert judgement to create a plant- and job-specific exposure matrix with yearly estimates (in arbitrary units) of exposure, declining by 10% for each decade from the start of TFE production until 2002 (Sleeuwenhoek and Cherrie, 2012). Only a few measurements of TFE air concentrations at the various plants were available to assist the exposure assessment (Sleeuwenhoek and Cherrie, 2012). The number of workers who had ever been exposed to TFE was 4773 (81.2%), while 1081 (18.4%) workers had never been exposed. Among workers who had ever been exposed to TFE, 4205 were also exposed to APFO. All workers exposed to APFO were also potentially exposed to TFE, mainly through accidental leaks, from opening of autoclaves, and from decomposition of PTFE. There was a high correlation between TFE and PFOA exposure intensities (Sleeuwenhoek and Cherrie, 2012), based on arbitrary units (Spearman correlation, 0.72). At two of the plants (Gendorf, Thornton-Cleveleys), previous exposure to vinyl chloride monomer might have occurred. No information was available on occupational exposure to agents known to promote the development of leukaemia. The largest TFE-production site (Parkersburg, West Virginia, USA) accounted for the largest number of unexposed workers.

Ascertainment of vital status (complete for 98.8% of the study population) and, where appropriate, cause of death was determined by epidemiology units at the company level (UK, USA), by university epidemiology departments (Germany, the Netherlands), or by local health units (Italy), through record-linking procedures or individual follow-up. [Record-linking procedures are expected to give a higher degree of completeness.] The mortality follow-up period was 1950–2008. Causes of death were recorded from death certificates according to the International Classification of Diseases (ICD) ICD-9 or ICD-10 classification. The mean values were 55 years for attained age, 9.2 years for duration of exposure to TFE, and 23 years for time since first exposure to TFE. For selected cancers, SMRs compared with national data were provided for ever APFO-exposed ($n = 4205$) and by cumulative APFO exposure, divided into four levels, among ever TFE-exposed and among three categories (low, medium, high) of cumulative TFE exposure ($n = 4773$). There were 534 deaths among men who had ever been exposed to APFO, of which 159 deaths were caused by cancer.

[The cohort included all TFE-production and polymerization sites worldwide at the time of the study and benefited from near-complete follow-up. Limitations were mainly related to the semiquantitative exposure assessment, with only a few TFE and no PFOA measurements available, no validation of estimated exposures by measurement, a low statistical power to detect less common cancers, and a high correlation between potential exposure to TFE monomer (classified as *probably carcinogenic to humans*, Group 2A; IARC, 2016) and PFOA. However, exposure to TFE among workers at the Parkersburg facility was considered very unlikely for the vast majority of workers, because processes involving TFE were conducted in a separate area of the plant with limited access, and strict hygiene-control practices (e.g. closed systems) were used to prevent emissions of this highly flammable and explosive compound. Moreover, possible exposure to other occupational and non-occupational carcinogens was not accounted for.]

2.1.7 Danish National Birth Cohort

The Danish National Birth Cohort was recruited for a nationwide cohort study that included data on about 100 000 pregnancies that occurred from 1996 through 2002 (Olsen et al., 2001). Approximately 50% of Danish women who were pregnant during this period were invited to participate when consulting their general practitioner during their first pregnancy visit, usually at weeks 6–12 of gestation; of those invited, about 60% agreed to participate in the cohort. Study participants completed questionnaires on lifestyle factors and environmental exposures, including diet, body size, alcohol intake, and smoking history, during a computer-administered interview at two time points during pregnancy as well as at two time points after pregnancy (6 and 18 months after delivery). Blood samples were collected once during the first trimester and once during the second trimester of pregnancy; cord blood was also collected at delivery.

A nested case–control study was designed to evaluate serum PFAS from blood samples collected during the first trimester of pregnancy in relation to risk of premenopausal breast cancer in mothers recruited to the Danish National Birth Cohort. Linkage to a nationwide cancer registry was used to identify 250 women who had received diagnoses of premenopausal breast cancer, with follow-up until 2010. These 250 women with breast cancer were matched to 233 randomly selected controls, with frequency-matching by age and limitation to those who were nulliparous (Bonefeld-Jørgensen et al., 2014). [The Working Group interpreted this nulliparity restriction to refer to women who, at the time of their blood sample during pregnancy, had not had a previous live birth.] In a follow-up analysis focused on gene–environment interaction, 72 of the original cases included as part of the study of Bonefeld-Jørgensen et al. (2014) were excluded because they had been withdrawn from the Danish National Patient Register for unknown reasons, resulting in a case group of 178, with a control group of 233 (Ghisari et al., 2017). [The Working Group noted that the removal of the 72 cases probably had no implication on the matching, since the age distribution among cases and controls appeared to be balanced after this removal (Ghisari et al., 2017).] Bonefeld-Jørgensen et al. (2014) conducted sensitivity analyses excluding these 72 cases and observed some differences in their findings. [The Working Group prioritized the results presented by Ghisari et al. (2017), given the withdrawal of some of the women from the study; the article by Bonefeld-Jørgensen et al. (2014) was, therefore, not tabulated as part of this monograph.]

The first-trimester blood samples (taken some time in weeks 6–14 of gestation) were stored and used for PFAS analysis. For cases, the average age at blood sampling was 30.6 years (range, 21–42 years), and the average at diagnosis was 41.1 years (range, 32–53 years). Serum concentrations of PFAS, including PFOA and PFOS, were assessed using LC-MS/MS. The association between PFAS and breast cancer risk was evaluated using unconditional logistic regression models, with PFAS concentrations transformed using a natural log transformation characterized as a continuous variable, and with adjustment for potential confounders (age at blood sampling, BMI before pregnancy, gravidity, oral contraceptive use, age at menarche, smoking during pregnancy, alcohol intake during pregnancy, maternal education, physical activity).

[The Working Group noted some strengths of this analysis, including a fairly large sample size of premenopausal cases of breast cancer and the measurement of PFAS serum concentration years before breast cancer diagnosis. The study included only a single PFAS concentration as a measure of exposure, assessed during the first trimester of pregnancy; in itself, this is not expected to introduce confounding because it was collected early in pregnancy (Sagiv et al., 2018). Further, the Working Group noted that

while only having a single measure of PFAS may be considered a limitation, there is some evidence, from analyses of repeat sampling of PFOA, that a single sample may represent long-term averages over a 5–8 year period, with potential misclassification resulting in only minor bias towards the null (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). However, a concern remained that single measurements of PFOA or PFOS, despite their long half-life, might not represent average exposure over the longer periods that may be relevant to cancer etiology. Although many key plausible confounders were adjusted for in the study, it would have been better, given temporal trends in PFAS, to match or adjust for the year of blood sampling and consider adjustment for other PFAS. Pregnancy might be an important window of susceptibility for breast cancer, but this design might also limit the comparability of these results with those obtained using measurements for serum samples collected from non-pregnant women. There was a lack of data on cancer subtypes (e.g. histology, hormone-receptor status), and results were presented for all cancer subtypes combined, which could mask cancer subtype-specific associations and bias the overall risk estimate towards the null. Other limitations include relatively small exposure contrasts in a population with background exposures, and the potential non-applicability of the findings to postmenopausal breast cancer. The reasoning behind the exclusion of the 72 women, who might, in fact, not have had breast cancer is a limitation and resulted in a smaller sample size, but the Working Group noted that although the results of analyses slightly differed with and without them, the conclusions remained similar.]

2.1.8 California Teachers Study

The California Teachers Study (CTS) is an ongoing prospective cohort study that includes women who were current and former public-school professionals in California, USA, who were enrolled in the California State Teachers Retirement System in 1995 (Bernstein et al., 2002). A self-administered baseline questionnaire was posted to 329 684 women, with approximately 40% responding (Bernstein et al., 2002). In 1995–1996, 133 479 women were enrolled in the cohort by completing the questionnaire. The baseline questionnaire covered menstrual and reproductive history, use of exogenous hormones, diet, smoking, alcohol use, height, weight, family history of cancer, and individual's medical history. The mean baseline age for the study participants was 54.1 years (standard deviation, 14.8 years); the ethnicity of the cohort was primarily non-Hispanic White (86.7%) (Bernstein et al., 2002).

Study participants were followed up annually to update details on cancer diagnoses, deaths, and residential moves. Participants were also sent more detailed follow-up questionnaires, focusing on exposures of interest. The study also uses linkages to state and national mortality files and reports from next of kin for dates and causes of death (Hurley et al., 2018).

A nested breast cancer case–control study (913 cases, 1270 controls) was conducted within the CTS and included blood sample collection and an interview-administered questionnaire (Hurley et al., 2018). Women were eligible if they had received a diagnosis of invasive breast cancer between 1 January 2006 and 1 August 2014, were aged < 80 years at the time of diagnosis, had no previous history of breast cancer at the time of entry into the CTS, and had resided in California continuously from the time of cohort entry to the time of diagnosis. Breast tumours were identified by linkage to the California Cancer Registry and were confirmed by pathology (99%). Thus,

as long as participants remained in California, they were actively followed up for cancer diagnosis. Controls were randomly sampled with frequency-matching by 5-year age group, race or ethnicity, and the California Cancer Registry regional entry of residence. Participation rates were 55% for controls and 65% for cases.

Phlebotomist-collected blood samples were stored and assayed for serum PFAS using LC-MS/MS. For the cases, blood samples were collected, on average, 35 months after a diagnosis of breast cancer (range, 9 months to 8.5 years). Samples collected before October 2011 were excluded, owing to concerns regarding time trends in PFAS levels and time trends in sample collection by case status, primarily affecting controls. [The Working Group noted that it was unlikely that this exclusion would introduce any bias, since it resulted largely in the exclusion of controls, rather than cases.] After exclusions, the final sample size was 902 cases and 858 controls. The associations between each PFAS detected in the serum samples and the risk of breast cancer were estimated using unconditional logistic regression (given the breaking of initial matching), adjusting for confounders (age at baseline, race or ethnicity, region of residence, blood draw date, the square of blood draw date, season of blood draw, total pack-years smoking, BMI, family history of breast cancer, age at first full-term pregnancy, menopausal status at blood draw, and pork consumption). Concentrations of PFAS were considered both as continuous variables ($\log_{10}$-transformed) and as categorical variables (based on tertiles of PFAS concentrations in the controls). Estimates were stratified by menopausal status at blood draw, estrogen receptor and progesterone receptor (ER/PR) status of the tumour, and other factors.

[The Working Group noted that the strengths of this study included a large number of cases identified through the cancer registry and population-based controls. Other strengths included adjustment for a large number of potential confounders and the stratified analysis by a number of important factors regarding breast cancer, including hormone-receptor status of the tumour. The primary limitation was the use of a single blood sample collected between 9 months and 8.5 years after diagnosis and presumably after at least initial treatment, both of which might affect blood PFAS concentration, and that this did not reflect the probable etiologically relevant period. This limitation rendered the study of minimal informativeness.]

2.1.9 Perfluorocarbon-production workers

Girardi and Merler (2019) studied the association between PFAS (including PFOA and PFOS) and mortality in a cohort of 462 male employees who had worked ≥ 6 months before 2009 in a factory in Italy. There were 14 658 person-years and 107 deaths, with an average follow-up time of 31.7 years. The factory had produced PFOA, POSF, and other chemicals (including one other PFAS, perfluorobutylsulfonyl fluoride) since 1968. Follow-up covered the period 1970 to 2018. Information on the underlying causes of death was obtained from the regional epidemiological department and, if not available, from the local health unit register for deaths after 1990, or from the complete death certificate, as recorded by the birth and death register of the municipality where the death had occurred before 1990. Results were given for a wide variety of outcomes, including all cancers combined and six specific cancer types: oesophagus, stomach, colon, liver, lung, all lymphoma or haematopoietic cancers, and NHL.

Measurements of PFOA serum concentration in workers, available for a subsample of the cohort (n = 120), were used to develop a regression model for job-specific levels across time. These models were then used to estimate a cumulative serum PFOA concentration for each cohort member. Employees were classified: (i) by three PFAS (either PFOA or PFOS) exposure categories (office workers, never in PFAS-exposed

department, ever in PFAS-exposed department); and (ii) by tertiles of estimated cumulative PFOA serum concentrations. SMRs were calculated for the exposed cohort compared with the regional population (adjusted for age, sex, calendar time). Poisson regression risk ratios were also calculated, taking workers of a nearby metalworking factory, who were working with the Italian train system, as referents. [The Working Group noted that there was some exposure to asbestos in the metalworking factory, which might have biased deaths from lung disease towards the null.] Additional analyses were conducted to calculate SMRs and risk ratios across categories of probability of PFAS exposure and tertiles of cumulative serum PFOA concentration using the regional population and the metalworking cohort as the referent, respectively.

Serum PFOA concentrations among 120 workers in the period 2000–2013 (696 measurements) showed a geometric mean of 4048 ng/mL (range, 19–91 900 ng/mL). For these same 120 workers (615 measurements), serum PFOS results showed a much lower geometric mean of 148.8 ng/mL (range, 10–3386 ng/mL). The intra-sample correlation between the PFOA and PFOS concentrations was high (Spearman correlation, $\rho = 0.59$; $P < 0.001$).

[The Working Group noted the exceptionally higher levels of PFOA exposure than in other occupational cohorts, with a resulting high exposure contrast, as a strength. The use of a JEM and some serum measurements to build a model of cumulative PFOA exposure that had evidence of a good fit to observed data, and comparisons with non-exposed workers, which might reduce confounding, were also strengths. While there was exposure to PFOS, serum PFOS concentrations were much lower than those of PFOA, and PFOS was not considered to be a potential confounder. Limitations were: a small occupational cohort with few deaths (107 deaths, 42 from cancer), no data on confounders (although the use of a worker comparison population might reduce confounding), a small number of deaths (7 each) from liver cancer and lymphohaematopoietic cancer (the two causes with positive trends with exposure), no data on some causes of death of major interest (e.g. cancers of the bladder, prostate, or testis). This study was of moderate informativeness, owing to the documented high exposure, but was limited by the small number of cancer outcomes.]

2.1.10 E3N cohort

E3N (Etude épidémiologique auprès de femmes de la Mutuelle générale de l'Education nationale) is a prospective population-based cohort study of 98 995 women in France that was initiated to identify risk factors for cancer and other chronic diseases in women (Clavel-Chapelon et al., 2015). In 1990, a questionnaire was sent to almost 500 000 women aged 40–65 years who were part of a national health insurance programme for workers, primarily teachers from the French national education system, inviting them to enrol in the study. Approximately 20% responded to the questionnaire, with 98 995 participants enrolling in the cohort.

At baseline, participants completed a self-administered questionnaire and consented to the study team accessing their health insurance records. Participants completed follow-up questionnaires every 2–3 years after baseline, with an average response rate of approximately 80%, and with limited loss to follow-up (< 3%). These questionnaires included information on a range of demographic and lifestyle factors and suspected risk factors for cancer. Between 1994 and 1999, approximately 25 000 participants (participation rate, 40%) donated blood samples. Sample aliquots were stored in liquid nitrogen in a biobank.

A nested prospective case–control study was conducted to evaluate serum PFOA and PFOS concentrations in relation to breast cancer risk

(Mancini et al., 2020a). Cases of breast cancer were identified by self-report, from the health insurance files, or through death certificates. Deaths could be reported by next of kin or ascertained from the health insurance files; cause of death was identified using the NDI. Pathology reports were available for most of the cases (93%), but self-reported cases without a pathology report were included in the analysis. In the cohort, 281 cases of breast cancer were identified that were diagnosed before 2013 and for which at least three aliquots of serum were available. Cases for which the dietary questionnaire had not been completed or diagnosed before the blood sampling or before completing the dietary questionnaire were excluded. The length of time between drawing of the blood sample and cancer diagnosis was not reported. From the eligible 240 cases of incident cancer remaining after exclusions, 194 cases of incident postmenopausal breast cancer were randomly selected for the study; this reduction was due to budgetary constraints. A control (also $n = 194$) was sampled from the cohort for each case, using a density-sampling approach based on not having a breast cancer at the time that the corresponding case was diagnosed, with matching by age (± 2 years), menopausal status and BMI at blood collection, and year of blood collection. Mean age at diagnosis was 68.8 years (range, 58.3–84.9 years). Information on tumour hormone-receptor expression was available for ER for 158 cases (77%), and for PR for 155 cases (80%). In total, 132 tumours were positive for ER (ER+) and 98 were positive for PR (PR+).

Concentrations of PFOA and PFOS were measured using LC-MS/MS. Both PFOA and PFOS, categorized in quartiles, were evaluated in relation to breast cancer risk using conditional logistic regression. [The Working Group noted that while collection of a single blood sample might be considered a limitation, there is some evidence, from repeat sampling of PFOA, that single samples may represent long-term averages over a 5–8 year period, with potential misclassification resulting in only minor bias to the null (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). However, there remains the concern that single measurements of PFOA or PFOS, despite the long half-life of these chemicals, might not represent average exposure over longer periods that might be relevant to cancer etiology.] Mancini et al. (2020a) explored several statistical models for confounder adjustment and considered a large number of confounders, selected a priori (total serum lipids, BMI, smoking status, physical activity, education level, history of benign breast disease, family history of breast cancer, parity or age at first full-term pregnancy, breastfeeding duration, age at menarche, age at menopause, use of oral contraceptives, current use of menopausal hormone therapy, adherence to a "Western" or "Mediterranean" diet). Stratified analyses evaluated how these associations varied by ER and PR status of the tumour.

[The Working Group noted that strengths included the prospective design and collection of blood specimens, the availability of blood data before diagnosis, extensive confounder control, limited loss to follow-up, and the availability of detailed diagnostic information (e.g. ER and PR status for nearly all cases of breast cancer). Limitations included relatively small exposure contrasts in a general population sample with background serum PFOA and PFOS concentrations and a lack of cases in premenopausal women. The response rate for the blood donation was low, which might affect the generalizability of the findings. The analyses by hormone-receptor subtype, while important, were limited by the small sample sizes, and there was a lack of information on the time between blood sampling and diagnosis.]

2.1.11 PLCO Cancer Screening Trial cohort

The PLCO was a large randomized controlled trial (about 150 000 adults; 76 685 men and 78 216 women), aged 55–74 years from 10 large cities in the USA), conducted in 1993–2001, and designed and sponsored by the National Cancer Institute. The goal was to determine the effects of screening on cancer-related mortality and secondary end-points in men and women aged 55–74 years, recruited between 1993 and 2001 (Prorok et al., 2000; Rhee et al., 2023a). The target populations for recruitment differed between the 10 clinical sites in the trial; recruitment methods included mass mailings using purchased mailing lists or lists of patients in local areas. Eligible women had not previously received diagnoses of cancer and had not undergone the screening tests in the 3 years preceding enrolment for testing in the trial. In the PLCO Trial, blood samples were to be collected from participants in the screening arm and stored for future etiological research (Hayes et al., 2000): blood samples were collected from 95% of screening-arm participants at baseline. Serum PFAS concentrations in the controls of the PLCO study were similar to those in the US NHANES study collected at about the same time, suggesting that the studied population was representative of the US population (Shearer et al., 2021).

Four separate nested case–control studies were conducted for the PLCO Trial cohort, investigating the association between PFOA or PFOS and cancers of the kidney, prostate, breast, and pancreas.

Shearer et al. (2021) conducted a nested case–control study within the PLCO cohort; this study involved 324 cases (216 men, 108 women) with RCC (the main subtype of kidney cancer) and 324 matched controls who were alive and free of RCC after the diagnosis dates of their corresponding matched case. Controls were matched individually on age at enrolment, sex, race and ethnicity, study centre, and year of blood draw. Exposure assessment was based on PFOA, PFOS, and other PFAS measured in serum collected 2–18 years before cancer diagnosis (mean, 8.8 years). Analyses using conditional logistic regression controlled for BMI, kidney function (estimated glomerular filtration rate, eGFR), smoking status (never, former, or current), history of hypertension, prior sample freeze–thaw cycles, and calendar year of blood draw. Analyses were conducted for eight different PFAS measured at baseline, including PFOA and PFOS. Additional analyses considered PFOA, PFOS, and PFHxS jointly. Analyses considered quartiles of serum concentrations, as well as continuous (log_2-transformed) serum levels. Geometric mean concentrations of PFOA among controls were 4.0 and 4.5 µg/L [ng/mL] for women and men, respectively; those for PFOS were 31.3 and 38.1 µg/L [ng/mL] for women and men, respectively, similar to serum concentrations for the general population at the time.

Steenland et al. (2022) included the cases and controls from Shearer et al. (2021) in a pooled analysis of PFOA and kidney cancer, combined with 103 cases and 511 matched controls from a PFOA-exposed cohort in West Virginia and Ohio previously reported by Barry et al. (2013). This pooled analysis was conducted to derive a dose–response curve between serum PFOA concentration and risk of kidney cancer using two of the largest studies of kidney cancer and PFOA exposure, and to conduct a risk assessment of excess lifetime risk of kidney cancer for specific PFOA serum concentrations and rates of drinking-water consumption.

Rhee et al. (2023a) studied 750 cases and 750 matched controls nested within the PLCO cohort. They looked at associations between a variety of PFAS, including PFOA and PFOS, measured at baseline, and subsequent prostate cancer. There were 750 men with aggressive prostate cancer (defined as stage III or IV or Gleason score ≥ 8, or Gleason score 7 and death from prostate cancer). Cases were diagnosed > 300 days after baseline

blood collection (median, 9 years). Controls were selected from among eligible participants who were alive and cancer-free at the time of the case diagnosis and were individually matched on age at baseline, race or ethnicity, calendar year of baseline blood collection, and characteristics of blood sample (e.g. whether thawed or not). Analyses were further adjusted for BMI, smoking status, family history of prostate cancer, history of diabetes, and serum concentrations of seven other PFAS. All eight PFAS were detected in more than 95% of samples; most PFAS were moderately correlated, with the highest correlation being between PFOA and PFOS (ρ = 0.70). Rhee et al. (2023a) also collected multiple serum samples (at baseline, and at 1- and 5-year follow-up) from a subset of controls (n = 60) and found that the variance between participants was generally markedly higher than the variance within participants; intraclass correlation coefficients across repeats for PFOA and PFOS were 0.73 and 0.85, respectively.

Chang et al. (2023) conducted a nested case–control study of breast cancer among women who were postmenopausal at baseline in the PLCO cohort. There were 621 cases and 621 controls, individually matched on age at baseline, date of blood draw, and menopausal hormone therapy use at baseline, who were alive and cancer-free past the follow-up time of their corresponding matched cases. Prediagnostic serum concentrations of PFOA and PFOS, measured 1 year after baseline, with a median of 5.6 years before case diagnosis, were the exposures of interest. In another study, intensity levels of PFOA and PFOS were assessed using the same untargeted metabolomics platform and were highly correlated with targeted measured serum concentrations of PFOA and PFOS (Spearman correlations, 0.77 and 0.76, respectively). Analyses were conducted for all cases combined by quartile of PFOA and PFOS intensity levels, and by ER (ER+, 435 cases) or PR (PR+, 299 cases) status, and joint ER/PR status. Models were adjusted for age at blood sampling, established breast cancer risk factors (age at menarche, age at first live birth, number of live births, age at menopause, duration of menopausal hormone therapy use, first-degree family history of female breast cancer, personal history of benign breast disease, BMI, smoking status, vigorous physical activity), natural log-transformed intensity levels of PFOA (for the PFOS model) or PFOS (for PFOA models), and variables, whose removal resulted in a ≥ 10% change in the odds ratios (study centre, race or ethnicity, and education).

Zhang et al. (2023) studied data for 360 cases of pancreatic ductal adenocarcinoma (the most common type of pancreatic cancer) and 360 matched controls in a nested case–control study in the PLCO cohort. The same study also involved another 251 cases and 251 controls from the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study, conducted in Finland. The PLCO case and control groups were matched on age, date of blood draw, sex, and race. Blood collected at PLCO baseline was analysed for PFOA and PFOS (median follow-up period, 9 years), using relative intensities rather than absolute concentrations (i.e. based on non-targeted analysis of PFOA and PFOS intensity levels). Previous work has shown that such rankings correlate well with measurements of absolute concentrations (Spearman correlation, 0.76) (Rhee et al., 2023c). Additionally, although untargeted analysis was used by Zhang et al. (2023), in previous PLCO studies, serum PFOA and PFOS concentrations were comparable to those measured among the general population in the NHANES survey in 1999–2000 (Rhee et al., 2023a). Data were analysed using conditional logistic regression, with continuous variables and quintiles, and controlling for smoking, BMI, age at blood draw, and diabetes. It did not appear that each PFAS was adjusted for the other.

[The Working Group noted strengths in all four PLCO nested case–control studies. First, all the studies involved large numbers of cases

and controls; second, they all used measured serum concentrations of PFOA and PFOS before cancer diagnosis, which is used as the exposure metric. Other strengths included consideration of a broad range of confounders, and data on tumour characteristics. Another strength was the mutual control for other PFAS under study (except in Zhang et al., 2023), and the availability of repeat samples in the prostate cancer study to assess reproducibility. Limitations in all four studies included the use of a single measurement to characterize long-term exposure, although the Working Group noted that in Rhee et al. (2023a) repeated measures of PFAS in a subsample indicated good reproducibility within individuals. Another weakness was the relatively low contrasts in exposure, typical of a general population study, making it more difficult to identify potentially corresponding health effect contrasts. While not necessarily a weakness, it was noted that the prostate cancer study by Rhee et al. (2023a) and the breast cancer study by Chang et al. (2023) were restricted to aggressive cancers and postmenopausal cases, respectively. However, for etiological research, it is probably more important to focus on aggressive than indolent prostate cancer, and postmenopausal breast cancer represents the great majority of breast cancer cases. Finally, the Working Group noted that the PLCO cohort had a higher percentage of White participants, e.g. 88–89% in Rhee et al. (2023a) and Chang et al. (2023), compared with the current US population (75%) (United States Census Bureau, 2022).]

2.1.12 Child Health and Development Studies pregnancy cohort

The Child Health and Development Studies (CHDS) pregnancy cohort included 20 754 pregnancies that resulted in 19 044 live births, including 9300 daughters. Between 1959 and 1967, pregnant women in the Oakland area of California, USA, receiving obstetric care through the Kaiser Foundation Health Plan were invited to participate (> 99% of all eligible women enrolled). The researchers obtained access to the medical records of all pregnant women recruited in the cohort, and to those of their children, and collected blood samples from the mothers (mostly, one blood sample per trimester and postpartum). Moreover, all mothers participated in an in-person interview during pregnancy (van den Berg et al., 1988).

To collect information on residence history and update residency and name changes over time, the CHDS cohort was linked to the California Department of Motor Vehicles. Data on the history of residential location of all women recruited in the CHDS cohort were used to identify the population at risk of cancer, corresponding with geographical surveillance by California's cancer registries. Deaths and cases of cancer were identified through linkage of the CHDS with the California Vital Status Records and the California Cancer Registry, respectively. Cases were also identified by self-report in a survey of CHDS daughters conducted in 2010–2013 (Cohn et al., 2020).

A nested breast cancer case–control study was conducted within the CHDS cohort, including 102 cases with breast cancer and 310 controls. Cases were identified through surveillance and self-report until March 2013 and were defined as CHDS daughters with cases of incident invasive or non-invasive breast cancer, which were diagnosed when they were aged < 52 years. Only cases of cancer for which a maternal perinatal blood sample was available for the analyses were selected; this led to the inclusion of 86% of all the cases of breast cancer identified. Three controls were matched to each case on birth year and trimester of maternal blood draw. Controls were selected randomly from among CHDS daughters not known to have received a diagnosis of breast cancer at the age of diagnosis of the corresponding case (Cohn et al., 2020).

After collection, serum samples had been stored at −20 °C, and concentrations of PFAS, including PFOA and PFOS, were measured using LC-MS/MS. The association between maternal PFAS serum levels and the daughter's risk of breast cancer was evaluated using age-matched conditional logistic regression, with PFOA and PFOS serum concentrations analysed as continuous variables after $\log_2$ transformation and adjusting for potential maternal confounders, such as maternal age, race, overweight in early pregnancy, parity, maternal history of breast cancer, maternal serum $\log_2$-transformed dichlorodiphenyldichloroethylene (*p,p′*-DDE), maternal serum $\log_2$-transformed dichlorodiphenyltrichloroethane (*o,p′*-DDT), and whether the daughter was breastfed (Cohn et al., 2020). Models evaluating PFOS also considered, a priori, inclusion of a PFOS precursor, (*N*-EtFOSAA), and total maternal cholesterol, both $\log_2$-transformed, and their product term (to test for interaction).

[The Working Group noted as a strength of the study that cases were likely to have been accurately determined via the California Cancer Registry.]

[The Working Group also noted that blood PFOA and PFOS concentrations decrease during pregnancy, owing to expanding plasma volume, decreased albumin concentration, and increased glomerular filtration rate. Nevertheless, it has been reported for previous studies that blood PFOA and PFOS concentrations measured during different pregnancy trimesters and postpartum in mothers, as well as measurements of cord blood, are well correlated (Glynn et al., 2012; Kato et al., 2014; Pan et al., 2017; Nielsen et al., 2020). This implies that blood PFOA and PFOS concentrations measured at different times during pregnancy and postpartum can be predictive of fetal exposure during pregnancy. Conversely, Cohn et al. (2020) analysed and compared, in the same study, PFOA and PFOS concentrations measured during pregnancy (22% of the samples) and in the early postpartum period (78% of the samples). This could have introduced a potential non-differential exposure misclassification bias, because not all blood samples were collected at the same time during pregnancy.]

[The Working Group noted that the study included examination of the prenatal exposure window, which is of interest with regard to the etiology of breast cancer in general. However, it was noted that no serum PFAS levels were directly available from study participants, namely, the CHDS daughters, and exposure was notably restricted to prenatal exposure, limiting the generalizability of the results and the possibility for comparison with other studies. Another weakness noted was that no individual information concerning the CHDS daughters was available in the study (Cohn et al., 2020), so that the analyses did not include important confounders. In the 1960s, PFAS contamination was expected to be still low in the USA general population (ATSDR, 2020) and this was reflected by the very low serum PFOA concentrations measured in women of the CHDS cohort, which affects the informativeness and the comparability of the results of the study (Cohn et al., 2020). In contrast, serum PFOS concentrations measured in women of the CHDS cohort were unexpectedly high, considering the time period of blood sampling; no explanation was provided by the authors for these high values. Also affecting generalizability was the restriction to cases diagnosed in daughters younger than 52 years. Additionally, the Working Group noted the lack of information on what percentage of the cohort moved out of the state and could not be followed up. Finally, there was a lack of data on cancer subtypes (e.g. histology, hormone-receptor status), and results were presented based on all cancer subtypes combined, which could mask cancer subtype-specific associations and bias the overall risk estimate towards the null. Overall, on the basis of these limitations, the Working

Group considered this study to be of minimal informativeness.]

2.1.13 The Ronneby Register cohort

Li et al. (2022a) investigated cancer incidence in residents in an area with high-level environmental exposure to, primarily, PFOS and PFHxS, in Sweden. The municipality of Ronneby, on the Baltic coast in the southern part of Sweden, had about 28 000 residents in 2013, and drinking-water was supplied by two waterworks. One of these, situated 2 km from a military airfield, supplied one third of the households of Ronneby municipality (a map of the area is given in Xu et al., 2021). In December 2013, measurements of PFAS in drinking-water from this waterworks revealed sum of PFAS concentrations above 10 000 ng/L [10 ng/mL], whereas the concentration for the other waterworks was below 90 ng/L [0.09 ng/mL] – but still higher than in the drinking-water of the neighbouring municipality of Karlshamn. It was found that PFOS and PFHxS accounted for > 90% of the total PFAS, while the PFOA contamination was relatively limited and strongly overlapping with the concentrations in the reference population. The source of the contamination was the use of PFAS-containing firefighting foam at the airfield from about 1985 until the waterworks was closed, by the end of 2013. No measurements were available before 2013, but the study authors assumed that levels in the drinking-water increased during the years after 1985 and decreased after the end of the exposure to contaminated water, in late 2013 (Li et al., 2018). The Ronneby Register cohort includes 60 507 individuals (men, 53%) who ever lived in Ronneby municipality in the period 1985–2013. Individual exposure was classified by coupling registry information on yearly residential address with information on which addresses had been supplied with contaminated water by the waterworks. Exposed individuals were those who had ever lived in the contaminated district (“ever-high”, n = 15 811; 26% of the Ronneby population). This group was subdivided by the calendar period and by the number of years living at an ever-high residence: “early-high” (1985–2004), “late-high” (2005–2013), “short-high” (1–10 years), and “long-high” (≥ 11 years). An internal referent was defined: inhabitants who had ever lived in Ronneby municipality in 1985–2013 but never at addresses receiving contaminated drinking-water. There were [44 696 (74%)] residents with never-high exposure (data derived from Li et al., 2022a, Table 4); the mean age at entry into the cohort was between 30 and 33 years, according to sex and exposure group.

The external reference groups included a regional population (the population of Blekinge County, excluding Ronneby municipality) and a national population (the whole Swedish population). The exposure classification was validated by measurements of several PFAS in the serum of 3084 people from Ronneby municipality (ever-high and never-high), sampled in 2014–2015, and in the serum of 226 people from a neighbouring municipality, sampled in 2016. The ratio of geometric mean levels of PFOS in the late-high group, relative to reference residents, was (239 ng/mL)/(3.9 ng/mL) = 61.3, that for PFHxS was (210 ng/mL)/(0.84 ng/mL) = 250, and that for PFOA was (13 ng/mL)/(1.5 ng/mL) = 8.7. Data on cancer occurrence during the follow-up period 1985–2016 were obtained from the Swedish Cancer Register (using ICD-7 codes). In all, 5702 first-occurring cancers were identified in the Ronneby Register cohort (n = 60 507), with 495 identified in people in the group with the highest exposure, the late-high group, including 374 in people who were in the long-high group. Age-, sex-, and calendar year-standardized incidence ratios (SIRs) were computed for a large number of cancer sites for residents who had never, or had ever, resided at addresses with contaminated water, compared with those residing in uncontaminated areas (external analysis). Internal comparisons based on Cox regression models

were made using the calendar year as the underlying timescale and were adjusted for age and sex. Information on lifestyle and health behaviours was not available, but annual data on highest attained education (from 1990 onwards), residence, work address, and demographic data were obtained from Swedish nationwide registers.

[The strengths of this cohort were the large general population sample with complete ascertainment and follow-up, owing to the use of high-quality Swedish population registers with complete population coverage, and a strong documented exposure contrast. A limitation was the mixed exposure profile, with high levels of PFOS and PFHxS, and somewhat elevated but significantly lower levels of PFOA, as well as the lack of individual serum measurements, or individual water contamination or consumption measurements, hence necessitating an ecological exposure assignment into groups by area and time of residence. Conversely, the group-level exposure assignments may have captured the large exposure contrasts in this population and were supported by a large number of serum measurements. The limited information on potential confounders may be a minor issue, since this is unlikely to be dependent on the water distribution system, which also fits with the sensitivity analysis adjusted for highest attained education (which has been shown to correlate with smoking in Sweden; Eek et al., 2010) showing no change of results. A lack of historical information on area-level contamination of drinking-water with PFAS, particularly during earlier years of the study period was, however, a limitation.]

2.1.14 Dongfeng-Tongji cohort

The Dongfeng-Tongji (DFTJ) cohort is an ongoing prospective study including over 41 000 retired workers recruited from an automotive company in Shiyan, China (Wang et al., 2013). The company is one of the three largest vehicle manufacturers in China and was founded in 1969, so that most first-generation workers had already retired when the DFTJ cohort was initiated. Participants in the DFTJ cohort were recruited in two waves: the first from September 2008 to June 2010, which included 27 009 participants; and the second from April to October 2013, which included 14 120 participants (Feng et al., 2022). The participation rate was approximately 87% during the first wave (Wang et al., 2013) and was not reported for the second wave; however, responders and non-responders reported similar sociodemographic characteristics (Feng et al., 2022). At inclusion, all participants answered face-to-face interviews, underwent physical examinations, and provided a blood sample. For each participant, 10 mL of peripheral venous blood was collected once at inclusion after overnight fasting. Plasma was separated from the blood sample and stored at −80 °C (Wang et al., 2013; Feng et al., 2022).

PFAS plasma levels were low in this cohort: PFOS had the highest median plasma concentrations (10.36 ng/mL), followed by PFOA (1.19 ng/mL).

Cases of incident breast cancer, diagnosed from September 2008 until the end of 2018 (median follow-up 9.6 years), were identified by reviewing participants' medical records or death certificates provided by the five hospitals owned by the automotive company and by the local Center for Disease Control and Prevention.

A case–cohort study was conducted among women included in the DFTJ cohort to investigate the association between plasma levels of six PFAS, including PFOA and PFOS, and breast cancer risk. Women with prevalent cases of cancer at baseline, those with insufficient blood specimens, and those who were lost to follow-up were excluded from the case–cohort study. Among the remaining 18 387 women, 226 were identified as incident breast cancer cases during follow-up. A subcohort of 990 women was randomly selected from the base cohort

(*n* = 18 387) according to strata determined by age, and among these women, 13 (1.31%) developed breast cancer during follow-up (Feng et al., 2022).

For all women included in the subcohort, the plasma concentrations of six PFAS, including PFOA and PFOS, were measured using LC-MS/MS. The association between PFAS and breast cancer risk was evaluated using Barlow-weighted Cox proportional hazard models adjusted for potential confounders selected a priori, such as age, BMI, smoking, drinking, marital status, education level, occupation type, batch to enter the cohort, parity, menopausal status, history of mastitis, use of hormone replacement therapy, and family history of cancer. PFAS concentrations were natural log-transformed and included one-by-one in separate models for six PFAS, modelled as continuous and categorical variables (in quartile groups identified based on the distribution of each PFAS in the subcohort) (Feng et al., 2022).

[The Working Group noted that this study represents a large cohort of retired Chinese workers with low-level exposure to PFOA, PFOS, and other PFAS compounds. Strengths are high baseline participation, good control for confounders obtained by interviews, limited loss to follow-up, ascertainment of diagnoses by medical records in five company-financed hospitals and death certificates. However, details were not provided on the probable completeness of diagnoses using these methods, nor the percentage of women whose diagnoses were confirmed only via death certificate, which would result in an unknown diagnosis date. Nevertheless, it cannot be predicted in which direction the risk estimates could be affected by the unknown diagnosis date. Other strengths included the availability of prediagnostic serum samples (an average of 9.6 years before diagnosis, but the range of time span from PFAS measurements to diagnosis was not provided). Blood samples were collected only once at baseline. The Working Group noted that there was some evidence, from analyses of repeat samples of PFOA, that single samples may represent long-term averages over a 5–8-year period, with potential misclassification resulting in only minor bias to the null (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). However, a concern remained that single PFOA or PFOS samples may not represent lifelong average exposure. Whether the selection of retired workers had an impact on risk estimates was uncertain, but there was no a priori reason to suggest any marked bias due to this selection. No cumulative lifelong exposure metric was available, and the very low exposure contrast for PFOA and PFOS limited the evaluation of exposure–response associations.]

2.1.15 NHANES 1999–2014 cohort

The NHANES is a continuously conducted and nationally representative cross-sectional survey. Participants are selected through a statistical process using census data to be representative of the noninstitutionalized population of the USA and are recruited via mailed letters inviting them to participate in NHANES. Between 1999 and 2014, individuals completed a household interview and a medical examination that included a blood sample collection and an assessment of anthropometric measures. Questionnaires assessed information including demographics, socioeconomic status, alcohol use and smoking history, diet, and medical history. [The Working Group noted that detailed information on the individuals included in the cohort, such as selection and participation rates, was not readily available.]

Among participants with stored blood samples, serum concentrations of PFOA and PFOS were quantified using LC-MS/MS (Wen et al., 2022).

The US National Center for Health Statistics has linked NHANES 1999–2014 cohort participants to the NDI to identify deaths and determine the underlying causes of death using probabilistic matching criteria based on identifiers such as social security numbers and date of birth. Participants were followed for cause-specific mortality until 31 December 2015. If the individual did not match to the NDI, this person was assumed to be alive as of the end of follow-up date. Mortality from all cancers combined was one of the cause-specific death categories included in the files available for public use, and no site-specific cancer mortality data were available. For 11 747 of the cohort participants aged ≥ 18 years at baseline and with blood samples analysed for PFAS, 1251 deaths were observed during the median follow-up of 81 months (interquartile range, IQR, 46–112 months). Of these deaths, 19.8% (248) were from cancer. The medians and 25th and 75th percentiles of serum PFOA and PFOS concentrations at baseline were 3.27 ng/mL (2.00, 5.00) and 11.60 ng/mL (6.40, 22.40), respectively, reflecting general population levels. [The Working Group noted that this study included only a single measure, which may be considered a limitation; however, there was some evidence, from analyses of repeat samples of PFOA, that single samples may represent long-term averages over a 5–8-year period, with potential misclassification resulting in only minor bias to the null (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). However, there remained the concern that single PFOA or PFOS samples may not represent average exposure over longer periods.]

Cox proportional hazards models were used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between PFOA and PFOS categorized in tertiles and overall cancer mortality adjusting for potential confounders (Wen et al., 2022). The confounders included were sex, age, race or ethnicity, smoking status, alcohol intake, physical activities, hypertension, diabetes, healthy eating index, creatinine clearance rate, serum total cholesterol, and serum cotinine. Analyses of PFOA and PFOS were adjusted for other PFAS.

[The Working Group noted some strengths in this study, including probably complete ascertainment of cancer mortality in this large population, which was selected to be a representative sample of the US population, making results generalizable in the USA, relatively good control over potential confounders, and adjustment for other PFAS in analyses of specific PFAS. Weaknesses noted were the unclear timing of the blood sampling relative to diagnosis or treatment; a short follow-up time for some of the participants, which may not reflect the relevant etiological window; and the use of a volunteer-based population that may be healthier than the general population, which may not be fully captured by sampling weights and thus may result in some selection bias. The study focused on all-cancer mortality, without data on cancer incidence or on mortality for specific cancers, making these data of limited informativeness for the Working Group's assessment of individual cancers. The observed estimates of association with cancer mortality would not be applicable to cancer incidence unless the cancer itself had a high rate of fatality.]

2.1.16 Multiethnic Cohort study

Kolonel et al. (2000) described the design and implementation of the Multiethnic Cohort (MEC) study, which was established to study diet and cancer in the USA. The MEC included a community sample of 215 251 citizens (men, 45%) aged 45–75 years who were enrolled during 1993–1996 in Hawaii and California (primarily Los Angeles County) when responding to a mailed 26-page baseline questionnaire on mainly dietary, demographic, and health issues.

The sampling frame was established using drivers' license files, voters' registration files, and Health Care Financing Administration files. The recruitment procedures aimed to obtain a balanced distribution of five specific ethnic groups comprising White, African-American, Latino, Japanese-American, and native Hawaiian people; therefore, less common groups were preferentially sampled. Response proportions spanned 20% to 49%, being lowest in Latino and highest in Japanese-American people. In the final sample, Japanese-American people comprised the largest group (28% in men and 25% in women) followed by White (24–22%), Latino (24–21%), African-American (13–19%) and native Hawaiian (6–7%). The participants represented a more educated subset of the general population. In addition to quantitative information about food and dietary components based upon portion size information, the questionnaire included data on smoking, drinking, obesity, and vigorous physical activity.

Goodrich et al. (2022) performed a case–control study of hepatocellular carcinoma (HCC) nested within the MEC. Incident cancers occurring during the 20 years of follow-up, including non-viral HCC (not of viral etiology), were identified from the early 1990s onwards by the Surveillance, Epidemiology and End Results (SEER) programme of the National Cancer Institute, which includes California. Additional information on health conditions was obtained from Medicare claims and California hospital discharge records (Goodrich et al., 2022). [It was uncertain whether the 50 cases of incident HCC constituted all the HCC cases arising during the follow-up period, given that the authors excluded HCC of viral etiology, and no information was provided about how the 50 controls matched on sex, age, race or ethnicity, and study area were selected.] Plasma concentrations of six PFAS (including PFOA and PFOS) were measured in prediagnostic fasting blood samples collected before diagnosis (median, 7.2 years; range, 0.9–16.4 years).] The unadjusted geometric mean of PFOS was 29.2 µg/L [ng/mL] in both cases and controls. For PFOA, concentrations were 4.21 µg/L [ng/mL] in cases and 4.78 µg/L [ng/mL] in controls, and for PFHxS values were 1.84 µg/L [ng/mL] in cases versus 2.07 µg/L [ng/mL] in controls.

The average age at blood collection was similar for cases (69.7 years) and controls (69.2 years). Men comprised 62% of the sample and 64% were residents of Hawaii. The prevalence of high BMI, high alcohol intake, and diabetes mellitus was much higher among cases than among controls.

Adjusted odds ratios for the association between plasma concentrations of each PFAS and risk of non-viral HCC were computed by conditional logistic regression, which accounted for the matching variables. Sensitivity analyses further adjusting for baseline BMI and baseline diabetes status were performed, but additional covariates (such as other PFAS) were not included in the statistical models. An additional sensitivity analysis considered ordinary logistic regression with covariates that included the matching variables. To account for possible non-linear associations, smoothing splines were inspected, and additional analyses contrasting risk above and below the 85th percentile (which corresponded to the 90th percentile in NHANES for PFOS) were carried out.

Rhee et al. (2023b) performed a nested case–cohort study of RCC within the MEC. The study identified 428 cases of incident RCC, which included all cases with prediagnostic serum samples available and diagnosed before 2018 using record linkages to the Hawaii Tumor Registry and the California Cancer Registry. The 428 controls, who were participants with no RCC diagnosis who were alive at the time of the case diagnosis, were identified using 1:1 matching on sex, race or ethnicity, study centre (Hawaii, California), age at serum collection (± 1 year), date of serum collection (± 1 year), time of serum collection (± 3 hours), and fasting status

(0 to < 6 hours, 6 to < 8 hours, 8 to < 10 hours, and ≥ 10 hours). Concentrations of nine PFAS (including PFOA and PFOS) were assessed in prediagnostic serum samples collected between 1994 and 2006.

Adjusted ORs and 95% CIs were estimated for the association between PFOA and PFOS (log_2-transformed and categorized in quartiles based on the distribution in the controls) and the risk of RCC using conditional logistic regression adjusting for the matching factors as well as BMI, eGFR, smoking status, and hypertension history. Analyses were conducted with further adjustment for other measured PFAS and with stratification by matching factors and other covariates, including race and ethnicity, sex, age at blood draw, calendar year of blood draw, years from blood draw to RCC diagnosis, and eGFR status.

[Strengths of the nested case–control studies conducted within the MEC included prediagnostic measurements of several PFAS compounds in a racially and ethnically diverse population, independent ascertainment of exposure and outcome with high accuracy, a strong focus on possible mechanistic pathways related to PFAS related metabolism, and baseline information on relevant potential confounders including education, socioeconomic level, and health behaviours such as smoking. Regarding the statistical analysis of the HCC study (Goodrich et al., 2022), risk estimates were not adjusted for date of blood sample collection, smoking, alcohol consumption, and other PFAS. Moreover, small numbers in combination with low exposure contrast limited the informativeness of this study. For the RCC study (Rhee et al., 2023b), strengths included the large sample size, adjustment for eGFR and other factors, and the consideration of multiple PFAS for adjustment. Although it was a strength to consider this association across multiple racial and ethnic groups, there was limited statistical power for some of these comparisons.]

2.1.17 Cohort of US Air Force servicemen

Purdue et al. (2023) performed a case–control study nested within a cohort of US Air Force servicemen (with prospectively collected blood specimens) to examine the risk of testicular germ cell tumours (TGCTs; the most common variety) according to adult serum concentrations of selected PFAS, including PFOA and PFOS. The US Air Force was using firefighting foams containing PFOS, PFOA, and other PFAS compounds since the late 1960s until 2018, when the use of long alkyl chain PFAS compounds was discontinued. The US Department of Defense (DoD) has identified over 200 Air Force installations with known or possible release of PFOS and PFOA and, in some airbases, these compounds have been measured in groundwater and drinking-water in amounts exceeding 70 parts per trillion (ppt), the 2016 US EPA lifetime health advisory threshold. The US DoD has since 1985 stored serum samples of members of the Air Force service (and other military branches) collected for the purposes of HIV testing before induction and at periodic medical examinations, overseas assignments, and major overseas deployments. From 2004, all service members had blood samples taken every second year. Samples were stored at −30° C at a central serum repository (Department of Defense Serum Repository, DoDSR), which contains sera from more than 10 million service members (also including US Army and US Navy personnel). DoDSR records have been linked to records of the Defense Medical Surveillance System, providing individual demographic, occupational, and health data.

Purdue et al. (2023) identified TGCT cases by linking DoDSR records with the DoD cancer registry, which contains data on patients diagnosed with cancer at military treatment facilities in the USA. In all, 530 male servicemen with active-duty status, at least one prediagnostic serum sample, no previous history of cancer and

aged < 40 years at the time of TGCT diagnosis were identified from 1988 through 2017. In cases with at least two prediagnostic samples and with the earliest sample collected ≥ 5 years before the TGCT diagnosis, a second prediagnostic sample collected as close to the 5-year prediagnostic lag-time as possible was analysed (n = 187). The median time between collection of selected samples was 4 years (range, 0.1–13.3 years). For each case, one randomly selected male control participant was identified among active-duty Air Force servicemen, with no history of cancer at the time of the case diagnosis, by matching on birth year, race or ethnicity (seven groups), year entering military service, and year of serum sampling. The first serum samples were collected 0.3–0.4 years (median values) after entry into military service and 0–20 years before the diagnosis of TGCT. Serum concentrations of nine PFAS, including linear and branched PFOA and PFOS isomers, were measured by LC-MS/MS. The PFAS serum concentrations of men in the Air Force service were comparable to those of men in the NHANES cohort, albeit slightly higher in earlier years and slightly lower in later years. Fewer than 1% of participants (n = 5) had occupational exposure as a firefighter during military service. Unadjusted and adjusted risk of TGCT was analysed separately for PFOA (sum of linear and branched isomers), PFOS (sum of linear and branched isomers), and other PFAS by conditional logistic regression of matched pairs grouped by quartiles of serum concentrations in controls. Besides matching factors, adjusted analyses accounted for military grade, number of deployments before diagnosis, and the six other PFAS.

[The Working Group noted several strengths of this study, including the nested design, a well-characterized source population, a large, matched dataset, measurements of PFOA and PFOS isomers, two repeated prediagnostic samples collected several years apart in a subset of the population, a reasonable exposure contrast (for PFOS, the upper quartile was > 42.2 ng/mL and the lower quartile was ≤ 18.3 ng/mL), and analyses accounting for effects of other PFAS compounds. Limitations were mainly the loss to follow-up of men leaving the military and missing serum samples for a large proportion of TGCT cases (217 out of 747 cases, 29%) that was not addressed in supplementary analyses. The completeness of TGCT ascertainment was not documented but may be a minor issue since an association between PFAS exposure and completeness of TGCT ascertainment seems unlikely. In most cases, only one serum measurement per person was available, and data on known strong determinants of TGCT (such as cryptorchidism) were lacking, but the association of such determinants with PFAS exposure seems unlikely. The Working Group noted that occupational exposure as a firefighter is classified as *carcinogenic to humans* (Group 1) (IARC, 2023), but the evidence for risk of testicular cancer was *limited*, and very few (n = 5) of the US Air Force servicemen cohort members had been exposed as a military firefighter.]

2.1.18 Finnish Maternity Cohort

The Finnish Maternity Cohort (FMC) is a national registry of women who donated serum during the first trimester of pregnancy. The registry was established in 1983 using residual serum from a national programme to screen for congenital infections (infections transmitted from mother to child during pregnancy) and is estimated to include > 90% of pregnancies among Finnish women during the period 1983–2016 (Pukkala et al., 2007; Holl et al., 2008; Lehtinen et al., 2017). Women donated serum at municipal maternity care units, usually between weeks 10 and 14 of gestation (Madrigal et al., 2024). The registry included each woman's personal identification number and data related to reproductive history, residence at the time of collection,

dates of sample collection and processing, and expected delivery date.

Madrigal et al. (2024) conducted a nested case–control study on the incidence of papillary thyroid cancer, which accounts for approximately 90% of thyroid cancers in Finland (Hakala et al., 2012), by linking the FMC to the nationwide Finnish Population Registry, Cancer Registry, and Medical Birth Register until 2016. The population registry provided information on emigration status and vital status. The cancer registry, which covers all incident cancer cases in Finland since 1953, included date of diagnosis, histology, and stage at diagnosis (Finnish Cancer Registry, 2023). The Medical Birth Register, established in 1987, includes data on gestational age, reproductive history, smoking status, BMI before pregnancy and at the prenatal visit, and information about the delivery and infant or fetus, but not prior history of breastfeeding (Finnish Institute for Health and Welfare, 2023). Information on BMI before pregnancy was largely missing. Madrigal et al. (2024) randomly selected 400 cases of papillary thyroid cancer from among all cases (total number not reported) diagnosed among women in the FMC who provided serum in 1986–2010 during their first pregnancy and for whom this pregnancy had resulted in a full-term live birth with delivery between 1987 and 2010. Cases were restricted to those whose age at sample collection was 18–39 years and who were diagnosed with thyroid cancer ≥ 3 years after delivery. First pregnancies only were used to avoid any changes in PFAS levels related to breastfeeding during a previous pregnancy. Age at cancer diagnosis ranged from 23 to 61 years (mean, 40.9 years). Living, cancer-free controls were individually matched on year of delivery (4–5-year increments) and age at first birth (3-year increments). Serum levels of PFAS and other persistent pollutants were analysed by the Environmental Health Unit Laboratory of the Finnish Institute for Health and Welfare using LC-MS/MS (Koponen et al., 2013; Koponen and Kiviranta, 2019). Analytes included 19 PFAS, 13 polychlorinated biphenyl congeners (PCBs), nine organochlorine pesticides, and three polybrominated diphenyl ethers. The Spearman rank correlation coefficient for levels of PFOA and PFOS was 0.61. Statistical analyses consisted of conditional logistic regression of continuous exposures (log_2-transformed) and of categories (25th, 50th, 75th, and 90th percentiles). For each PFAS detected in > 60% of the controls, including PFOA and PFOS, analyses were conducted with no covariates; with adjustment for any PFAS, PCBs, or organochlorine pesticides correlated (Spearman correlation, 0.3–0.61); and with adjustment for smoking.

[The Working Group noted several strengths of this study, including collection of serum before thyroid cancer diagnosis; adjustment in the analysis for other PFAS, PCBs, and organochlorine pesticides correlated ($\rho = 0.3–0.61$) with the analyte of interest; follow-up of the cohort covering the peak years of thyroid cancer incidence; and the availability of data in the Medical Birth Register on several potential confounders. The Working Group noted that one would expect only minor misclassification of long-term exposure because of reliance on a single prediagnostic sample according to a simulation study (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). PFAS concentrations might have been lower than pre-pregnancy levels because of increased plasma volume (Chapman et al., 1998) and glomerular filtration rates (Shankar et al., 2011) in the first trimester; however, a study of PFAS and birth outcomes suggests that little confounding may have occurred (Sagiv et al., 2018). The controls were not matched on the exact year of delivery but on increments of 4–5 years, which might affect comparison of PFAS levels because of temporal trends, although, given the estimated half-lives for PFOA and PFOS, such an effect was thought to be minimal. The Working Group did not

consider that the study had important surveillance bias among women diagnosed under age 40 years, when women may have frequent reproductive health-related visits; given that neither the women nor their medical providers were aware of their PFAS serum levels, these levels are not expected to affect thyroid cancer surveillance, and there were no large differences in stage at diagnosis by age at diagnosis. Analyses were not adjusted for pre-pregnancy BMI (a risk factor for thyroid cancer), which was missing for 85% of the women, nor were there data available on medical or environmental exposure to radiation. Finally, the study population had low-level exposure with a small exposure contrast.]

2.1.19 The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study

The Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study was a randomized chemoprevention trial the primary aim of which was to evaluate the effects of supplementation with alpha-tocopherol and beta-carotene on lung cancer incidence (Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). Secondary aims included evaluating the effects on other major cancers and overall and cause-specific mortality. The participants in the ATBC trial were White male smokers, aged 50–69 years at recruitment, who were identified in the Central Population Register as residing in south-western Finland, who responded to a questionnaire on their smoking history and willingness to participate, and who attended two clinic visits at which they completed a baseline study questionnaire and had trained nurses measure height, weight, blood pressure, heart rate, and visual acuity. The questionnaires included medical, smoking, and occupational history, and dietary habits over the past 12 months. Excluded from the study were people with a previous diagnosis of cancer other than non-melanoma skin cancer or carcinoma in situ; chronic renal insufficiency; cirrhosis of the liver; chronic alcoholism; receiving anticoagulant therapy; other medical conditions that might limit participation for 6 years; and current use of the vitamin supplements under investigation in the trial (Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). An overnight fasting blood sample was collected at the initial clinic visit, with serum stored at −70° C. Recruitment began in 1985 and continued until 1988 when a total of 29 246 men were randomized to one of four treatment groups in a 2 × 2 factorial design. After late exclusions of 113 men found not to be eligible, the final study population numbered 29 133. Follow-up consisted of three annual clinic visits, with cancer cases ascertained through the Finnish Cancer Registry and deaths through the Central Population Register. The intervention continued until 30 April 1993, with publication of the trial results in 1994 (Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). After the cessation of the trial, researchers continued to follow the cohort for 20 years, regularly updating data on mortality and cancer incidence.

Zhang et al. (2023) reported on two nested case–control studies on the incidence of pancreatic ductal adenocarcinoma, the most common type of pancreatic cancer. One study was conducted within the ATBC cohort, together with a parallel study conducted within the PLCO cohort (see description of this study above). Within the ATBC study, 251 cases of pancreatic ductal adenocarcinoma were ascertained until December 2011. A total of 251 controls were incidence-density sampled and matched to the cases on age at blood draw (± 5 years) and date of blood draw (within 30 days). Relative serum levels of PFOA and PFOS were measured using untargeted ultra-performance liquid chromatography-tandem mass spectrometry and/or gas chromatography-mass spectrometry. PFOS measurements were available only for 130 cases and controls. Statistical analyses consisted of conditional logistic regression to calculate odds

ratios and 95% confidence intervals per standard deviation increase of $\log_{10}$-transfomed PFOA or PFOS levels and quintiles based on the distribution of the controls, with adjustment for age at blood draw, smoking (years smoked and cigarettes per day), diabetes, and BMI.

[The Working Group noted that the strengths of the ATBC study included prediagnostic blood samples, detailed information on potential confounders collected through questionnaires and, for height and weight, by trained staff, and excellent case ascertainment. In addition, the numbers of cases in the ATBC (n = 251) and PLCO (n = 360) studies reported by Zhang et al. (2023) were large. The Working Group noted that the use of a single blood sample collected at baseline would be expected to result in only minor misclassification of long-term exposure, according to a simulation study (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). The Working Group noted that the study limitations included the low-level exposure with a small exposure contrast. The restriction of participants to White male smokers may affect the generalizability of the results. Furthermore, the study relied upon relative quantification of PFOA and PFOS; however, in previous research, relative measures have correlated well with targeted absolute concentration measurements (Rhee et al., 2023c).]

2.1.20 New York Mount Sinai Hospital Bio*Me* biobank cohort

Bio*Me* is a biobank linked to medical records within the Institute for Personalized Medicine at the Icahn School of Medicine at Mount Sinai, New York, USA. The collection of plasma samples, medical records, and questionnaire data from patients at Mount Sinai who lived in New York City and the larger metropolitan area started in 2007 and is currently ongoing (Icahn School of Medicine at Mount Sinai, 2023). Participants are enrolled from ambulatory care practices across the Mount Sinai Health System in New York City (Bar-Mashiah et al., 2022). No eligibility criteria were established, to make the cohort as inclusive as possible. As of September 2019, 52 500 patients were enrolled, and about 600 new patients are being enrolled each month (Icahn School of Medicine at Mount Sinai, 2023; van Gerwen et al., 2023). There did not appear to be follow-up of patients other than that conducted through the Mount Sinai hospital or ambulatory network.

van Gerwen et al. (2023) identified 88 cases of thyroid cancer within the Bio*Me* biobank for whom the time between plasma collection and thyroid cancer diagnosis was ≥ 1 year (n = 31; defined as longitudinal cases) or < 1 year (n = 57; defined as cross-sectional cases). The authors did not specify how cases were identified. Of the 88 identified cases of thyroid cancer, 74 were papillary thyroid cancer, as confirmed in pathology reports. Further inclusion or exclusion criteria (e.g. previous cancer) were not specified. Controls were pair-matched to cases on sex, age, race or ethnicity, BMI, smoking status, and calendar year of blood sample collection for PFAS measurement. Eighteen individual PFAS (including PFOA and PFOS) were measured using untargeted methods with liquid chromatography-high resolution mass spectrometry. Analyses were conducted for all thyroid cancer cases, for only papillary thyroid cancer, and for overall cases, stratified by time of blood sample collection in relation to diagnosis (longitudinal cases, n = 31, or cross-sectional cases, n = 57). Median age at sample collection was 43.5 years for cases and controls. Most of the population were women (83%). The mean time between sample collection and cancer diagnosis was 1.5 years for all thyroid cancer cases combined, 4.0 years for the longitudinal cases, and 0.1 years for the cross-sectional cases.

[The Working Group noted that the strengths of the study included the availability

of histological data for the cases and analyses adjusted for age, sex, race, and BMI, and sample storage time, and, for some analyses, adjustment for other specific PFAS compounds. Also, plasma samples were collected ≥ 1 year before diagnosis for a subset, albeit small, of the cases. Limitations included the small sample size, particularly for cases for which plasma was collected > 1 year before diagnosis (longitudinal cases), with the remainder having plasma collected < 1 year before diagnosis or at diagnosis (cross-sectional cases). In addition, the study was based on the use of untargeted assay methods, which limits direct comparisons with other studies. Also, thyroid cancer might be detected among asymptomatic patients who sought medical care for unrelated reasons, which raises a concern for detection bias, given that the cases were recruited in ambulatory practice, especially with such short follow-up. However, the Working Group noted that, since the case and control participants were recruited from within the same network of Mount Sinai ambulatory care practices, a generally comparable medical screening pattern could be assumed among cases and controls; thus, detection bias was unlikely, also considering that patients and practitioners were unaware of PFAS measurements.]

2.1.21 Cancer Prevention Study II LifeLink cohort

The American Cancer Society (ACS) Cancer Prevention Study II (CPS-II) enrolled 1 185 106 participants from 50 US states and the District of Columbia who completed a questionnaire, and mortality was ascertained using the NDI. A subset of this cohort, the CPS-II Nutrition Cohort, started in 1992–1993 by including 184 194 participants aged 50–74 years from 21 states followed with biennial questionnaires for cancer incidence, further verified through medical records or cancer registry files. Between 1998 and 2001, the CPS-II LifeLink Cohort started by recruiting 39 371 members from 20 states from within the CPS-II Nutrition Cohort. Those participants were required to be alive at the time of recruitment into the CPS-II LifeLink Cohort, since participation included a baseline questionnaire and a blood sample collection. Participants in the CPS-II LifeLink Cohort are followed for cancer incidence within the CPS-II Nutrition Cohort (Winquist et al., 2023).

Winquist et al. (2023) performed a study with a case–cohort design within the CPS-II LifeLink Cohort. Participants in the CPS-II LifeLink Cohort were eligible to participate in the case–cohort study if they were men or postmenopausal women who were cancer-free (excluding non-melanoma skin cancer) at the time of blood collection. The median age at the time of enrolment in LifeLink was 71 years for men and 69 years for women. From these eligible participants, the case group was defined as individuals with first primary cancers of kidney (n = 158), bladder (n = 401), prostate (men only, n = 1610), female breast (n = 786), or pancreas (n = 172); or haematopoietic malignancies (n = 635) as of 30 June 2015. The median follow-up time for the members of the subcohort was 14.3 years. The comparison subcohort included 499 women and 500 men (representing 3% of the LifeLink cohort meeting the inclusion criteria). PFOA and PFOS were measured together with other PFAS using LC-MS/MS. Several covariates were available, and the analyses were adjusted for identified cancer risk factors associated with PFAS exposure. Notably, the main models were not adjusted for BMI, because BMI was considered to be on the causal pathway. Of the participants in the comparison cohort, 98% were non-Hispanic White and 79% were aged ≥ 65 years at blood collection. Some participants identified as cases were included in the comparison subcohort (4 kidney cancers, 9 bladder cancers, 11 breast cancers, 58 prostate cancers, 7 pancreatic cancers, 16 haematological malignancies).

[The Working Group noted as strengths the large number of cases and the collection of blood samples before diagnosis. Because of the design as a survivor cohort, and the long time period that had elapsed between enrolment in the CPS-II Nutrition Cohort and enrolment in the LifeLink cohort, it was likely that this study would not have included some persons who may have had cancer related to PFOA or PFOS, especially those who developed cancers earlier in life in a susceptible exposed population. This survivor bias would have biased the results downwards (i.e. towards the null or even towards inverse associations). Indeed, participants in this cohort were cancer-free survivors of the CPS-II Nutrition Cohort whose blood was collected in 1998–2001, when most of them were aged > 65 years. Although using a single sample to measure PFAS is a potential limitation, there is some evidence, from analyses of repeat samples of PFOA, that single samples may represent long-term averages over a 5–8-year period, with potential misclassification resulting in only minor bias to the null (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). However, there remains the concern that single PFOA or PFOS samples may not represent average exposure over longer periods, which is particularly relevant here given the older age at blood draw. Thus, it is reasonable to assume that most of the CPS Nutrition Cohort would have been exposed to PFOA or PFOS well before the time of blood collection. The Working Group considered this study to be of minimal informativeness.]

2.1.22 Case–control studies in West Virginia and Ohio

Vieira et al. (2013) conducted two case–control studies of 18 different incident cancers during the years 1996–2005 among residents of 13 counties in Ohio and West Virginia, USA, which included both contaminated and non-contaminated water districts near the same polymer-production plant in Parkersburg, West Virginia, which was the source of contamination in the population studied by Barry et al. (2013). This cohort was described in Section 2.1.5 above. The two case–control studies were included in the same publication (Vieira et al., 2013).

The source population in the study by Vieira et al. was cancer registries of Ohio and West Virginia, and the study included counties outside the contaminated water districts studied by the C8 Science Panel. The source population in Barry et al. was the population living in six contaminated water districts near the plant in Parkersburg, West Virginia, who had participated in the C8 Health Project baseline study of 69 000 residents in the water districts and had provided blood samples in which PFOA was measured.

The final data set consisted of 7869 cases from Ohio and 17 238 cases from West Virginia, from 13 counties, in whom cancer had been diagnosed at age ≥ 15 years, with 18 cancer categories (i.e. bladder, brain, female breast, cervix, colon or rectum, kidney, leukaemia, liver, lung, melanoma of the skin, multiple myeloma, NHL, ovary, pancreas, prostate, testis, thyroid, and uterus).

In the first case–control study conducted by Vieira et al., cases (of the 18 cancers of interest) and controls (controls were all other cancers apart from the cancer of interest, and excluding kidney, pancreatic, testicular, and liver cancers) were compared with regard to residence in a contaminated or non-contaminated water district. This study included cases from both West Virginia and Ohio. Odds ratios were calculated for residence versus non-residence in contaminated water districts, adjusted for age, sex, diagnosis year, smoking status (current, past, and unknown, with never smoker as the referent) and insurance provider (government-insured Medicaid, uninsured, and unknown, with privately insured as the referent). Analyses were done for each of the six contaminated water

districts versus non-contaminated districts (the districts had different degrees of contamination, and serum levels for a large number of residents of each contaminated district were known), and for all six contaminated districts combined.

These same authors also conducted a separate case–control study among Ohio residents only. The Ohio registry provided more residential detail than did the West Virginia registry, enabling geocoding of exact addresses. Exposure in the case–control study in Ohio was based on estimated individual serum levels of PFOA at specific addresses at specific points in time. The individual serum PFOA levels were estimated using linked environmental and toxicokinetics models (Shin et al., 2011a, b). The environmental models estimated air and water concentrations of PFOA between 1951 and 2008, integrating emissions data from the facilities, fate, and transport characteristics of PFOA, and addresses of case and control participants, and then, using estimated water consumption and PFOA serum half-life data, annual serum levels for those drinking the contaminated water were estimated. The authors assumed that the serum levels estimated 10 years before case diagnosis (and analogously for matched controls) were the exposure of interest. Odds ratios were calculated, relative to the unexposed, for the low (3.7–12.8 µg/L [ng/mL]), medium (12.9–30.7 µg/L [ng/mL]), high (30.8–109 µg/L [ng/mL]), and very high (110–655 µg/L [ng/mL]) exposure categories. The second study used the same set of potential confounders as the first study (see above), but additionally considered race.

[The Working Group noted that there was probably some overlap between the cancer cases considered in the study by Vieira et al. (2013) and those in Barry et al. (2013), although the extent of overlap was unknown. The Working Group noted that the strengths of this study were the good case ascertainment via cancer registries, the large number of incident cancers from cancer registries, and the reasonably large number of exposed cases of many specific cancers in people in the contaminated water districts (although small numbers were sometimes an issue for analyses of rarer cancers by category of exposure). The case–control study in Ohio benefited from being able to estimate serum levels for individuals on the basis of a model that was shown to provide a good prediction of the observed levels for 30 000 residents of the six contaminated water districts at one point in time (2005–2006) (Spearman correlation, 0.71; Winquist et al., 2013). The Working Group also noted limitations, including the assignment of an ecological exposure (by water district) in the first case–control study, as well as the use of estimated individual serum levels in the second case–control study (data from Ohio only) based on a model. In this second case–control study, a limitation was also the somewhat arbitrary assumption that the estimated serum levels 10 years before case diagnosis were the most relevant, as well as the assumption that the case and control participants had remained in the same residence for 10 years. Another limitation was the fairly small number of potential confounders available in the analyses. A potential limitation for both studies was the use of people with cancer as the controls, although the authors excluded those cancers thought to be potentially positively associated with PFOA. Use of cancer controls might bias estimates to the null, if any of the included cancers were in fact associated with PFOA. The use of cancer controls also might not reflect the general population with regard to potential confounders such as socioeconomic status and diet, but these potential differences in confounders were considered unlikely to have substantive effects in this population with very high exposure.]

2.2 Cancers of the urinary tract

See Table 2.2.

2.2.1 Kidney cancer

Three occupational cohort studies (Steenland and Woskie, 2012; Consonni et al., 2013; Raleigh et al., 2014), two population-based cohort studies (Barry et al., 2013; Li et al., 2022a), two population-based nested case–control studies (Shearer et al., 2021; Rhee et al., 2023b), one population-based case–cohort study (Winquist et al., 2023) and one population-based case–control study (Vieira et al., 2013) investigated the association between PFOA or PFOS exposure and mortality from and/or relative risk of kidney cancer. Some addressed PFOA exposures in settings where co-exposure to other PFAS compounds beyond background levels was unlikely, indicating that associations, if any, would primarily be due to PFOA (Steenland and Woskie, 2012; Barry et al., 2013; Vieira et al., 2013; Raleigh et al., 2014). Other studies addressing general populations with background exposure to multiple PFAS compounds provided PFAS-specific estimates (Winquist et al., 2023) or estimated PFOA associations after controlling for co-exposure to other PFAS compounds (Shearer et al., 2021; Rhee et al., 2023b).

Raleigh et al. (2014) investigated mortality and cancer incidence in an occupational cohort including 4668 employees working for ≥ 365 days from 1947 through 2002 at an APFO facility in Cottage Grove, Minnesota (in the Minneapolis metropolitan area), USA, and 4359 employees working for ≥ 365 days before 1999 at a tape and abrasives production facility (reference group). Individual cumulative airborne exposure to APFO was estimated. The study updated earlier studies of the same cohort (Gilliland and Mandel, 1993; Lundin et al., 2009) (see Section 2.1.1). There was no indication of increased risk of kidney cancer on the basis of either mortality data (24 deaths across the exposed and reference populations) or incidence data (35 cases).

[The Working Group noted that study strengths were complete ascertainment of the cohort, very limited loss to cancer follow-up, and quantitative cumulative exposure assessment with a large exposure contrast. Co-exposure to TFE (IARC Group 2A; with *inadequate* evidence in humans, but *sufficient* evidence in experimental animals, with evidence that it is a potent carcinogen in rats and mice, IARC, 2016) was addressed explicitly and found to be minimal. The small number of cases created uncertain risk estimates. Non-differential misclassification of exposure may have caused bias towards the null, and risk estimates with reference to unexposed workers should be interpreted with caution.]

Steenland and Woskie (2012) studied cause-specific mortality among 5791 fluoropolymer-production workers (men, 81%) in a polymer-production plant in Parkersburg, West Virginia, USA. The study was an extension by an additional 6 years of the cohort study by Leonard et al. (2008) and with a comprehensive quantitative exposure assessment. The cohort was described in detail earlier (Section 2.1.3). The mean and median estimated PFOA serum concentrations in workers from the Parkersburg plant were 350 ng/mL and 403 ng/mL, respectively, compared with a median of 4 ng/mL in the population of the USA. Mortality rates for exposed workers were compared with those for other workers from the same company in the region and the USA population.

The SMR (with other workers from the same company as the referent) for kidney cancer in the highest quartile of estimated cumulative serum PFOA concentration was 2.66 (95% CI, 1.15–5.24; 8 cases) with no lag, 2.82 (95% CI, 1.13–5.81; 7 cases) after a lag of 10 years, and 3.67 (95% CI, 1.48–7.57; 7 cases) after a lag of 20 years. Exposure–response analyses indicated a positive trend for kidney cancer in analyses with no lag and less consistently with a 10-year lag or a

Table 2.2 Epidemiological studies on exposure to PFOA and PFOS and cancers of the urinary tract

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Raleigh et al. (2014) MN, USA Enrolment, 1947–2002/follow-up, 1947–2008 (mortality), 1988–2008 (incidence) Cohort	9027 (4668 exposed workers, 4359 reference workers); Cottage Grove (MN) PFOA cohort; workers employed for ≥ 1 yr during 1947–2002 at an APFO facility (Cottage Grove; *n* = 4668); reference workers without any exposure to APFO, employed at a tape and abrasives production facility located in the same suburban geographical area and managed by the same company (Saint Paul; *n* = 4359) Exposure assessment method: see Table 2.1	Kidney, mortality	Exposed to APFO (SMR, MN referent):			Age, sex, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Reference population sharing similar socioeconomic characteristics. *Other limitations*: See Table 2.1. Lacking data on workers who left Minnesota or Wisconsin; small numbers; no accounting for health behaviours.
			Unexposed (Saint Paul plant)	18	1.23 (0.73–1.95)		
			Exposed (Cottage Grove plant)	6	0.53 (0.20–1.16)		
		Kidney, mortality	Estimated cumulative airborne APFO exposure quartile (SMR, MN referent):			Age, sex, calendar period	
			1st quartile ($< 2.6 \times 10^{-5}$ µg/m^3-years)	1	0.32 (0.01–1.77)		
			2nd quartile (2.6×10^{-5} to $< 1.4 \times 10^{-4}$ µg/m^3-years)	2	0.74 (0.09–2.69)		
			3rd quartile (1.4×10^{-4} to $< 7.3 \times 10^{-4}$ µg/m^3-years)	2	0.66 (0.08–2.38)		
			4th quartile ($\geq 7.3 \times 10^{-4}$ µg/m^3-years)	1	0.42 (0.01–2.34)		

Table 2.2 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Raleigh et al. (2014) MN, USA Enrolment, 1947–2002/follow-up, 1947–2008 (mortality), 1988–2008 (incidence) Cohort (cont.)		Kidney, incidence	Estimated cumulative airborne APFO exposure quartile (HR):			Age, sex*, year of birth	*Other comments*: *The Working Group assumed that the models were also adjusted for sex, as reported in the methods of Raleigh et al. (2014).
			Unexposed (Saint Paul plant)	19	1		
			1st quartile (< 2.9×10^{-5} µg/m^3-years)	4	1.07 (0.36–3.16)		
			2nd quartile (2.9×10^{-5} to < 1.5×10^{-4} µg/m^3-years)	4	1.07 (0.36–3.17)		
			3rd quartile (1.5×10^{-4} to < 7.9×10^{-4} µg/m^3-years)	4	0.98 (0.33–2.92)		
			4th quartile (≥ 7.9×10^{-4} µg/m^3-years)	4	0.73 (0.21–2.48)		
		Urinary bladder, mortality	Exposed to APFO (SMR, MN referent):			Age, sex, calendar period	
			Unexposed (Saint Paul plant)	8	0.62 (0.27–1.22)		
			Exposed (Cottage Grove plant)	8	0.89 (0.38–1.76)		
		Urinary bladder, mortality	Estimated cumulative airborne APFO exposure quartile (SMR, MN referent):			Age, sex, calendar period	
			1st quartile (< 2.6×10^{-5} µg/m^3-years)	1	0.40 (0.01–2.25)		
			2nd quartile (2.6×10^{-5} to < 1.4×10^{-4} µg/m^3-years)	2	0.93 (0.11–3.38)		

Table 2.2 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Raleigh et al. (2014) MN, USA Enrolment, 1947–2002/follow-up, 1947–2008 (mortality), 1988–2008 (incidence) Cohort (cont.)		Urinary bladder, mortality (cont.)	3rd quartile (1.4×10^{-4} to $< 7.3 \times 10^{-4}$ μg/m³-years)	4	1.61 (0.44–4.13)	Age, sex, calendar period	
			4th quartile ($\geq 7.3 \times 10^{-4}$ μg/m³-years)	1	0.53 (0.01–2.97)		
		Urinary bladder, incidence	Estimated cumulative airborne APFO exposure quartile (HR):			Age, sex*, year of birth	
			Unexposed (Saint Paul Plant)	43	1		
			1st quartile ($< 2.9 \times 10^{-5}$ μg/m³)	7	0.81 (0.36–1.81)		
			2nd quartile (2.9×10^{-5} to $< 1.5 \times 10^{-4}$ μg/m³)	6	0.78 (0.33–1.85)		
			3rd quartile (1.5×10^{-4} to $< 7.9 \times 10^{-4}$ μg/m³)	15	1.50 (0.80–2.81)		
			4th quartile ($\geq 7.9 \times 10^{-4}$ μg/m³)	12	1.66 (0.86–3.18)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Alexander and Olsen (2007) Decatur (AL), USA Enrolment, 1961–1997/follow-up, 1970–2002 (mortality and incidence) Cohort	1588; Decatur (AL) PFOS cohort; production workers in the Alexander et al. (2003) cohort; a questionnaire was administered to living cohort members (response rate, 73.9%) to identify incident cases of bladder cancer; bladder cancer decedents were identified using underlying cause of death from death certificates; analyses excluded 495 living cohort members who did not return the questionnaire Exposure assessment method: see Table 2.1	Urinary bladder, incidence	PFOS exposure category (SIR, US referent):			Age, sex, calendar year	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Use of incidence data with 74% participation rate in survey; use of cumulative exposure with internal comparisons, good exposure contrast; attempt to validate self-reported cancer for survey respondents. *Other limitations*: See Table 2.1. Occupational cohort with only 11 cases of bladder cancer, 2 in the highest category of exposure; bladder cancer incidence identified by survey of cohort (6 cases) and death certificates (5 deaths); no cancer registry matching; no ability to validate 5 bladder cancers identified by death certificate, mostly male (82%); only partial data on smoking.
			Never exposed	2	0.61 (0.07–2.19)		
			Ever exposed (low or high)	9	1.70 (0.77–3.22)		
			Ever high	6	1.74 (0.64–3.79)		
			Ever low	7	2.26 (0.91–4.67)		
			High for ≥ 1 yr	3	1.12 (0.23–3.27)		
			High or low for ≥ 1 yr	6	1.31 (0.48–2.85)		
		Urinary bladder, incidence	Cumulative PFOS exposure (years of employment in high PFOS-exposed jobs; SIR, US referent):			Age, sex, calendar year	
			0 to < 1	2	1.07 (0.12–3.85)		
			1 to < 5	4	0.95 (0.25–2.43)		
			5 to < 10	3	2.72 (0.55–73.95)		
			≥ 10	2	1.43 (0.16–5.15)		
		Urinary bladder, incidence	Cumulative PFOS exposure (years of employment in high PFOS-exposed jobs; RR):			Age, sex	
			0 to < 1	2	1		
			1 to < 5	4	0.83 (0.15–4.65)		
			5 to < 10	3	1.92 (0.30–12.06)		
			≥ 10	2	1.52 (0.21–10.99)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Steenland and Woskie (2012) Parkersburg (WV), USA Enrolment, 1948–2002/follow-up, 1952–2008 (mortality) Cohort	5791 workers; Parkersburg (WV, USA), polymer-production PFOA occupational cohort; workers (men, 81%) at a polymer-manufacturing facility who had potential exposure to fluoropolymers with sufficiently detailed work histories Exposure assessment method: see Table 2.1	Kidney, mortality	PFOA-exposed workers (SMR):			Age, sex, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Ability to evaluate associations with PFOA in a population exposed to levels much higher than in the general population. *Other limitations*: See Table 2.1. Restriction to mortality rates and small numbers of kidney cancer; reverse causation due to reduced glomerular function is an unresolved issue, but there was no excess of kidney disease found in in Steenland et al. (2015), who studied a subset of these workers (*n* = 3717).
			Other workers referent (same region and company)	12	1.28 (0.66–2.24)		
			US referent	12	1.09 (0.56–1.9)		
		Kidney, mortality	Cumulative serum exposure, no lag (SMR, other workers referent, same region and company):				
			1st quartile (0 to < 904 ppm-years)	1	1.07 (0.02–3.62)		
			2nd quartile (904 to < 1520 ppm-years)	3	1.37 (0.28–3.99)		
			3rd quartile (1520 to < 2700 ppm-years)	0	0.00 (0.00–1.42)		
			4th quartile (≥ 2700 ppm-years)	8	2.66 (1.15–5.24)		
			Trend-test *P*-value, 0.02				
		Kidney, mortality	Cumulative serum exposure, 10-yr lag (SMR, other workers referent, same region and company):				
			1st quartile (0 to < 798 ppm-years)	2	1.05 (0.13–3.79)		
			2nd quartile (798 to < 1379 ppm-years)	2	0.87 (0.11–3.15)		
			3rd quartile (1379 to < 2384 ppm-years)	1	0.44 (0.01–2.44)		
			4th quartile (≥ 2384 ppm-years)	7	2.82 (1.13–5.81)		
			Trend-test *P*-value, 0.02				

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Steenland and Woskie (2012) Parkersburg (WV), USA Enrolment, 1948–2002/follow-up, 1952–2008 (mortality) Cohort (cont.)		Kidney, mortality	Cumulative serum exposure, 20-yr lag (SMR, other workers referent, same region and company):			Age, sex, calendar period	
			1st quartile 1 (0 to < 515 ppm-years)	3	1.34 (0.28–3.91)		
			2nd quartile (515 to < 1057 ppm-years)	1	0.46 (0.01–2.55)		
			3rd quartile (1057 to < 1819 ppm-years)	0	0.00 (0.00–2.03)		
			4th quartile (≥ 1819 ppm-years)	7	3.67 (1.48–7.57)		
			Trend-test *P*-value, 0.003				
		Urinary bladder, mortality	PFOA-exposed workers (SMR):				
			Other workers referent (same region and company)	10	1.08 (0.52–1.99)		
			US referent	10	0.95 (0.46–1.75)		
		Urinary bladder, mortality	Cumulative serum exposure, no lag (SMR, other workers referent, same region and company):				
			1st quartile (0 to < 904 ppm-years)	2	1.24 (0.15–4.47)		
			2nd quartile (904 to < 1520 ppm-years)	6	2.49 (0.97–5.78)		
			3rd quartile (1520 to < 2700 ppm-years)	1	0.39 (0.01–2.17)		
			4th quartile (≥ 2700 ppm-years)	1	0.36 (0.10–2.01)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Steenland et al. (2015) Parkersburg (WV), USA Enrolment, 1948–2002/follow-up, 1951 to interview date in 2008–2011 (incidence) Cohort	3713 workers; a subset of Parkersburg (WV, USA), polymer-production PFOA cohort in Steenland and Woskie (2012); polymer-production workers (men, 80%) who responded (self or next-of-kin) to a questionnaire about health outcomes and who had measured or estimated occupational and residential exposure estimates; 29 incident cases of bladder cancer Exposure assessment method: see Table 2.1	Urinary bladder, incidence	Cumulative PFOA exposure, 10-yr lag (RR):			Age, sex, race, education, BMI, time-varying smoking, time-varying alcohol consumption, year of birth	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Ability to evaluate associations with PFOA in a population exposed to levels much higher than in the general population. *Other limitations*: See Table 2.1. Few bladder cancers (n = 29).
			1st quartile (< 0.8 μg/mL-years)	NR	1		
			2nd quartile (0.8 to < 3.44 μg/mL-years)	NR	0.55 (0.12–2.61)		
			3rd quartile (3.44 to < 7.04 μg/mL-years)	NR	0.47 (0.10–2.21)		
			4th quartile (≥ 7.04 μg/mL-years)	NR	0.31 (0.06–1.54)		
			Trend-test *P*-value, 0.03				

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Eriksen et al. (2009) Denmark Enrolment, 1 December 1993 to 31 May 1997/follow-up, 1 December 1993 to 1 July 2006 Case–cohort	Case–cohort within the Diet, Cancer and Health cohort Cases: 332 cases of cancer of the urinary bladder Comparison cohort: 772 (680 men, 92 women); subcohort of participants randomly selected without cancer at the end of follow-up Exposure assessment method: see Table 2.1	Urinary bladder, incidence	Baseline plasma PFOA concentration (IRR):			Age, sex, smoking status, smoking intensity, smoking duration, years of school attendance, occupation associated with bladder cancer risk (rubber industry; textile industry; metal processing; glass industry; truck, bus, taxi drivers; painter, hairdresser; waiter; cook)	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Large cohort with numerous incident cancers (n = 1240) followed 0–12 yr after baseline enrolment; control of confounders; use of internal comparison. *Other limitations*: See Table 2.1. Low exposure contrast in a population with background exposure levels.
			1st quartile	84	1		
			2nd quartile	82	0.71 (0.46–1.07)		
			3rd quartile	83	0.92 (0.61–1.39)		
			4th quartile	83	0.81 (0.53–1.24)		
			Continuous (per 1 ng/mL increase)	332	1.00 (0.95–1.05)		
		Urinary bladder, incidence	Baseline plasma PFOS concentration (IRR):				
			1st quartile	83	1		
			2nd quartile	84	0.76 (0.50–1.16)		
			3rd quartile	83	0.93 (0.61–1.41)		
			4th quartile	82	0.70 (0.46–1.07)		
			Continuous (per 10 ng/mL increase)	332	0.93 (0.83–1.03)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Barry et al. (2013) Mid-Ohio Valley (OH and WV), USA Enrolment, August 2005 to August 2006/ follow-up, 1952–2011 (incidence) Cohort	32 254 (28 541 community members and 3713 workers); C8 Science Panel Study; included people enrolled in the C8 Health Project who lived, worked, or attended school for ≥ 1 yr between 1950 and 3 December 2004 in a contaminated-water district in the vicinity of a chemical plant using PFOA in manufacturing processes (Parkersburg, WV; polymer-production facility), as well as a subset of those from the original Parkersburg (WV), polymer-production PFOA occupational cohort who worked at the plant between 1948 and 2002 Exposure assessment method: see Table 2.1	Kidney, incidence	Estimated cumulative PFOA serum concentration (ng/mL), no lag (HR):			Age, time-varying smoking, time-varying alcohol consumption, sex, education, birth year (5-yr calendar intervals)	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Large cohort and strong exposure contrast, lagged analyses, adjustment for several covariates. *Other limitations*: See Table 2.1. Self-reported cancer data. Co-exposure to other PFAS in residents not evaluated.
			1st quartile	NR	1		
			2nd quartile	NR	1.23 (0.70–2.17)		
			3rd quartile	NR	1.48 (0.84–2.60)		
			4th quartile	NR	1.58 (0.88–2.84)		
			Continuous (per unit on natural log scale)	105	1.10 (0.98–1.24)		
			Trend-test *P*-value, 0.18				
		Kidney, incidence	Estimated cumulative PFOA serum concentration (ng/mL), 10-yr lag (HR):				
			1st quartile	NR	1		
			2nd quartile	NR	0.99 (0.53–1.85)		
			3rd quartile	NR	1.69 (0.93–3.07)		
			4th quartile	NR	1.43 (0.76–2.69)		
			Continuous (per unit on natural log scale)	105	1.09 (0.97–1.21)		
			Trend-test *P*-value, 0.34				
		Urinary bladder, incidence	Estimated cumulative PFOA serum concentration (ng/mL), no lag (HR):				
			Continuous (per unit on natural log scale)	105	1.00 (0.89–1.12)		
		Urinary bladder, incidence	Estimated cumulative PFOA serum concentration (ng/mL), 10-yr lag (HR):				
			Continuous (per unit on natural log scale)	105	0.98 (0.88–1.10)		

Table 2.2 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Consonni et al. (2013) USA, UK, Italy, Germany, the Netherlands Enrolment, 1950–2002/follow-up, 1950–2008 (mortality) Cohort	5879 male workers (4205 APFO-exposed); the pooled international TFE cohort includes male workers who were ever employed or employed for 6 or 12 mo at one or more of six TFE-production sites in North America and Europe in 1950–2002; the principal occupational exposures were TFE and APFO (facilitating production of PTFE) Exposure assessment method: see Table 2.1	Kidney and other organs in the urinary tract, mortality	Cumulative APFO exposure (SMR, national referent):			Age, calendar period, country	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. The cohort includes all TFE production sites worldwide during the entire period of production and benefits from almost complete enrolment and follow-up data. *Other limitations*: See Table 2.1. Low statistical power to detect less-common cancers; high exposure correlations between TFE monomer and PFOA which precluded evaluation of effects of the individual compounds.
			Ever APFO-exposed	10	1.69 (0.81–3.11)		
			< 16 unit-year	3	1.57 (0.32–4.59)		
			16–138 unit-year	3	1.50 (0.31–4.39)		
			139+ unit-year	4	2.00 (0.54–5.12)		
			Trend-test *P*-value, 0.28				
		Urinary bladder, mortality	SMR (national referent):				
			Ever APFO-exposed	3	0.55 (0.11–1.60)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Shearer et al. (2021) USA Enrolment, 1993–2001; follow-up (from blood draw): median, 8.8 yr (incidence) Nested case–control	Nested within the PLCO cohort (see Table 2.1) Cases: 324; cancer source not reported Controls: 324; density-sampled on calendar time and individually matched on age categories, sex, race and ethnicity, study centre, and year of blood draw Exposure assessment method: see Table 2.1	Kidney (RCC), incidence	Serum PFOA (OR):			Age, sex, race/ ethnicity, study centre, study year of blood draw, BMI, smoking status, history of hypertension, glomerular filtration rate, previous freeze–thaw cycle, calendar year of blood draw	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. High specificity of the outcome. Adjustment for kidney function to exclude reverse causation and for relevant potential confounders. *Other limitations*: See Table 2.1. Exposure assessment at a single time point likely attenuated risk estimates; no lagged analyses and no analyses of risk according to cumulative exposure; external validity is limited by a study population defined by phlebotomy and including mainly non-Hispanic Whites.
			< 4.0 µg/L	47	1		
			≥ 4.0 to 5.5 µg/L	83	1.47 (0.77–2.80)		
			> 5.5 to 7.3 µg/L	69	1.24 (0.64–2.41)		
			> 7.3 to 27.2 µg/L	125	2.63 (1.33–5.20)		
			Continuous (per unit on $\log_2$ scale)	324	1.71 (1.23–2.37)		
			Trend-test *P*-value, 0.007				
		Kidney (RCC), incidence	Serum PFOA (OR):			Age, sex, race/ ethnicity, study centre, study year of blood draw, BMI, smoking status, history of hypertension, glomerular filtration rate, previous freeze–thaw cycle, calendar year of blood draw, PFOS serum concentration, PFHxS serum concentration	
			< 4.0 µg/L	47	1		
			≥ 4.0 to 5.5 µg/L	83	1.41 (0.69–2.90)		
			> 5.5 to 7.3 µg/L	69	1.12 (0.52–2.42)		
			> 7.3 to 27.2 µg/L	125	2.19 (0.86–5.61)		
			Continuous (per unit on $\log_2$ scale)	324	1.68 (1.07–2.63)		
			Trend-test *P*-value, 0.13				

Table 2.2 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Shearer et al. (2021) USA Enrolment, 1993–2001; follow-up (from blood draw): median, 8.8 yr (incidence) Nested case–control (cont.)		Kidney (RCC), incidence	Serum PFOA (OR):			Age, sex, race/ethnicity, glomerular filtration rate, BMI, history of hypertension, smoking status, previous freeze–thaw cycle, calendar year of blood draw, study year of blood draw, study centre	
			Time from blood draw, ≥ 8 yr: Continuous (per unit on log_2 scale)	NR	1.66 (1.25–2.19)		
		Kidney (RCC), incidence	Serum PFOS (OR):				
			≤ 26.3 μg/L	60	1		
			> 26.3 to 38.4 μg/L	82	1.67 (0.84–3.30)		
			> 38.4 to 49.9 μg/L	61	0.92 (0.45–1.88)		
			> 49.9 to 154.2 μg/L	121	2.51 (1.28–4.92)		
			Continuous (per unit on log_2 scale)	324	1.39 (1.04–1.86)		
			Trend-test *P*-value, 0.009				
		Kidney (RCC), incidence	Serum PFOS (OR):			Age, sex, race/ethnicity, study centre, study year of blood draw, BMI, smoking status, history of hypertension, glomerular filtration rate, previous freeze–thaw cycle, calendar year of blood draw, PFOA serum concentration, PFHxS serum concentration	
			≤ 26.3 μg/L	60	1		
			> 26.3 to 38.4 μg/L	82	1.24 (0.59–2.57)		
			> 38.4 to 49.9 μg/L	61	0.53 (0.22–1.24)		
			> 49.9 to 154.2 μg/L	121	1.14 (0.45–2.88)		
			Continuous (per unit on log_2 scale)	324	0.92 (0.60–1.42)		
			Trend-test *P*-value, 0.64				

Table 2.2 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Steenland et al. (2022) USA Enrolment, 1993–2014 (PLCO); August 2005 to August 2006 (C8 Panel study); follow-up, 1993–2014 (PLCO), 1952–2011 (C8 Panel study) Nested case–control	Cases: PLCO, 324; C8 Panel study, 103; all cases of RCC; cases from the PLCO cohort were the same as those identified in Shearer et al. (2021); cases from the C8 study were identified in the C8 panel cohort study (Barry et al., 2013) using the topographical code C64.9 and excluding urothelial carcinomas (e.g. morphology codes 8120, 8130), to capture mostly RCCs Controls: PLCO, 324; C8 panel study, 511; for the PLCO component, controls were the same as those identified in Shearer et al. (2021); for the C8 component, up to 5 controls per case were selected, matched on sex, race, year of birth (within 5 yr); controls were required to have survived past the age at which the case was diagnosed Exposure assessment method: see Table 2.1 for Shearer et al. (2021) and Barry et al. (2013)	Kidney (RCC), incidence	Serum PFOA, 2-piece linear spline (not transformed) model (log odds):			Age, sex, race/ethnicity, study centre (PLCO), year of blood draw (PLCO), birth year (C8), BMI, hypertension	*Exposure assessment method*: See Table 2.1 for Shearer et al. (2021) and Barry et al. (2013). *Other strengths*: See Table 2.1. Pooled analysis of large and informative studies on kidney cancer. *Other limitations*: See Table 2.1. Cumulative serum levels were not available in the PLCO study.
			Continuous (per ng/mL increase up to the knot (9.5 ng/mL))	427	0.135 (0.071–0.198)		
		Kidney (RCC), incidence	Serum PFOA, 2-piece linear spline (natural log-transformed) model (best-fitting) (log odds):				
			Continuous (per unit increase up to the knot (ln PFOA = 2.55))	427	0.656 (0.333–0.979)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Li et al. (2022a) Ronneby, southern Sweden Enrolment, 1985–2013/follow-up, 1985–2016 (incidence) Cohort	60 507; the Ronneby Register Cohort included all individuals who ever lived in Ronneby municipality in 1985–2013; one third of the households received PFAS-contaminated drinking-water from a waterworks situated near a military airfield where PFAS-containing firefighting foam was used in 1985–2013 (15 811 individuals considered “ever-high”); subsets with long-term exposure (≥ 11 yr) in the latest part of the follow-up period (2005–2013) were considered to be more highly exposed Exposure assessment method: see Table 2.1	Kidney, incidence	Men, residential exposure to highly PFAS-contaminated drinking-water (SIR, Blekinge county excluding Ronneby referent):			Age, calendar year	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Complete registration of a large cohort; no loss to follow-up; long follow-up period. *Other limitations*: See Table 2.1. Mixed PFAS exposure (mainly PFOS, PFHxS and PFOA); no adjustment for known determinants of kidney cancer such as hypertension and overweight; relatively few cases producing uncertain risk estimates.
			Never	46	0.67 (0.49–0.90)		
			Ever	17	0.86 (0.50–1.38)		
		Kidney, incidence	Women, residential exposure to highly PFAS-contaminated drinking-water (SIR, Blekinge county excluding Ronneby referent):			Age, calendar year	
			Never	43	1.17 (0.84–1.57)		
			Ever	16	1.47 (0.84–2.39)		
		Kidney, incidence	Residential exposure to highly PFAS-contaminated drinking-water (HR):			Calendar year, age, sex	
			Never	89	1		
			Ever	33	1.27 (0.85–1.91)		
		Kidney, incidence	Time period of residential exposure to highly PFAS-contaminated drinking-water (HR):			Calendar year, age, sex	
			Never	89	1		
			Early (1985–2004)	19	1.05 (0.64–1.73)		
			Late (2005–2013)	14	1.85 (1.00–3.40)		
		Kidney, incidence	Duration of residential exposure to highly PFAS-contaminated drinking-water (HR):			Calendar year, age, sex	
			Never	89	1		
			Short (1–10 yr)	15	1.11 (0.64–1.92)		
			Long (≥ 11 yr)	18	1.47 (0.87–2.49)		
		Urinary bladder, incidence	Men, residential exposure to highly PFAS-contaminated drinking-water (SIR, Blekinge county excluding Ronneby referent):			Age, calendar year	
			Never	166	0.94 (0.80–1.09)		
			Ever	57	1.10 (0.84–1.43)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Li et al. (2022a) Ronneby, southern Sweden Enrolment, 1985–2013/follow-up, 1985–2016 (incidence) Cohort (cont.)		Urinary bladder, incidence	Women, residential exposure to highly PFAS-contaminated drinking-water (SIR, Blekinge county excluding Ronneby referent):			Age, calendar year	
			Never	35	0.69 (0.48–0.95)		
			Ever	17	1.13 (0.66–1.80)		
		Urinary bladder, incidence	Residential exposure to highly PFAS-contaminated drinking-water (HR):			Calendar year, age, sex	
			Never	200	1		
			Ever	74	1.30 (0.99–1.69)		
		Urinary bladder, incidence	Time period of residential exposure to highly PFAS-contaminated drinking-water (HR):			Calendar year, age, sex	
			Never	200	1		
			Early (1985–2004)	46	1.20 (0.87–1.66)		
			Late (2005–2013)	28	1.50 (0.98–2.29)		
		Urinary bladder, incidence	Duration of residential exposure to highly PFAS-contaminated drinking-water (HR):			Calendar year, age, sex	
			Never	200	1		
			Short (1–10 yr)	39	1.23 (0.87–1.73)		
			Long (≥ 11 yr)	35	1.39 (0.95–2.02)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rhee et al. (2023b) CA and HI, USA Enrolment, 1993–1996/follow-up, through 2018 Nested case–control	Nested within the MEC cohort Cases: 428; all RCC cases identified as of 2018 in the MEC study, with available pre-diagnostic serum sample; incident cases identified through linkage with the SEER HI registry and the CA state cancer registry Controls: 428; controls were MEC participants alive at the time of the matched case diagnosis and matched 1:1 to cases on sex, race or ethnicity, study centre, age and date at serum collection, time of serum collection, and fasting status Exposure assessment method: see Table 2.1	Kidney (RCC), incidence	PFOA serum concentration (OR):			Sex, race/ ethnicity, study centre, age at serum collection, date of serum collection, time of serum collection, fasting status, smoking status, BMI, history of hypertension, eGFR, PFOS (log_2-transformed), PFHxS (log_2-transformed), PFNA (log_2-transformed), FOSA detected	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Large sample size; consideration of multiple PFAS adjustment; stratification by race/ ethnicity. *Other limitations*: See Table 2.1. Some of the stratified analysis by race/ethnicity have limited statistical power.
			1st quartile (≤ 3.27 μg/L)	107	1		
			2nd quartile (> 3.27 to 4.47 μg/L)	99	1.26 (0.80–1.97)		
			3rd quartile (> 4.47 to 6.22 μg/L)	122	1.26 (0.78–2.05)		
			4th quartile (> 6.22 μg/L)	100	1.04 (0.60–1.81)		
			Continuous (per unit on log_2 scale)	428	0.89 (0.67–1.18)		
			Trend-test *P*-value, 0.75				
		Kidney (RCC), incidence	White participants, PFOA serum concentration (OR):			Sex, study centre, age at serum collection, date of serum collection, time of serum collection, fasting status, smoking status, BMI, history of hypertension, eGFR, PFOS (log_2-transformed), PFHxS (log_2-transformed), PFNA (log_2-transformed), FOSA detected	
			1st quartile (≤ 3.27 μg/L)	19	1		
			2nd quartile (> 3.27 to 4.47 μg/L)	15	2.08 (0.62–6.98)		
			3rd quartile (> 4.47 to 6.22 μg/L)	24	3.63 (0.84–15.8)		
			4th quartile (> 6.22 μg/L)	22	2.94 (0.56–15.5)		
			Continuous (per unit on log_2 scale)	80	2.12 (0.87–5.18)		
			Trend-test *P*-value, 0.48				

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rhee et al. (2023b) CA and HI, USA Enrolment, 1993–1996/follow-up, through 2018 Nested case–control (cont.)		Kidney (RCC), incidence	African-American participants, PFOA serum concentration (OR):			Sex, study centre, age at serum collection, date of serum collection, time of serum collection, fasting status, smoking status, BMI, history of hypertension, eGFR, PFOS (log_2-transformed), PFHxS (log_2-transformed), PFNA (log_2-transformed), FOSA detected	
			1st quartile (≤ 3.27 μg/L)	24	1		
			2nd quartile (> 3.27 to 4.47 μg/L)	15	1 (0.23–4.33)		
			3rd quartile (> 4.47 to 6.22 μg/L)	17	1.01 (0.24–4.23)		
			4th quartile (> 6.22 μg/L)	16	1.08 (0.23–5.13)		
			Continuous (per unit on log_2 scale)	72	1.01 (0.51–1.98)		
			Trend-test *P*-value, 0.91				
		Kidney (RCC), incidence	Japanese-American participants, PFOA serum concentration (OR):				
			1st quartile (≤ 3.27 μg/L)	14	1		
			2nd quartile (> 3.27 to 4.47 μg/L)	25	2.62 (0.79–8.69)		
			3rd quartile (> 4.47 to 6.22 μg/L)	37	2.65 (0.77–9.15)		
			4th quartile (> 6.22 μg/L)	31	3.29 (0.84–12.88)		
			Continuous (per unit on log_2 scale)	107	1.00 (0.47–2.13)		
			Trend-test *P*-value, 0.22				

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rhee et al. (2023b) CA and HI, USA Enrolment, 1993–1996/follow-up, through 2018 Nested case–control (cont.)		Kidney (RCC), incidence	Native Hawaiian participants, PFOA serum concentration (OR):			Sex, study centre, age at serum collection, date of serum collection, time of serum collection, fasting status, smoking status, BMI, history of hypertension, eGFR, PFOS ($\log_2$-transformed), PFHxS ($\log_2$-transformed), PFNA ($\log_2$-transformed), FOSA detected	
			1st quartile (≤ 3.27 μg/L)	12	1		
			2nd quartile (> 3.27 to 4.47 μg/L)	10	0.3 (0.04–2.31)		
			3rd quartile (> 4.47 to 6.22 μg/L)	17	0.28 (0.03–2.39)		
			4th quartile (> 6.22 μg/L)	11	0.08 (0.01–0.94)		
			Continuous (per unit on $\log_2$ scale)	50	0.57 (0.21–1.55)		
			Trend-test *P*-value, 0.04				
		Kidney (RCC), incidence	PFOA serum concentration (OR):			Sex, race/ ethnicity, study centre, age at serum collection, date of serum collection, time of serum collection, fasting status, smoking status, BMI, history of hypertension, eGFR, PFOS ($\log_2$-transformed), PFHxS ($\log_2$-transformed), PFNA ($\log_2$-transformed), FOSA detected	
			Calendar year blood drawn, before 2002: Continuous (per unit on $\log_2$ scale)	90	1.49 (0.77–2.87)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rhee et al. (2023b) CA and HI, USA Enrolment, 1993–1996/follow-up, through 2018 Nested case–control (cont.)		Kidney (RCC), incidence	PFOA serum concentration (OR):			Sex, race/ ethnicity, study centre, age at serum collection, date of serum collection, time of serum collection, fasting status, smoking status, BMI, history of hypertension, eGFR, PFOS (log_2-transformed), PFHxS (log_2-transformed), PFNA (log_2-transformed), FOSA detected	
			Calendar year blood drawn, in 2002 or later: Continuous (per unit on log_2 scale)	336	0.80 (0.56–1.13)		
		Kidney (RCC), incidence	PFOS serum concentration (OR):				
			1st quartile (< 16.65 µg/L)	118	1		
			2nd quartile (16.65 to < 25.05 µg/L)	105	1.05 (0.66–1.66)		
			3rd quartile (25.05 to < 36.40 µg/L)	100	0.99 (0.58–1.68)		
			4th quartile (≥ 36.40 µg/L)	105	0.93 (0.51–1.72)		
			Continuous (per unit on log_2 scale)	428	0.95 (0.74–1.23)		
			Trend-test *P*-value, 0.72				
		Kidney (RCC), incidence	PFOS serum concentration (OR):				
			Calendar year blood drawn, before 2002: Continuous (per unit on log_2 scale)	90	0.77 (0.40–1.48)		
		Kidney (RCC), incidence	PFOS serum concentration (OR):				
			Calendar year blood drawn, in 2002 or later: Continuous (per unit on log_2 scale)	336	0.96 (0.73–1.28)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Winquist et al. (2023) 20 states, USA Enrolment, 1998–2001/follow-up, through 30 June 2015 Case–cohort	Case–cohort within the CPS-II Lifelink Cohort (see Table 2.1) Cases: 3762 overall (kidney cancer, 158, of which 109 were RCC, and urinary bladder, 401); incident cases from the CPS-II Lifelink Cohort (surviving CPS-II Nutrition cohort participants) with a first cancer diagnosis of kidney, urinary bladder detected through self-report or NDI linkage, and verified through medical-record review or cancer registry Controls: 999; a sex-stratified simple random sample of 499 women and 500 men (~3% of the eligible cohort); stratification sampling was to ensure an adequate number of subcohort participants in sex-specific analyses (for breast and prostate cancers) Exposure assessment method: see Table 2.1	Kidney, incidence	Serum PFOA concentration (HR):			Sex, year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Large number of cases. *Other limitations*: See Table 2.1. Survivor cohort with blood collected from persons mostly over aged 65, thus the study would not include persons who may have had PFOA- or PFOS-related cancer developed earlier in life, resulting in bias towards the null or even towards inverse associations.
			1st quartile (< 3.900 ng/mL)	39	1		
			2nd quartile (3.900 to < 5.200 ng/mL)	39	0.93 (0.56–1.56)		
			3rd quartile (5.200 to < 7.300 ng/mL)	39	0.83 (0.49–1.40)		
			4th quartile (≥ 7.300 ng/mL)	39	1.20 (0.71–2.04)		
			Continuous (per unit on log_2 scale)	156	1.08 (0.88–1.33)		
		Kidney, incidence	Women, serum PFOA concentration (HR):			Year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 3.900 ng/mL)	17	1		
			2nd quartile (3.900 to < 5.200 ng/mL)	13	0.80 (0.34–1.87)		
			3rd quartile (5.200 to < 7.300 ng/mL)	17	1.04 (0.45–2.44)		
			4th quartile (≥ 7.300 ng/mL)	18	1.94 (0.87–4.35)		
			Continuous (per unit on log_2 scale)	65	1.33 (0.97–1.83)		
		Kidney, incidence	Men, serum PFOA concentration (HR):				
			1st quartile (< 3.900 ng/mL)	22	1		
			2nd quartile (3.900 to < 5.200 ng/mL)	26	0.87 (0.43–1.75)		
			3rd quartile (5.200 to < 7.300 ng/mL)	22	0.65 (0.31–1.35)		
			4th quartile (≥ 7.300 ng/mL)	21	0.81 (0.39–1.68)		
			Continuous (per unit on log_2 scale)	91	0.89 (0.66–1.20)		

Table 2.2 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Winquist et al. (2023) 20 states, USA Enrolment, 1998–2001/follow-up, through 30 June 2015 Case–cohort (cont.)		Kidney, incidence	Serum PFOS concentration (HR):			Sex, year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 13.000 ng/mL)	35	1		
			2nd quartile (13.000–< 18.000 ng/mL)	39	0.92 (0.54–1.57)		
			3rd quartile (18.000-< 26.000 ng/mL)	42	0.97 (0.58–1.63)		
			4th quartile (≥ 26.000 ng/mL)	40	1.14 (0.67–1.92)		
			Continuous (per unit on log_2 scale)	156	1.03 (0.84–1.26)		
		Kidney, incidence	Women, serum PFOS concentration (HR):			Year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 13.000 ng/mL)	19	1		
			2nd quartile (13.000 to < 18.000 ng/mL)	10	0.37 (0.14–0.94)		
			3rd quartile (18.000 to < 26.000 ng/mL)	17	0.76 (0.35–1.66)		
			4th quartile (≥ 26.000 ng/mL)	19	0.93 (0.40–2.15)		
			Continuous (per unit on log_2 scale)	65	1.06 (0.70–1.59)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Winquist et al. (2023) 20 states, USA Enrolment, 1998–2001/follow-up, through 30 June 2015 Case–cohort (cont.)		Kidney, incidence	Men, serum PFOS concentration (HR):			Year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 13.000 ng/mL)	16	1		
			2nd quartile (13.000 to < 18.000 ng/mL)	29	1.72 (0.82–3.61)		
			3rd quartile (18.000 to < 26.000 ng/mL)	25	1.39 (0.66–2.93)		
			4th quartile (≥ 26.000 ng/mL)	21	1.33 (0.62–2.85)		
			Continuous (per unit on log_2 scale)	91	1.00 (0.79–1.28)		
		Kidney (RCC), incidence	Serum PFOA concentration (HR):			Sex, year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 3.900 ng/mL)	27	1		
			2nd quartile (3.900 to < 5.000 ng/mL)	25	1.00 (0.54–1.87)		
			3rd quartile (5.000 to < 7.400 ng/mL)	28	0.74 (0.40–1.36)		
			4th quartile (≥ 7.400 ng/mL)	27	1.21 (0.65–2.27)		
			Continuous (per unit on log_2 scale)	107	1.06 (0.83–1.35)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Winquist et al. (2023) 20 states, USA Enrolment, 1998–2001/follow-up, through 30 June 2015 Case–cohort (cont.)		Kidney (RCC), incidence	Women, serum PFOA concentration (HR):			Year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 3.900 ng/mL)	8	1		
			2nd quartile (3.900 to < 5.000 ng/mL)	8	1.33 (0.42–4.19)		
			3rd quartile (5.000 to < 7.400 ng/mL)	13	1.66 (0.54–5.12)		
			4th quartile (≥ 7.400 ng/mL)	13	3.14 (1.04–9.54)		
			Continuous (per unit on log_2 scale)	42	1.54 (1.05–2.26)		
		Kidney (RCC), incidence	Men, serum PFOA concentration (HR):				
			1st quartile (< 3.900 ng/mL)	19	1		
			2nd quartile (3.900–< 5.000 ng/mL)	17	0.79 (0.36–1.74)		
			3rd quartile (5.000–< 7.400 ng/mL)	15	0.45 (0.20–1.01)		
			4th quartile (≥ 7.400 ng/mL)	14	0.64 (0.28–1.46)		
			Continuous (per unit on log_2 scale)	65	0.80 (0.57–1.11)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Winquist et al. (2023) 20 states, USA Enrolment, 1998–2001/follow-up, through 30 June 2015 Case–cohort (cont.)		Kidney (RCC), incidence	Serum PFOS concentration (HR):			Sex, year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 13.000 ng/mL)	25	1		
			2nd quartile (13.000 to < 19.000 ng/mL)	29	0.82 (0.45–1.49)		
			3rd quartile (19.000 to < 26.000 ng/mL)	24	0.96 (0.51–1.80)		
			4th quartile (≥ 26.000 ng/mL)	29	1.13 (0.61–2.07)		
			Continuous (per unit on log_2 scale)	107	1.08 (0.84–1.38)		
		Kidney (RCC), incidence	Women, serum PFOS concentration (HR):			Year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 13.000 ng/mL)	11	1		
			2nd quartile (13.000 to < 19.000 ng/mL)	6	0.40 (0.12–1.35)		
			3rd quartile (19.000 to < 26.000 ng/mL)	10	0.89 (0.32–2.46)		
			4th quartile (≥ 26.000 ng/mL)	15	1.29 (0.45–3.74)		
			Continuous (per unit on log_2 scale)	42	1.30 (0.77–2.20)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Winquist et al. (2023) 20 states, USA Enrolment, 1998–2001/follow-up, through 30 June 2015 Case–cohort (cont.)		Kidney (RCC), incidence	Men, serum PFOS concentration (HR):			Year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 13.000 ng/mL)	14	1		
			2nd quartile (13.000 to < 19.000 ng/mL)	23	1.25 (0.57–2.74)		
			3rd quartile (19.000 to < 26.000 ng/mL)	14	1.10 (0.46–2.60)		
			4th quartile (≥ 26.000 ng/mL)	14	0.98 (0.42–2.29)		
			Continuous (per unit on log_2 scale)	65	0.97 (0.73–1.29)		
		Urinary bladder, incidence	Serum PFOA concentration (HR):			Sex, year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 3.800 ng/mL)	95	1		
			2nd quartile (3.800 to < 5.100 ng/mL)	97	0.84 (0.56–1.26)		
			3rd quartile (5.100 to < 6.700 ng/mL)	99	0.87 (0.58–1.30)		
			4th quartile (≥ 6.700 ng/mL)	105	0.86 (0.58–1.27)		
			Continuous (per unit on log_2 scale)	396	0.93 (0.77–1.13)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Winquist et al. (2023) 20 states, USA Enrolment, 1998–2001/follow-up, through 30 June 2015 Case–cohort (cont.)		Urinary bladder, incidence	Women, serum PFOA concentration (HR):			Year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 3.800 ng/mL)	25	1		
			2nd quartile (3.800 to < 5.100 ng/mL)	25	1.23 (0.60–2.52)		
			3rd quartile (5.100 to < 6.700 ng/mL)	12	0.68 (0.27–1.70)		
			4th quartile (≥ 6.700 ng/mL)	20	0.81 (0.37–1.78)		
			Continuous (per unit on log_2 scale)	82	0.91 (0.63–1.31)		
		Urinary bladder, incidence	Men, serum PFOA concentration (HR):				
			1st quartile (< 3.800 ng/mL)	70	1		
			2nd quartile (3.800 to < 5.100 ng/mL)	72	0.80 (0.49–1.32)		
			3rd quartile (5.100 to < 6.700 ng/mL)	87	0.92 (0.57–1.49)		
			4th quartile (≥ 6.700 ng/mL)	85	0.87 (0.54–1.40)		
			Continuous (per unit on log_2 scale)	314	0.93 (0.74–1.17)		

Table 2.2 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Winquist et al. (2023) 20 states, USA Enrolment, 1998–2001/follow-up, through 30 June 2015 Case–cohort (cont.)		Urinary bladder, incidence	Serum PFOS concentration (HR):			Sex, year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 13.000 ng/mL)	95	1		
			2nd quartile (13.000 to < 18.000 ng/mL)	92	0.81 (0.54–1.21)		
			3rd quartile (18.000 to < 25.000 ng/mL)	106	1.07 (0.72–1.60)		
			4th quartile (≥ 25.000 ng/mL)	103	0.96 (0.64–1.44)		
			Continuous (per unit on log_2 scale)	396	1.01 (0.86–1.20)		
		Urinary bladder, incidence	Women, serum PFOS concentration (HR):			Year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 13.000 ng/mL)	27	1		
			2nd quartile (13.000– < 18.000 ng/mL)	17	0.51 (0.24–1.05)		
			3rd quartile (18.000– < 25.000 ng/mL)	20	0.65 (0.33–1.30)		
			4th quartile (≥ 25.000 ng/mL)	18	0.63 (0.29–1.35)		
			Continuous (per unit on log_2 scale)	82	0.82 (0.58–1.16)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Winquist et al. (2023) 20 states, USA Enrolment, 1998–2001/follow-up, through 30 June 2015 Case–cohort (cont.)		Urinary bladder, incidence	Men, serum PFOS concentration (HR):			Year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	
			1st quartile (< 13.000 ng/mL)	68	1		
			2nd quartile (13.000– < 18.000 ng/mL)	75	0.92 (0.57–1.49)		
			3rd quartile (18.00– < 25.000 ng/mL)	86	1.20 (0.75–1.94)		
			4th quartile (≥ 25.000 ng/mL)	85	1.10 (0.68–1.78)		
			Continuous (per unit on $\log_2$ scale)	314	1.06 (0.78–1.28)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Vieira et al. (2013) OH and WV, USA 1996–2005 (incidence) Case–control	Cases: study 1: kidney cancer, 751, and bladder cancer, 1350; study 2: kidney cancer, 246, and bladder cancer, 395; index cancer cases were retrieved from cancer registries covering a community sample with relatively high exposure to PFOA because of contamination of drinking-water from the Parkersburg (WV), polymer-production plant; 18 different cancers were analysed (bladder, brain, female breast, cervix, colon/ rectum, kidney, leukaemia, liver, lung, melanoma of the skin, multiple myeloma, NHL, ovary, pancreas, prostate, testis, thyroid, and uterus)	Kidney, incidence	Study 1. Residence in a PFOA-contaminated water district (OH and WV) (OR):			Age, sex, diagnosis year, insurance provider, smoking status	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Well-ascertained cases based on cancer registries. *Other comments*: See Table 2.1. Substantial overlap of the study population addressed by a C8 Science Panel Project by Barry et al. (2013).
			Unexposed	657	1		
			Any exposed water district	94	1.1 (0.9–1.4)		
		Kidney, incidence	Study 2. Individual-level annual PFOA serum exposure, assuming 10-yr residency and latency (OH only) (OR):			Age, race, sex, diagnosis year, insurance provider, smoking status	
			Unexposed	187	1		
			Low (3.7–12.8 μg/L)	11	0.8 (0.4–1.5)		
			Medium (12.9–30.7 μg/L)	17	1.2 (0.7–2.0)		
			High (30.8–109 μg/L)	22	2.0 (1.3–3.2)		
			Very high (110–655 μg/L)	9	2.0 (1.0–3.9)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Vieira et al. (2013) OH and WV, USA 1996–2005 (incidence) Case–control (cont.)	Controls: study 1: 23 548 (for kidney), 22 198 (for bladder) other cancers; study 2: 7339 (for kidney), 6944 (for bladder); for each cancer site evaluated, controls were cases of cancer at all other sites, (excluding sites in the kidney, testis, pancreas, and liver, which have been associated with PFOA in studies in experimental animals or humans) Exposure assessment method: See Table 2.1	Urinary bladder, incidence	Study 1. Residence in a PFOA-contaminated water district (OH and WV) (OR):			Age, sex, diagnosis year, insurance provider, smoking status	
			Unexposed	1213	1		
			Any exposed water district	137	0.8 (0.7–1.0)		
		Urinary bladder, incidence	Study 2. Individual-level annual PFOA serum exposure, assuming 10-yr residency and latency (OH only) (OR):			Age, race, sex, diagnosis year, insurance provider, smoking status	
			Unexposed	326	1		
			Low (3.7–12.8 μg/L)	23	0.9 (0.6–1.4)		
			Medium (12.9–30.7 μg/L)	21	0.9 (0.6–1.4)		
			High (30.8–109 μg/L)	21	1.2 (0.8–2.0)		
			Very high (110–655 μg/L)	4	0.6 (0.2–1.5)		

AL, Alabama; APFO, ammonium perfluorooctanoate; approx., approximately; BMI, body mass index; CA, California; CI, confidence interval; CPS-II, Cancer Prevention Study II; eGFR, estimated glomerular filtration rate; FOSA, perfluorooctane sulfonamide; HI, Hawaii; HR, hazard ratio; IRR, incidence rate ratio; MEC, Multiethnic Cohort; MN, Minnesota; NDI, National Death Index; NR, not reported; OH, Ohio; OR, odds ratio; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; ppm, parts per million; PTFE, polytetrafluoroethylene; RCC, renal cell carcinoma; RR, rate ratio; SEER, Surveillance, Epidemiology, and End Results; SIR, standardized incidence ratio; SMR, standardized mortality ratio; TFE, tetrafluoroethylene; UK, United Kingdom; US, United States; USA, United States of America; WV, West Virginia; yr, year(s).

20-year lag. Numbers were small (1–7 cases in each quartile of exposure with a 10-year lag), but the test for trend across quartiles was significant.

[The Working Group noted that the detailed historical exposure assessment using blood samples to model serum PFOA levels across time was an improvement in the exposure assessment. Results were limited by restriction to mortality rates and by small numbers of fatal kidney cancer. Possible co-exposure to TFE (IARC Group 2A) could not be excluded but seemed of minor importance. Earlier indications that PFOA may be associated with kidney disease and reduced glomerular filtration and thereby introduce reverse causation in studies on PFAS and kidney cancer have not been corroborated in later studies (Dhingra et al., 2016, 2017).]

Barry et al. (2013) evaluated the risk of kidney cancer, among other cancers, in 32 254 adult community residents in the Mid-Ohio Valley, USA, exposed to drinking-water contaminated with PFOA as a result of chemical plant emissions and in workers at a local chemical plant producing PTFE (C8 Health Project). The cohort was described in detail earlier (Section 2.1.5). Briefly, information on cancer occurrence was obtained by interview in 2005–2011 for the period from 1952 onwards. Cumulative exposure to PFOA in community residents was assessed using serum measurements in 2005–2006, historical regional and occupational data from several sources, and PFOA toxicokinetics. The estimated annualized serum PFOA concentrations matched well with measured levels (Spearman correlation, 0.71, comparing predicted levels with 2005–2006 measured levels, Winquist et al., 2013). Cumulative PFOA serum estimates in workers were estimated using a chemical plant-specific JEM.

Estimated cumulative serum PFOA concentration was associated with risk of kidney cancer; the hazard ratio for a one-unit increase in natural log-transformed serum PFOA was 1.10 (95% CI, 0.98–1.24; $P = 0.10$; 105 cases). Quartile analysis also indicated positive trends with increasing exposure. The adjusted hazard ratio for the fourth quartile versus the first was 1.58 (95% CI, 0.88–2.84; linear trend test, $P = 0.18$). Risk estimates based upon 10-year lagged analyses were slightly attenuated in the fourth quartile but not in the third quartile.

[The Working Group noted that this study presented improvements over other cohort studies because of its large study population; the large exposure contrast including both high-level occupational PFOA exposure, environmental PFOA exposure, and PFOA background exposure; the comprehensive exposure modelling using biological measurements in combination with environmental data, also taking PFOA toxicokinetics and variation across time into account; and the statistical analyses adjusting for a number of covariates and including lagged analyses. Although the study almost entirely included residents alive in 2005, the participation in the C8 Health Project was high, and the cohort was largely representative of the target population (Winquist et al., 2013). Moreover, a simulation study did not indicate that failure to include residents who died from kidney cancer before enrolment would bias risk estimates towards null (Barry et al., 2015). Nevertheless, capture of a larger part of the at-risk population would have added additional value to this study.]

Consonni et al. (2013) investigated cause-specific mortality rates in an international occupational cohort of 5879 male TFE workers, of whom 4205 were exposed to APFO. An individual semiquantitative estimate of cumulative TWA exposure to APFO was assigned from a study-specific JEM. The cohort was described in detail earlier (Section 2.1.6). Using national data as the referent, the risk of cancer of the kidney and urinary organs other than bladder (ICD-9 code 189) was elevated (SMR, 1.69; 95% CI, 0.81–3.11) but with no indication of an exposure–response relation (Consonni et al., 2013).

[The Working Group noted that the informativeness of this study was limited because of the small numbers of exposed men with cancer (*n* = 10), the semiquantitative exposure assessment with few measurements available, and the high correlation between TFE and PFOA exposure. However, exposure to TFE at the Parkersburg facility – the largest facility of the study – was considered to be very low because of strict hygiene controls for this flammable and explosive compound.]

Shearer et al. (2021) conducted a general population-based case–control study, nested within the PLCO cohort, addressing the risk of RCC according to prediagnostic serum concentrations of eight PFAS compounds, including PFOA and PFOS. The PLCO cohort was described in detail earlier (Section 2.1.11). In brief, 324 cases of RCC and 324 individually matched controls with baseline serum samples were enrolled in 1993–2001 from the screening arm of a multicentre randomized cancer screening trial in USA that included approximately 150 000 citizens (approximately half, 74 000, randomly assigned to the screening arm of the trial, provided blood samples at the baseline screening examination; Hayes et al., 2000). The adjusted risk of RCC was increased in individuals with higher PFOA serum concentration. The adjusted odds ratio in the highest exposure quartile (> 7.3–27.2 μg/L [ng/mL]) versus the lowest (< 4.0 μg/L [ng/mL]) was 2.63 (95% CI, 1.33–5.20) and, using a continuous exposure metric, the approximate risk related to a doubling of the serum concentration was 1.71 (95% CI, 1.23–2.37). Several potential confounders were controlled either by matching or by including variables in the models. The estimates of relative risk for PFOA changed little when PFOS and PFHxS were included in multivariable analysis and did not vary by kidney function (*P* for heterogeneity, 0.97), duration of time since blood sampling (*P* for heterogeneity, 0.32), and prior freeze–thaw cycles of the specimen (*P* for heterogeneity, 0.63). There was no indication of risk modification by sex (*P* for heterogeneity, 0.87) or age (*P* for heterogeneity, 0.66); although estimates did not differ significantly, associations seemed stronger among those with normal BMI (*P* for heterogeneity, 0.74), those without a history of hypertension (*P* for heterogeneity, 0.31), and among former and current smokers (*P* for heterogeneity, 0.24).

The adjusted risk of RCC was also increased in individuals with higher PFOS serum concentrations with a significant exposure–response trend, but the risk was attenuated when adjusted for PFOA and PFHxS serum concentrations.

[The Working Group noted that this general population study was distinguished from other case–control studies by having PFOA analyses adjusted for other PFAS compounds, by benefiting from blood samples collected on average 8.8 years before diagnosis, and by adjustment for several potential confounders, which added strongly to the reliability of the results. Although using a single sample to measure PFAS was a potential limitation, there is some evidence, from analyses of repeat samples of PFOA, that single samples may represent long-term averages over a 5–8-year period, with potential misclassification resulting in only minor bias to the null (see Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). Exposure assessment at a single time point precludes analyses of risk according to cumulative exposure in specified exposure windows; however, some insight was obtained by analyses stratified by years from blood collection to diagnosis. Overall, this study within a general population added substantially to the evaluation of risk of kidney cancer after exposure to PFOA and PFOS.]

Steenland et al. (2022) conducted a pooled analysis of two studies on PFOA and RCC, described above (Barry et al., 2013; Shearer et al., 2021). Both studies were based upon quantitative assessment of PFOA serum concentrations and

enabled exposure–response modelling for the purposes of assessment of lifetime excess risk and setting of limit values. The pooled analysis included 427 cases and 835 controls. The best-fitting dose–response model for the pooled data was a two-piece linear spline model with natural log-transformed serum PFOA and a knot at 2.55 (serum PFOA concentration, approximately 12.5 ng/mL). The log odds of RCC increased up to the knot and was flat thereafter. [The Working Group noted that the focus of this paper was on risk assessment and calculation of limit values and on quantitative exposure–response modelling that is important for causal inference.]

Li et al. (2022a) investigated the risk of kidney cancer in a community sample with a high level of exposure in Sweden, with follow-up from 1985 (when PFAS contamination of waterworks started) until the end of 2016 (Section 2.1.13). Exposure was categorized according to period and duration of living in a contaminated area. PFOA constituted only a minor part of the PFAS contamination, which was dominated by PFOS and PFHxS. SIRs, calculated separately for men and women, were adjusted for age and calendar year using regional reference rates. The SIR for kidney cancer for participants who had ever resided in an area with high PFAS contamination was elevated in women, but not in men. Internal comparisons of exposed and unexposed residents, adjusted for age, sex, and calendar year, revealed an increased risk among those who had ever been exposed (HR, 1.27; 95% CI, 0.85–1.91), with slightly higher risk in residents with longer and more recent exposure. [Major strengths included the complete registration of the cohort, no loss to follow-up, and a long follow-up period. Major limitations were the ecological exposure assessment based upon residence without individual estimates related to PFOS exposure, and the relatively few cases of kidney cancer, producing uncertain risk estimates.]

Rhee et al. (2023b) conducted a nested case–control study of prediagnostic serum concentrations of nine PFAS among 428 cases of RCC and 428 individually matched controls within the MEC (see Section 2.1.16). The MEC included more than 215 000 men and women aged 45–75 years at baseline (1993–1996) and represents a very racially, ethnically, and socioeconomically diverse population. Cohort members were living in Hawaii and California (primarily Los Angeles County), USA. Cases were ascertained via cancer registries in California and Hawaii. The controls were individually matched to cases on race, ethnicity, sex, age at serum sample, date of serum sampling, study centre (Hawaii or California), fasting status at time of sample, and time of day of sampling. The controls were not diagnosed with RCC at the time when their matched case was diagnosed. Eleven PFAS were measured, including PFOA, PFOS, PFHxS, and PFNA. Exposure levels were similar to those measured in the general population in NHANES. Analyses were conducted by conditional logistic regression maintaining the matched pairs, were mutually adjusted for all PFAS, and controlled for the matching factors, as well as for smoking status, eGFR, history of hypertension, and BMI. Analyses were carried out modelling the exposure both as categorical (using quartiles) or as continuous (using $\log_2$) serum levels. PFOA and PFOS were correlated (Spearman correlation, $\rho = 0.61$), and PFNA was correlated with both PFOA and PFOS ($\rho = 0.57$ and $\rho = 0.48$, respectively). The legacy PFAS (PFOA, PFOS, PFHxS, and PFNA) were detected in ≥ 97% of study participants. PFOA was not associated with renal cancer in the overall study group, with the OR for quartile 4 versus quartile 1 being 1.04 (95% CI, 0.60–1.81) and the OR for continuous $\log_2$ PFOA being 0.89 (95% CI, 0.67–1.18). However, a positive association was observed for White participants, with an OR per $\log_2$ PFOA concentration of 2.12 (95% CI, 0.87–5.18) and higher ORs for upper quartiles (ranging from 2.1 to 3.6 for quartiles 2 to 4 versus

quartile 1). There was also a suggestive association for those sampled before 2002 (OR per $\log_2$ PFOA concentration, 1.49; 95% CI, 0.77–2.87).

[The Working Group considered this nested case–control study to be informative, given the large sample size, the adjustment for multiple PFAS, the multiple racial or ethnic groups studied, the good cancer ascertainment via registries, and the availability of serum levels before diagnosis. The limitations were mainly the small sample sizes for different racial or ethnic groups.]

Winquist et al. (2023) conducted a case–cohort study within the prospective CPS-II LifeLink Cohort of the ACS, with measurements of PFOA, PFOS, and several other PFAS in prediagnostic serum samples collected during 1998–2001 (Section 2.1.21). Overall, there was no increased risk of kidney cancer or RCC with increasing serum PFOA. In women, serum PFOA concentration was positively associated with RCC (HR per doubling of serum PFOA, 1.54; 95% CI, 1.05–2.26), whereas no association was observed in men. [The Working Group noted several strengths, including the case–cohort design, the large sample size, the good cancer ascertainment via registries and examination by histological subtype, and availability of prediagnostic serum samples. The limitations were mainly the low exposure levels and narrow exposure contrast. There may be a survivor bias downwards for kidney cancer, for which a relatively high proportion of cases are diagnosed before age 65 years, because of the gap between exposure and enrolment.]

Vieira et al. (2013) conducted a case–control study in Ohio and West Virginia, USA, to investigate the risk of 18 cancers, including kidney cancer, in a community sample with relatively high exposure to PFOA due to contamination of drinking-water from the polymer-production plant in Parkersburg, West Virginia, USA (Section 2.1.22). Incident cancers diagnosed in 1996–2005 were identified from cancer registries. The control population was people with other cancers, except cancers of the kidney, testis, liver, or pancreas. Logistic regression was used to estimate odds ratios, which were adjusted for age, sex, diagnosis year, smoking status, and insurance provider. For the Ohio subset with individual-level serum estimates of exposure, the adjusted odds ratios (AOR) for kidney cancer were higher in the highest exposure categories, with indications of a dose–response relation: AOR, 0.8 (95% CI, 0.4–1.5; 11 cases), 1.2 (95% CI, 0.7–2.0; 17 cases), 2.0 (95% CI, 1.3–3.2; 22 cases), and 2.0 (95% CI, 1.0–3.9; 9 cases) versus unexposed for low, medium, high, and very high exposure categories, respectively. Estimates of PFOA annual serum levels 10 years before diagnosis were 3.7–12.8 μg/L [ng/mL], 12.9–30.7 μg/L [ng/mL], 30.8–109 μg/L [ng/mL], and 110–655 μg/L [ng/mL] for these four categories.

For the combined populations of Ohio and West Virginia without individual-level exposure estimates, the odds ratio for kidney cancer was 1.1 (95% CI, 0.9–1.4; 94 exposed cases) for participants exposed to contaminated water districts relative to unexposed participants.

[The Working Group noted that some of the cancer cases were overlapping cases from the study by Barry et al. (2013). Strengths were the large study population with a strong exposure contrast and estimates of individual-level exposure for a subset of the population. Misclassification of exposure was likely to be non-differential, resulting in attenuated risk estimates (if truly deviating from unity). Limitations of the main analysis applying individual-level exposure estimates were mainly related to modelled exposure data.]

The Working Group conducted a random-effects meta-analysis to estimate the rate ratio (RR) per unit (linear) of serum PFOA, by following the same methodology outlined in Bartell and Vieira (2021). Details of the methodology are outlined in Annex 3 (Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.

int/636). The studies by Raleigh et al. (2014) and Consonni et al. (2013) were not included because of the lack of serum measurements. Categorical rate ratios based on contrasting the upper category (usually quartiles) with the referent were used, along with the assumed midpoints of the upper category and referent, to regress the log of the rate ratios on the midpoints to obtain a single linear continuous coefficient that estimates the change in log rate ratio per 10 ng/mL of (linear) PFOA. When including the studies by Steenland and Woskie (2012), Barry et al. (2013), Vieira et al. (2013), Shearer et al. (2021), Rhee et al. (2023b), and Winquist et al. (2023), the meta-analysis described above gave a result for an increase in the meta-rate ratio (meta-RR) per increase of 10 ng/mL of PFOA as 1.15 (95% CI, 0.97–1.37; I^2 = 0.91). In a sensitivity analysis excluding the studies by Steenland and Woskie (2012) and Vieira et al. (2013), given the concern regarding overlap with Barry et al. (2013), this sensitivity analysis gave a result for an increase in the meta-rate ratio per increase of 10 ng/mL PFOA as 1.21 (95% CI, 0.94–1.57; I^2 = 0.95). [The Working Group noted that a general limitation of the meta-analysis was the assumption of a linear exposure–response relation, although it has been observed that in studies with continuous exposure coefficients, (Barry et al., 2013; Shearer et al., 2021; Rhee et al., 2023b; Winquist et al., 2023) a logarithmic transformation of PFOA levels seems to fit the data better than do the untransformed PFOA levels. Other main limitations of the meta-analysis were: (i) the estimate of the linear coefficient using assumed midpoints of only two categories (uppermost and lowest); (ii) the use of average duration of exposure to transform cumulative exposure in the studies by Barry et al., Steenland and Woskie, and Vieira et al. to an assumed average exposure; and (iii) the assumption in the studies by Rhee et al., Shearer et al., and Winquist et al. that a single PFOA measurement is a good estimate of long-term lifetime average exposure (beyond a 5–8-year duration discussed in Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). Given these limitations, as well as the high heterogeneity across studies with different strengths and weaknesses, the Working Group chose to not rely primarily on the meta-analysis of exposure–response relation to determine the hazard identification for kidney cancer in humans.]

2.2.2 Bladder cancer

See Table 2.2.

Raleigh et al. (2014) studied bladder cancer mortality and incidence among 4668 APFO-exposed workers at an APFO-production facility in Minnesota, USA (see Section 2.1.1 for more details). APFO is the ammonium salt of PFOA; the two substances are usually considered chemically equivalent in aqueous biological media such as the human body (Vierke et al., 2012). Raleigh et al. also studied 4359 unexposed workers at a different plant. Workers at both plants were employed for ≥ 1 year at their respective plants. For bladder cancer mortality, using the Minnesota population as the referent, the SMR for unexposed workers was 0.62 (95% CI, 0.27–1.22; 8 deaths). The SMRs for exposed workers, divided into quartiles of estimated APFO air exposure, were 0.40, 0.93, 1.61, and 0.53, based on 1, 2, 4, and 1 death, respectively. Bladder cancer incidence was ascertained using the Minnesota state cancer registry. Bladder cancer incidence hazard ratios, by quartile of estimated cumulative APFO air exposure, using unexposed workers as the referent, were 0.81 (95% CI, 0.36–1.81; 7 cases), 0.78 (95% CI, 0.33–1.85; 6 cases), 1.50 (95% CI, 0.80–2.81; 15 cases), and 1.66 (95% CI, 0.86–3.18; 12 cases).

Alexander et al. (2003) studied a cohort of 2083 production workers (145 deaths) who were exposed to PFOS at a plant in Decatur, Alabama, USA, that produced speciality films and

fluorochemicals, and who had worked ≥ 1 year at the plant between 1961 and 1997. Based on serum measurements for a sample of workers, a JEM was developed for all workers whereby jobs were classified into three exposure groups. Alexander and Olsen (2007) further followed this PFOS cohort, focusing on bladder cancer incidence; cases were identified using both questionnaire (6 cases) and death certificates (5 cases). Groups with no, low, and high exposure were estimated to have serum PFOS levels of [110–290 ng/mL], [390–890 ng/mL], and [1300–1970 ng/mL], respectively. Among those with any PFOS exposure, the bladder cancer SIR was 1.70 (95% CI, 0.77–3.22; 9 cases) compared with US cancer rates. Using a US population as the referent, the SIRs according to increasing cumulative exposure were 1.07 (95% CI, 0.12–3.85), 0.95 (95% CI, 0.25–2.43), 2.72 (95% CI, 0.55–73.95) and 1.43 (95% CI, 0.16–5.15). Comparing the three groups with the highest cumulative exposure with the group with the lowest cumulative exposure (internal referent, two cases), relative risks by increasing exposure were 0.83 (95% CI, 0.15–4.65; 4 cases), 1.92 (95% CI, 0.30–12.06; 3 cases), and 1.52 (95% CI, 0.21–10.99; 2 cases). A further study of medical care for some of these employees was conducted by Olsen et al. (2004), but this study was limited to certain categories of workers eligible for employer-provided care during the period 1993–1998. [The Working Group considered this study to provide only minimal information for estimating cancer incidence in this cohort.]

Steenland and Woskie (2012) studied cancer mortality among 5791 workers exposed to PFOA at a polymer-production plant in Parkersburg, West Virginia, USA (see Section 2.1.3 for more details). Compared with other non-exposed workers at other plants in the same company, the authors found an SMR for bladder cancer of 1.08 (95% CI, 0.52–1.99; 10 deaths). By quartile of estimated cumulative exposure, SMRs were 1.24 (95% CI, 0.15–4.47; 2 deaths), 2.49 (95% CI, 0.97–5.78; 6 deaths), 0.39 (95% CI, 0.01–2.17; 1 death), and 0.36 (95% CI, 0.10–2.01; 1 death). Steenland et al. (2015) followed a subset ($n = 3713$) of the PFOA-exposed workers in Steenland and Woskie (2012) for bladder cancer incidence. Bladder cancers were found via interview and confirmed via medical records, or via matching to local cancer registries. These authors found, when analysing estimated cumulative serum exposure by quartiles with a 10-year lag and using the lowest quartile as the referent in an internal comparison, RRs of 0.55 (95% CI, 0.12–2.61), 0.47 (95% CI, 0.10–2.21), and 0.31 (95% CI, 0.06–1.54), respectively, based on 29 cases of incident bladder cancer.

Eriksen et al. (2009) conducted a case–cohort study (713, 332, 128, and 67 patients with prostate, bladder, pancreatic, and liver cancer, respectively, and 772 cancer-free participants selected randomly from the full cohort) in a general-population national cohort of 57 053 people in Denmark. Analysis of bladder cancer incidence was done using baseline-measured plasma level of both PFOA and PFOS (Section 2.1.4 for more details). All participants had no previous diagnosis of cancer at the beginning of follow-up. Follow-up for cancer patients ranged from 0 to 12 years (median, 7 years). Analyses of IRRs by quartile of PFOA measured at baseline, using quartile 1 (84 cases) as the referent, were 0.71 (95% CI, 0.46–1.07), 0.92 (95% CI, 0.61–1.39), and 0.81 (95% CI, 0.53–1.24), respectively, based on 82, 83, and 83 cases, respectively. Corresponding RRs for PFOS measured at baseline, using quartile 1 (83 cases) as the referent, were 0.76 (95% CI, 0.50–1.16), 0.93 (95% CI, 0.61–1.41), and 0.70 (95% CI, 0.46–1.07), based on 84, 83, and 82 cases, respectively.

Barry et al. (2013) analysed bladder cancer incidence in a cohort of 32 254 participants with both low and high exposure to drinking-water containing PFOA (with high exposure being similar to the high levels in occupational cohorts), who were living near the plant

in Parkersburg, West Virginia, USA (see Section 2.1.5 for more details). The median PFOA concentration measured in all cohort members in 2005–2006 was 26.1 µg/L [ng/mL], and the mean was 86.6 µg/L [ng/mL] (the US general population concentration was about 4 µg/L [ng/mL] at the time) (Winquist et al., 2013). Approximately 12% of participants in this study had worked in the Parkersburg plant that was the source of the PFOA contamination. Cancer incidence was determined via interview, with confirmation from medical records or from linkage with Ohio and West Virginia cancer registries. Hazard ratios were estimated per unit of increase in natural log-transformed cumulative serum level (a continuous variable), with serum levels over time estimated by a model with good correlation (Spearman correlation, 0.71) to observed serum levels that were available in 2005–2006 for all cohort members (Winquist et al., 2013). Hazard ratios were 1.00 (95% CI, 0.89–1.12) with no lag and 0.98 (95% CI, 0.88–1.10) with a 10-year lag (0 exposure assigned during most recent 10 years), based on 105 cases of incident bladder cancer. [The Working Group noted that among the non-occupational studies with bladder cancer outcomes, the larger studies with the best-characterized individual exposure were those of Eriksen et al. (2009) and Barry et al. (2013). The former was a study of a general population with low background levels of exposure, whereas the latter included both low-exposure participants and participants with very high exposures similar to occupational levels. Hence, the exposure contrasts were much smaller in the former than the latter, but the results of these two larger studies were nonetheless concordant in finding no association with bladder cancer for either PFOA or PFOS (Barry et al. did not study PFOS).]

Consonni et al. (2013) conducted an international cohort study of mortality in male workers at six TFE-production sites who were concomitantly exposed to APFO (or equivalently PFOA, as APFO breaks down to PFOA when soluble). The Spearman correlation between APFO and TFE in this study was 0.72 (see Section 2.1.6 for more details). Restricting the cohort to workers who had ever had exposure to APFO, in the supplemental data, the authors reported a bladder cancer SMR (versus national rates) of 0.55 (95% CI, 0.11–1.60; 3 deaths). [The Working Group noted that the small numbers of cases of incident bladder cancer and of deaths from bladder cancer in each of the three occupational cohorts reported in five papers (Alexander et al., 2003; Alexander and Olsen, 2007; Steenland and Woskie, 2012; Consonni et al., 2013; Steenland et al., 2015) limited the ability to evaluate associations between PFOA and PFOS and bladder cancer. Analyses of bladder cancer incidence in other studies noted below had better statistical precision. However, exposure contrasts between high and no or low exposure were often much reduced in the non-occupational studies.]

Li et al. (2022a) studied bladder cancer incidence in Ronneby, Sweden, among 60 507 residents among whom one third of households had been exposed to relatively high levels of both PFOS and PFOA in drinking-water contaminated by nearby military firefighting operations. [The Working Group noted that, although the authors were unable to estimate separate effects of the two exposures, the PFOS level was more than tenfold that of PFOA, on the basis of a subset of the participants with measured levels of these compounds in serum (see Section 2.1.13 for more details).] For men who had never resided in a high-exposure area, the SIR for bladder cancer incidence (the area surrounding Ronneby was used as the referent) was 0.94 (95% CI, 0.80–1.09; 166 cases), whereas for women it was 0.69 (95% CI, 0.48–0.95; 35 cases). For men ever living in a high-exposure area, the SIR was 1.10 (95% CI, 0.84–1.43; 57 cases), and for women the corresponding estimate was 1.13 (95% CI, 0.66–1.80; 17 cases). When Ronneby residents with ever-high exposure were compared with those with

never-high exposure, the bladder cancer hazard ratio was 1.30 (95% CI, 0.99–1.69). When Ronneby residents with ever-high exposure were further subdivided into "early-high" (lower exposure) and "late-high" (higher exposure), hazard ratios compared with the "never-high" exposure group were 1.20 (95% CI, 0.87–1.66) and 1.50 (95% CI, 0.98–2.29), respectively. [The Working Group noted that the study by Li et al. also had large exposure contrasts but was somewhat weakened by the fact that exposure was assigned ecologically depending on whether or not the participants lived in the Ronneby area.]

Winquist et al. (2023) studied 39 371 surviving participants in the CPS-II Nutrition Cohort (enrolled in 1991–1992) who resided in urban or suburban areas of 20 states in the USA and who had been recruited for participation in the CPS-II LifeLink Cohort. CPS-II LifeLink participants completed a LifeLink cohort baseline questionnaire and provided a blood sample in 1998–2001 (median age: 70 years overall, 71 years for men, 69 years for women) (Section 2.1.21). Using a case–cohort approach, 396 cases of incident bladder cancer were identified and verified among those without previous cancer and compared with a randomly sampled subcohort of 500 men and 499 women. PFOA, PFOS, and several other PFAS compounds were measured in the collected blood samples. In the subsample, PFOS was present at the highest concentrations (median, 18.0 ng/mL), followed by PFOA (median, 5.2 ng/mL); levels were similar to those reported in NHANES. Cases were compared with the subcohort at risk at time of case occurrence via Cox regression. Hazard ratios for bladder cancer incidence were adjusted for sex, year of serum sample collection, age at serum collection; race and education from the 1982 baseline survey; smoking status and alcohol consumption from the 1997 survey (but not adjusted for other PFAS). Overall, for the sexes combined, there were no clear associations between PFOA or PFOS and bladder cancer. The hazard ratios for PFOA quartiles 2 to 4 versus quartile 1 in relation to bladder cancer were 0.84 (95% CI, 0.56–1.26), 0.87 (95% CI, 0.58–1.30), and 0.86 (95% CI, 0.58–1.27), and there was no continuous (using $\log_2$ of serum levels) trend (HR, 0.93; 95% CI, 0.77–1.13; $P = 0.478$). For PFOS, quartile analyses showed hazard ratios of 0.81 (95% CI, 0.54–1.21), 1.07 (95% CI, 0.72–1.60), and 0.96 (95% CI, 0.64–1.44), and there was no evidence for an association, with a continuous hazard ratio of 1.01 (95% CI, 0.86–1.20; $P = 0.890$). Sex-specific analyses also showed no clear association for either PFOA or PFOS.

[The study by Winquist et al. (2023) had low exposure contrasts, with a single baseline sample, a moderate number of cases, and good case ascertainment. A weakness was that this was a survivor cohort, with median age at enrolment of approximately 70 years and follow-up starting at time of blood draw approximately 8–9 years after enrolment, which would preclude the identification of bladder cancer cases during this period (eligibility for follow-up after serum sample excluded any prior cancer, fatal or not), resulting in a potential downward bias and minimal informativeness.]

Vieira et al. (2013) conducted two case–control studies of incident bladder cancer among residents of 13 counties in Ohio and West Virginia, USA, including both contaminated and non-contaminated water districts near the same plant in Parkersburg, West Virginia, which was the source of contamination in the population studied by Barry et al. (2013) (see Section 2.1.22). In the first case–control study, cases and controls (all other cancer cases excluding kidney, pancreatic, testicular, and liver cancers), obtained from both Ohio and West Virginia cancer registries, were compared with regard to residence in a contaminated or non-contaminated water district. The bladder cancer OR for exposed residents in a contaminated water district was 0.8 (95% CI, 0.7–1.0; 137 exposed cases) versus residents in non-contaminated water districts. These same authors also conducted a separate

case–control study among Ohio residents; the cases were people with bladder cancer, and the controls were people with other cancers in the Ohio counties, again excluding kidney, pancreatic, testicular, and liver cancers. Exposure in the second study was based on estimated individual annual serum levels of PFOA at specific addresses at specific points in time, 10 years before the diagnosis dates for cases and controls. Relative to the unexposed, ORs for the participants with low, medium, high, and very high-exposure 10 years before diagnosis were 0.9 (95% CI, 0.6–1.4; 23 cases), 0.9 (95% CI, 0.6–1.4; 21 cases), 1.2 (95% CI, 0.8–2.0; 21 cases), and 0.6 (95% CI, 0.2–1.5; 4 cases), respectively, in the second case–control study.

[The Working Group noted that the study by Vieira et al. (2013) included participants with the same large exposure contrasts as in Barry et al. (2013), but it was also somewhat weakened by small numbers in high-exposure groups and assignment of either group-level exposure or broadly estimated individual exposure 10 years before diagnosis.]

2.3 Cancers of the male genital tract

See Table 2.3.

2.3.1 Testicular cancer

Of the studies listed in Table 2.3, associations between PFOA and/or PFOS exposure and testicular cancer were evaluated in two cohort studies (Barry et al., 2013; Li et al., 2022a), one prospective nested case–control study (Purdue et al., 2023), and one cancer registry-based case–control study (Vieira et al., 2013) that probably had some overlap with the Barry et al. (2013) cohort study. Two occupational cohort mortality studies (Steenland and Woskie, 2012; Consonni et al., 2013) reported SMRs based on 1 death from testicular cancer; a third occupational cohort (Raleigh et al., 2014) identified 5 cases of incident testicular cancer among PFOA-exposed workers but did not report estimates of association with testicular cancer. [Given the small numbers of deaths attributable to testicular cancer and lack of risk estimates in the latter studies, the Working Group focused on the investigations by Barry et al. (2013), Li et al. (2022a), Purdue et al. (2023), and Vieira et al. (2013) in the following summary.] In addition, the Working Group conducted an analysis of data from studies carried out in the Veneto region of Italy (an area in which drinking-water is contaminated with PFAS).

Barry et al. (2013) evaluated the risk of testicular cancer in a study of 32 254 community residents and workers exposed to PFOA from a fluoropolymer-production plant in the Mid-Ohio Valley, USA (see the description of the C8 Science Panel study in Section 2.1.5). In analyses that included 17 validated incident testicular cancer cases, the authors observed evidence of an exposure–response association with estimated cumulative PFOA serum concentrations (unlagged analysis: adjusted HR for 1-unit increase in natural log-transformed levels, 1.34; 95% CI, 1.00–1.79). The corresponding hazard ratio in analyses comparing those in the highest and lowest PFOA exposure quartiles was 3.17 (95% CI, 0.75–13.45); in the categorical analysis, $P = 0.04$ for the exposure–response trend based on the within-category midpoints. The patterns of associations were similar, albeit slightly attenuated, in analyses with exposures lagged by 10 years (continuous: HR 1.28; 95% CI, 0.95–1.73; categorical, quartile 4 versus quartile 1: HR, 2.36; 95% CI, 0.41–13.65; P for trend, 0.02). Of the 17 cases of testicular cancer with complete covariate data that were included in these analyses, 15 were reported among community members and 2 among workers; in analyses excluding those employed at the plant, stronger associations were observed (continuous with no lag: HR, 1.73; 95% CI, 1.24–2.40; continuous with 10-year lag: HR, 1.53; 95% CI, 1.09–2.15).

Table 2.3 Epidemiological studies on exposure to PFOA and PFOS and cancers of the male genital tract

Reference, location, enrolment/ follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Raleigh et al. (2014) MN, USA Enrolment, 1947–2002/ follow-up, 1947–2008 (mortality), 1988–2008 (incidence) Cohort	9027 men (3716 exposed, 3834 reference); Cottage Grove (MN), PFOA cohort; workers employed for ≥ 1 yr during 1947–2002 at an APFO facility (Cottage Grove; $n = 4668$); reference workers without any exposure to APFO employed at a tape and abrasives production facility located in the same suburban geographical area and managed by the same company (Saint Paul; $n = 4359$) Exposure assessment method: see Table 2.1	Prostate, mortality	Exposed to APFO (SMR, MN referent):			Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. *Other limitations*: See Table 2.1. Risk estimates not reported for testicular cancer due to the small number of incident cases among exposed workers ($n = 5$).
			Unexposed (Saint Paul plant)	48	1.03 (0.76–1.37)		
			Exposed (Cottage Grove plant)	24	0.83 (0.53–1.23)		
		Prostate, mortality	Estimated cumulative airborne APFO exposure quartile (SMR, MN referent):				
			1st quartile (< 2.6 × 10^{-5} µg/ m^3-year)	5	0.66 (0.21–1.54)		
			2nd quartile (2.6 × 10^{-5} to < 1.4 × 10^{-4} µg/ m^3-year)	8	1.15 (0.50–2.27)		
			3rd quartile (1.4 × 10^{-4} to < 7.3 × 10^{-4} µg/ m^3-year)	3	0.37 (0.08–1.07)		
			4th quartile (≥ 7.3 × 10^{-4} µg/ m^3-year)	8	1.29 (0.56–2.54)		
		Prostate, mortality	Estimated cumulative airborne APFO exposure quartile (HR):			Age, year of birth	
			Unexposed (Saint Paul plant)	NR	1		
			1st quartile (< 2.9 × 10^{-5} µg/ m^3-year)	NR	0.34 (0.25–1.60)		
			2nd quartile (2.9 × 10^{-5} to < 1.5 × 10^{-4} µg/ m^3-year)	NR	1.12 (0.53–2.37)		

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Raleigh et al. (2014) MN, USA Enrolment, 1947–2002/ follow-up, 1947–2008 (mortality), 1988–2008 (incidence) Cohort (cont.)		Prostate, mortality (cont.)	3rd quartile (1.5×10^{-4} to < 7.9×10^{-4} µg/ m³-year)	NR	0.36 (0.11–1.17)	Age, year of birth	
			4th quartile (≥ 7.9×10^{-4} µg/ m³-year)	NR	1.32 (0.61–2.84)		
		Prostate, incidence	Estimated cumulative airborne APFO exposure quartile (HR):				
			Unexposed (Saint Paul plant)	253	1		
			1st quartile (< 2.9×10^{-5} µg/ m³-year)	42	0.80 (0.57–1.11)		
			2nd quartile (2.9×10^{-5} to < 1.5×10^{-4} µg/ m³-year)	42	0.85 (0.61–1.19)		
			3rd quartile (1.5×10^{-4} to < 7.9×10^{-4} µg/ m³-year)	49	0.89 (0.66–1.21)		
			4th quartile (≥ 7.9×10^{-4} µg/ m³-year)	55	1.11 (0.82–1.49)		

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Steenland and Woskie (2012) Parkersburg (WV), USA Enrolment, 1948–2002/ follow-up, 1952–2008 (mortality) Cohort	5791; Parkersburg (WV), polymer-production occupational PFOA cohort; workers (men, 81%) at a polymer manufacturing facility who had potential exposure to fluoropolymers with sufficiently detailed work histories Exposure assessment method: see Table 2.1	Testis, mortality	PFOA-exposed workers (SMR):			Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Ability to evaluate associations in a high PFOA-exposed population. *Other limitations*: See Table 2.1. Limited statistical power to assess mortality from testicular and prostate cancers.
			Other workers referent (same region and company)	1	1.80 (0.05–10.03)		
			US referent	1	0.74 (0.02–4.12)		
		Prostate, mortality	PFOA-exposed workers (SMR):				
			Other workers referent (same region and company)	21	0.76 (0.47–1.16)		
			US referent	21	0.72 (0.45–1.10)		
		Prostate, mortality	Cumulative serum exposure, no lag (SMR, other workers referent, same region and company):				
			1st quartile (0 to < 904 ppm-years)	6	1.07 (0.39–2.34)		
			2nd quartile (904 to < 1520 ppm-years)	6	0.82 (0.30–1.78)		
			3rd quartile (1520 to < 2700 ppm-years)	5	0.65 (0.21–1.51)		
			4th quartile (≥ 2700 ppm-years)	4	0.57 (0.16–1.46)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Steenland et al. (2015) Parkersburg (WV), USA Enrolment, 1948–2002/follow-up, 1951-interview date in 2008–2011 (incidence) Cohort	3713 (2955 male); A subset of Parkersburg (WV), polymer-production PFOA cohort in Steenland and Woskie (2012); polymer-production workers (mean, 80%) who responded (self or next-of-kin) to a questionnaire about health outcomes and who had measured or estimated occupational and residential exposure estimates; 129 incident cases of prostate cancer Exposure assessment method: See Table 2.1	Prostate, incidence	Cumulative PFOA exposure, no lag (RR):			Age, race, education, BMI, time-varying smoking, time-varying alcohol consumption, year of birth	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Ability to evaluate associations between PFOA and prostate cancer incidence in a high PFOA-exposed population. *Other limitations*: See Table 2.1. Possibility of selection bias given that the investigation included only 62% of the target population; inability to evaluate risk of testicular cancer and other relatively less common malignancies.
			1st quartile (< 3.03 μg/mL-years)	NR	1		
			2nd quartile (3.03 to < 6.16 μg/mL-years)	NR	1.81 (0.69–4.78)		
			3rd quartile (6.16 to < 11.42 μg/mL-years)	NR	2.45 (0.96–6.25)		
			4th quartile (≥ 11.42 μg/mL-years)	NR	1.88 (0.72–4.88)		
			Trend-test *P*-value, 0.11				
		Prostate, incidence	Cumulative PFOA exposure, 10-yr lag (RR):				
			1st quartile (< 0.8 μg/mL-years)	NR	1		
			2nd quartile (0.8 to < 3.44 μg/mL-years)	NR	1.92 (0.56–6.58)		
			3rd quartile (3.44 to < 7.04 μg/mL-years)	NR	1.89 (0.57–6.34)		
			4th quartile (≥ 7.04 μg/mL-years)	NR	2.15 (0.64–7.26)		
			Trend-test *P*-value, 0.10				

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Eriksen et al. (2009) Denmark Enrolment, December 1993 to May 1997/ follow-up, 1 December 1993 to 1 July 2006 Case–cohort	Case–cohort within the Diet, Cancer and Health cohort (see Table 2.1) Cases: 713 incident cases of prostate cancer Comparison cohort: 772 (680 men, 92 women); subcohort of participants randomly selected without cancer at the end of follow-up Exposure assessment method: see Table 2.1	Prostate, incidence	Baseline plasma PFOA concentration (IRR):			Age, years of school attendance, BMI, dietary fat intake, fruit and vegetable intake	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Large number of prostate cancer cases and non-cases. *Other limitations*: See Table 2.1. Results not reported for different time intervals between serum collection and diagnosis of prostate cancer; lack of mutually adjusted analyses of PFOS and PFOA.
			1st quartile	179	1		
			2nd quartile	178	1.09 (0.78–1.53)		
			3rd quartile	178	0.94 (0.67–1.32)		
			4th quartile	178	1.18 (0.84–1.65)		
			Continuous (per 1 ng/mL increase)	713	1.03 (0.99–1.07)		
		Prostate, incidence	Baseline plasma PFOS concentration (IRR):				
			1st quartile	179	1		
			2nd quartile	178	1.35 (0.97–1.87)		
			3rd quartile	180	1.31 (0.94–1.82)		
			4th quartile	176	1.38 (0.99–1.93)		
			Continuous (per 10 ng/mL increase)	713	1.05 (0.97–1.14)		

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Barry et al. (2013) Mid-Ohio Valley (OH and WV) Enrolment, August 2005 to August 2006/ follow-up, 1952 to 2011 (incidence) Cohort	32 254 (28 541 community members and 3713 workers); C8 Science Panel Study; included people enrolled in the C8 Health Project who lived, worked, or attended school for ≥ 1 yr between 1950 and 3 December 2004 in a contaminated-water district in the vicinity of a chemical plant (Parkersburg (WV), polymer-production) using PFOA in manufacturing, as well as a subset of those from the original Parkersburg (WV), polymer production occupational cohort who worked at the plant between 1948 and 2002 Exposure assessment method: see Table 2.1	Testis, incidence	Estimated cumulative PFOA serum concentration (ng/mL), no lag (HR):			Age, time-varying smoking, time-varying alcohol consumption, education, birth year (5-yr calendar intervals)	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Relatively high participation from those in the C8 Health Project; validation of diagnosed cancers. *Limitations*: See Table 2.1. Limited statistical power to assess risk of testicular cancer.
			1st quartile	NR	1		
			2nd quartile	NR	1.04 (0.26–4.22)		
			3rd quartile	NR	1.91 (0.47–7.75)		
			4th quartile	NR	3.17 (0.75–13.45)		
			Continuous (per unit on natural log scale)	17	1.34 (1.00–1.79)		
			Trend-test *P*-value, 0.04				
		Testis, incidence	Estimated cumulative PFOA serum concentration (ng/mL), 10-yr lag (HR):				
			1st quartile	NR	1		
			2nd quartile	NR	0.87 (0.15–4.88)		
			3rd quartile	NR	1.08 (0.20–5.90)		
			4th quartile	NR	2.36 (0.41–13.65)		
			Continuous (per unit on natural log scale)	17	1.28 (0.95–1.73)		
			Trend-test *P*-value, 0.02				
		Testis, incidence	Community residents: estimated cumulative PFOA serum concentration (ng/mL), no lag (HR):				
			1st quartile	NR	1		
			2nd quartile	NR	0.80 (0.16–3.97)		
			3rd quartile	NR	3.07 (0.61–15.36)		
			4th quartile	NR	5.80 (0.97–34.58)		
			Continuous (per unit on natural log scale)	15	1.73 (1.24–2.40)		
			Trend-test *P*-value, 0.05				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Barry et al. (2013) Mid-Ohio Valley (OH and WV) Enrolment, August 2005 to August 2006/ follow-up, 1952 to 2011 (incidence) Cohort (cont.)		Testis, incidence	Community residents: estimated cumulative PFOA serum concentration (ng/mL), 10-yr lag (HR):			Age, time-varying smoking, time-varying alcohol consumption, education, birth year (5-yr calendar intervals)	
			1st quartile	NR	1		
			2nd quartile	NR	0.98 (0.13–7.14)		
			3rd quartile	NR	1.54 (0.19–12.21)		
			4th quartile	NR	4.66 (0.52–41.63)		
			Continuous (per unit on natural log scale)	15	1.53 (1.09–2.15)		
			Trend-test *P*-value, 0.02				
		Prostate, incidence	Estimated cumulative PFOA serum concentration (ng/mL), no lag (HR):				
			Continuous (per unit on natural log scale)	446	0.99 (0.93–1.04)		
		Prostate, incidence	Estimated cumulative PFOA serum concentration (ng/mL), 10-yr lag (HR):				
			Continuous (per unit on natural log scale)	446	0.99 (0.94–1.05)		

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Consonni et al. (2013) USA, UK, Italy, Germany, Netherlands Enrolment, 1950–2002/ follow-up, 1950–2008 Cohort	5879 male workers (APFO-exposed, 4205); the pooled international TFE cohort includes male workers who were ever employed or employed for 6 or 12 mo at one or more of six TFE-production sites in North America and Europe from 1950 to 2002; the principal occupational exposures were TFE and APFO (aiding production of PTFE) Exposure assessment method: see Table 2.1	Testis, mortality	SMR (national referent):			Age, calendar period, country	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. *Other limitations*: See Table 2.1. Limited statistical power to assess mortality from testicular and prostate cancers.
			Ever APFO-exposed	1	1.35 (0.03–7.49)		
		Prostate, mortality	SMR (national referent):			Age, calendar period, country	
			Ever APFO-exposed	3	0.24 (0.05–0.70)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rhee et al. (2023a) USA Enrolment, 1993–2001; follow-up (from blood draw), median, 9 yr (incidence) Nested case–control	Nested within the PLCO cohort (see Table 2.1 of Shearer et al., 2021) Cases: 750; aggressive prostate cancer (defined as stage III or IV, Gleason score ≥ 8, or Gleason score 7 and death from prostate cancer) diagnosed > 300 days after blood collection Controls: 750; alive and cancer-free at time of case diagnosis, and individually matched to cases on age at baseline, race/ethnicity, study centre, calendar and study year of blood collection, and prior freeze–thaw cycle Exposure assessment method: see Table 2.1	Prostate (aggressive/advanced), incidence	Serum PFOA concentration (OR):			Age, race/ethnicity, study centre, calendar year of blood collection, study year of blood collection, prior freeze–thaw	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. *Other limitations*: See Table 2.1.
			< 2.90 µg/L	194	1		
			2.90 to < 3.80 µg/L	155	0.75 (0.55–1.03)		
			3.80 to < 4.67 µg/L	130	0.65 (0.47–0.91)		
			4.67 to < 6.50 µg/L	149	0.69 (0.49–0.97)		
			≥ 6.50 µg/L	122	0.57 (0.39–0.82)		
			Continuous (per unit increase on $\log_2$ scale)	750	0.82 (0.71–0.96)		
			Trend-test *P*-value, 0.005				
		Prostate (aggressive/advanced), incidence	Serum PFOA concentration (OR):			Age, race/ethnicity, study centre, calendar year of blood collection, study year of blood collection, prior freeze–thaw, BMI, smoking status, family history of prostate cancer, history of diabetes, PFOS, PFHxS, PFNA, *N*-EtFOSAA, FOSA, *N*-MeFOSAA, PFHpS	
			< 2.90 µg/L	194	1		
			2.90 to < 3.80 µg/L	155	0.75 (0.53–1.07)		
			3.80 to < 4.67 µg/L	130	0.72 (0.49–1.07)		
			4.67 to < 6.50 µg/L	149	0.67 (0.44–1.03)		
			≥ 6.50 µg/L	122	0.54 (0.32–0.91)		
			Continuous (per unit increase on $\log_2$ scale)	750	0.79 (0.63–0.99)		
			Trend-test *P*-value, 0.02				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rhee et al. (2023a) USA Enrolment, 1993–2001; follow-up (from blood draw), median, 9 yr (incidence) Nested case–control (cont.)		Prostate (aggressive/ advanced), incidence	No. of years from blood draw to diagnosis (OR for a 1-unit increase in serum PFOA on log_2 scale):			Age, race/ ethnicity, study centre, calendar year of blood collection	
			< 1 to 3 yr	115	0.67 (0.51–0.87)		
			> 3 yr	635	0.90 (0.79–1.03)		
		Prostate (aggressive/ advanced), incidence	Serum PFOS concentration (OR):			Age, race/ ethnicity, study centre, calendar year of blood collection, study year of blood collection, prior freeze–thaw	
			< 19.10 µg/L	170	1		
			19.10 to < 25.50 µg/L	145	0.86 (0.62–1.18)		
			25.50 to < 33.50 µg/L	168	0.99 (0.72–1.37)		
			33.50 to < 47.12 µg/L	136	0.80 (0.58–1.12)		
			≥ 47.12 µg/L	131	0.74 (0.51–1.06)		
			Continuous (per unit increase on log_2 scale)	750	0.93 (0.83–1.05)		
			Trend-test *P*-value, 0.08				

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rhee et al. (2023a) USA Enrolment, 1993–2001; follow-up (from blood draw), median, 9 yr (incidence) Nested case–control (cont.)		Prostate (aggressive/advanced), incidence	Serum PFOS concentration (OR):			Age, race/ethnicity, study centre, calendar year of blood collection, study year of blood collection, prior freeze–thaw, BMI, smoking status, family history of prostate cancer, history of diabetes, PFOA, PFHxS, PFNA, *N*-EtFOSAA, FOSA, *N*-MeFOSAA, PFHpS	
			< 19.10 µg/L	170	1		
			19.10 to < 25.50 µg/L	145	0.93 (0.64–1.37)		
			25.50 to < 33.50 µg/L	168	1.07 (0.69–1.66)		
			33.50 to < 47.12 µg/L	136	0.88 (0.53–1.46)		
			≥ 47.12 µg/L	131	0.84 (0.45–1.58)		
			Continuous (per unit increase on log_2 scale)	750	0.99 (0.79–1.23)		
			Trend-test *P*-value, 0.34				
		Prostate (aggressive/advanced), incidence	No. of years from blood draw to diagnosis (OR for a 1-unit increase in serum PFOS on log_2 scale):			Age, race/ethnicity, study centre, calendar year of blood collection	
			< 1 to 3 yr	115	0.85 (0.70–1.04)		
			> 3 to 5 yr	89	0.94 (0.74–1.18)		
			> 5 to 9 yr	155	0.98 (0.82–1.19)		
			> 9 yr	391	0.95 (0.84–1.09)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Li et al. (2022a) Ronneby, southern Sweden Enrolment, 1985–2013/follow-up, 1985–2016 (incidence) Cohort	60 507 (including 31 938 men); the Ronneby Register Cohort included all individuals who ever lived in Ronneby municipality in 1985–2013; one third of the households received PFAS-contaminated drinking-water from a waterworks situated near a military airfield where PFAS-containing firefighting foam was used in 1985–2013 (15 811 individuals with exposure considered "ever-high"); subsets with long-term exposure (≥ 11 yr) in the latest part of the follow-up period (2005–2013) were considered more highly exposed Exposure assessment method: see Table 2.1	Testis, incidence	Residential exposure to highly PFAS-contaminated drinking-water (SIR, Blekinge county excluding Ronneby referent):			Age, calendar year	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Complete ascertainment of community members in the cohort and follow-up through register-based linkages; high contrast in PFAS exposures within the cohort. *Other limitations*: See Table 2.1. Limited ability to assess potential effects of PFOS and PFOA individually; limited statistical power to assess risk of testicular cancer.
			Never	30	0.85 (0.57–1.21)		
			Ever	14	1.28 (0.70–2.15)		
		Testis, incidence	Residential exposure to highly PFAS-contaminated drinking-water (HR):				
			Never	31	1		
			Ever	14	1.38 (0.73–2.61)		
		Testis, incidence	Time period of residential exposure to highly PFAS-contaminated drinking-water (HR):				
			Never	31	1		
			Early (1985–2004)	9	1.35 (0.64–2.84)		
			Late (2005–2013)	5	1.46 (0.55–3.83)		
		Testis, incidence	Duration of residential exposure to highly PFAS-contaminated drinking-water (HR):				
			Never	31	1		
			Short (1–10 yr)	9	1.32 (0.63–2.79)		
			Long (≥ 11 yr)	5	1.51 (0.56–4.03)		
		Prostate, incidence	Residential exposure to highly PFAS-contaminated drinking-water (SIR, Blekinge county excluding Ronneby referent):				
			Never	712	1.14 (1.05–1.22)		
			Ever	181	0.96 (0.82–1.11)		
		Prostate, incidence	Residential exposure to highly PFAS-contaminated drinking-water (HR):				
			Never	712	1		
			Ever	181	0.83 (0.71–0.98)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Li et al. (2022a) Ronneby, southern Sweden Enrolment, 1985–2013/follow-up, 1985–2016 (incidence) Cohort (cont.)		Prostate, incidence	Time period of residential exposure to highly PFAS-contaminated drinking-water (HR):			Calendar year, age	
			Never	712	1		
			Early (1985–2004)	114	0.88 (0.72–1.08)		
			Late (2005–2013)	67	0.76 (0.59–0.98)		
		Prostate, incidence	Duration of residential exposure to highly PFAS-contaminated drinking-water (HR):				
			Never	712	1		
			Short (1–10 yr)	95	0.96 (0.78–1.20)		
			Long (≥ 11 yr)	86	0.72 (0.58–0.91)		
Purdue et al. (2023) USA Enrolment, 1988–2017/follow-up, through 2018 Nested case–control	Nested within a cohort of active-duty US Air Force servicemen (see Table 2.1) Cases: 530 overall (187 with two samples); TGCT diagnosed in the Department of Defence Cancer Registry	Testis, incidence	Serum PFOA (first/only sample) concentration (OR):			Date of birth, race/ethnicity, year entered military service, sample availability, military grade, number of deployments	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. *Other limitations*: See Table 2.1.
			≤ 4.45 ng/mL	161	1		
			4.46–5.87 ng/mL	115	0.7 (0.4–1.0)		
			5.88–7.85 ng/mL	121	0.7 (0.5–1.0)		
			> 7.85 ng/mL	133	0.8 (0.5–1.2)		
			Trend-test *P*-value, 0.46				
		Testis, incidence	Serum PFOA (first/only sample) concentration (OR):			Date of birth, race/ethnicity, year entered military service, sample availability, military grade, number of deployments, other PFAS (PFOS, PFHxS, PFNA, PFDA, PFUnDA, *N*-MeFOSAA)	
			≤ 4.45 ng/mL	161	1		
			4.46–5.87 ng/mL	115	0.7 (0.4–1.0)		
			5.88–7.85 ng/mL	121	0.7 (0.4–1.1)		
			> 7.85 ng/mL	133	0.8 (0.5–1.4)		
			Trend-test *P*-value, 0.86				

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Purdue et al. (2023) USA Enrolment, 1988–2017/follow-up, through 2018 Nested case–control (cont.)	Controls: 530 overall (187 with two samples); one control per case, density-sampled with replacement among eligible US Air Force servicemen on active duty and cancer-free as of the case diagnosis date and matched by date of birth, race/ethnicity (seven groups), year entering military service, year of baseline serum sample collection, and year of second sample collection (if applicable) Exposure assessment method: see Table 2.1	Testis, incidence	Serum PFOA (second sample) concentration (OR):			Date of birth, race/ethnicity, year entered military service, sample availability, military grade, number of deployments	
			≤ 4.25 ng/mL	55	1		
			4.26–5.65 ng/mL	52	1.0 (0.6–1.8)		
			5.66–7.55 ng/mL	39	0.7 (0.4–1.4)		
			> 7.55 ng/mL	41	0.7 (0.4–1.5)		
			Trend-test *P*-value, 0.35				
		Testis, incidence	Serum PFOA (second sample) concentration (OR):			Date of birth, race/ethnicity, year entered military service, sample availability, military grade, number of deployments, other PFAS (PFOS, PFHxS, PFNA, PFDA, PFUnDA, *N*-MeFOSAA)	
			≤ 4.25 ng/mL	55	1		
			4.26–5.65 ng/mL	52	1.0 (0.5–2.0)		
			5.66–7.55 ng/mL	39	0.6 (0.3–1.4)		
			> 7.55 ng/mL	41	0.6 (0.2–1.6)		
			Trend-test *P*-value, 0.22				
		Testis, incidence	Serum PFOS (first/only sample) concentration (OR):			Date of birth, race/ethnicity, year entered military service, sample availability, military grade, number of deployments	
			≤ 18.3 ng/mL	131	1		
			18.4–29.3 ng/mL	116	1.0 (0.6–1.5)		
			29.4–42.2 ng/mL	153	1.4 (0.8–2.3)		
			> 42.2 ng/mL	130	1.2 (0.7–2.0)		
			Trend-test *P*-value, 0.64				

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Purdue et al. (2023) USA Enrolment, 1988–2017/follow-up, through 2018 Nested case–control (cont.)		Testis, incidence	Serum PFOS (first/only sample) concentration (OR):			Date of birth, race/ethnicity, year entered military service, sample availability, military grade, number of deployments, other PFAS (PFOA, PFHxS, PFNA, PFDA, PFUnDA, *N*-MeFOSAA)	
			≤ 18.3 ng/mL	131	1		
			18.4–29.3 ng/mL	116	1.2 (0.7–1.9)		
			29.4–42.2 ng/mL	153	1.9 (1.0–3.4)		
			> 42.2 ng/mL	130	1.8 (0.9–3.6)		
			Trend-test *P*-value, 0.15				
		Testis, incidence	Serum PFOS (second sample) concentration (OR):			Date of birth, race/ethnicity, year entered military service, sample availability, military grade, number of deployments	
			≤ 13.2 ng/mL	42	1		
			13.3–21.2 ng/mL	38	1.1 (0.6–1.9)		
			21.3–33.5 ng/mL	50	1.9 (0.9–4.1)		
			> 33.5 ng/mL	57	2.6 (1.1–6.4)		
			Trend-test *P*-value, 0.02				

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Purdue et al. (2023) USA Enrolment, 1988–2017/follow-up, through 2018 Nested case–control (cont.)		Testis, incidence	Serum PFOS (second sample) concentration (OR):			Date of birth, race/ethnicity, year entered military service, sample availability, military grade, number of deployments, other PFAS (PFOA, PFHxS, PFNA, PFDA, PFUnDA, *N*-MeFOSAA)	
			≤ 13.2 ng/mL	42	1		
			13.3–21.2 ng/mL	38	1.5 (0.7–3.3)		
			21.3–33.5 ng/mL	50	2.8 (1.1–7.0)		
			> 33.5 ng/mL	57	4.6 (1.4–15.1)		
			Trend-test *P*-value, 0.009				
		Testis (seminoma), incidence	Serum PFOS (first/only sample) concentration (OR):				
			Below median	NR	1		
			Above median	NR	1.8 (1.0–3.3)		
		Testis (seminoma), incidence	Serum PFOS (second sample) concentration (OR):				
			Below median	NR	1		
			Above median	NR	2.8 (1.2–6.3)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Winquist et al. (2023) 20 US states Enrolment 1998–2001; follow-up through 30 June 2015 Case–cohort	Case–cohort study within the CPS-II Lifelink Cohort (see Table 2.1) Cases: 3762 overall (1610 prostate cancers); incident cases from the CPS-II Lifelink Cohort (surviving CPS-II Nutrition cohort participants) with first cancer diagnosis of prostate (men only) detected through self-report or NDI linkage and verified through medical records review or cancer registry; all participants with incident cancers Comparison cohort: 999; a sex-stratified simple random sample of 499 women and 500 men (~3% of the eligible cohort); stratification sampling was to ensure an adequate number of subcohort participants in sex-specific analyses (for breast and prostate cancers) Exposure assessment method: see Table 2.1	Prostate, incidence	Serum PFOA concentration (HR):			Year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. *Other limitations*: See Table 2.1.
			1st quartile (< 4.000 ng/mL)	398	1		
			2nd quartile (4.000 to < 5.300 ng/mL)	391	0.82 (0.60–1.11)		
			3rd quartile (5.300 to < 6.900 ng/mL)	405	0.93 (0.68–1.27)		
			4th quartile (≥ 6.900 ng/mL)	405	0.83 (0.61–1.14)		
			Continuous (per unit on log_2 scale)	1599	0.93 (0.79–1.08)		
		Prostate, incidence	Serum PFOS concentration (HR):				
			1st quartile (< 14.000 ng/mL)	389	1		
			2nd quartile (14.000 to < 19.000 ng/mL)	392	0.94 (0.70–1.26)		
			3rd quartile (19.000 to < 26.000 ng/mL)	410	1.11 (0.81–1.50)		
			4th quartile (≥ 26.000 ng/mL)	408	1.08 (0.80–1.46)		
			Continuous (per unit on log_2 scale)	1599	1.00 (0.88–1.14)		

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Vieira et al. (2013) OH and WV, USA 1996–2005 (incidence) Case–control	Cases: study 1: 134 cancers of the testis, 3678 cancers of the prostate; Study 2: 61 cancers of the testis, 1155 cancers of the prostate; index cancer cases were retrieved from cancer registries covering a community sample with relatively high exposure to PFOA because of contamination of drinking-water from the Parkersburg (WV), polymer-production plant Controls: NR; for each cancer site evaluated, controls were cases of cancer at all other sites among men, with the exclusion of four cancers of a priori interest (kidney, testis, pancreas, and liver) that have been associated with PFOA in studies in experimental animals or humans Exposure assessment method: see Table 2.1	Testis, incidence	Study 1: residence in a PFOA-contaminated water district (OH and WV) (OR):			Age, diagnosis year, insurance provider, smoking status	*Exposure assessment critique*: See Table 2.1 *Other strengths*: See Table 2.1. *Other limitations*: See Table 2.1. *Other comments*: Substantial overlap with Barry et al. (2013).
			Unexposed	116	1		
			Any exposed water district	18	1.0 (0.6–1.8)		
			Little Hocking	8	5.1 (1.6–15.6)		
			Lubeck	2	0.9 (0.2–4.5)		
			Tuppers Plains	2	0.4 (0.1–2.0)		
			Belpre	1	0.6 (0.1–5.0)		
			Pomeroy	0	NC		
			Mason	5	0.5 (0.2–1.5)		
		Testis, incidence	Analysis 2: individual-level annual PFOA serum exposure, assuming 10-yr residency and latency (OH only) (OR):			Age, race, diagnosis year, insurance provider, smoking status	
			Unexposed	50	1		
			Low (3.7–12.8 µg/L)	1	0.2 (0.0–1.6)		
			Medium (12.9–30.7 µg/L)	3	0.6 (0.2–2.2)		
			High (30.8–109 µg/L)	1	0.3 (0.0–2.7)		
			Very high (110–655 µg/L)	6	2.8 (0.8–9.2)		
		Prostate, incidence	Analysis 1: Residence in a PFOA-contaminated water district (OH and WV) (OR):			Age, diagnosis year, insurance provider, smoking status	
			Unexposed	3244	1		
			Any exposed water district	434	0.9 (0.8–1.1)		
			Little Hocking	36	1.4 (0.9–2.3)		
			Lubeck	78	1.2 (0.9–1.6)		
			Tuppers Plains	56	0.8 (0.6–1.1)		
			Belpre	56	0.8 (0.6–1.1)		
			Pomeroy	12	1.3 (0.6–2.6)		
			Mason	196	0.9 (0.7–1.0)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Vieira et al. (2013) OH and WV, USA 1996–2005 (incidence) Case–control (cont.)		Prostate, incidence	Analysis 2: individual-level annual PFOA serum exposure, assuming 10-yr residency and latency (OH only) (OR):			Age, race, diagnosis year, insurance provider, smoking status	
			Unexposed	941	1		
			Low (3.7–12.8 µg/L)	71	1.1 (0.8–1.5)		
			Medium (12.9–30.7 µg/L)	65	0.8 (0.6–1.0)		
			High (30.8–109 µg/L)	47	0.8 (0.5–1.1)		
			Very high (110–655 µg/L)	31	1.5 (0.9–2.5)		
Hardell et al. (2014) Örebro County, Sweden 2007–2011 Case–control	Cases: 201; patients with prostate cancer admitted for treatment at the University Hospital in Örebro between 2007 and 2011 Controls: 186; cancer-free controls from Örebro County who were identified from the Swedish population registry and matched to cases on age Exposure assessment method: quantitative measurements; analytical method was state-of-the-art; a single blood sample was collected during the same time period for cases and matched controls; blood was collected before	Prostate, incidence	Serum PFOA concentration (OR):			Age, BMI, year of sampling	*Exposure assessment critique*: Key strengths were that whole blood levels represent the combined exposure through all exposure pathways; measurement error low. Key limitations were that if prostate cancer alters ADME of PFAS there could be possible differential exposure misclassification, as blood collection of cases was at or after diagnosis; single samples at time of case hospitalization may not reflect exposure at crucial windows in cancer development.
			≤1.9 ng/mL (median for controls)	93	1		
			> 1.9 ng/mL	108	1.1 (0.7–1.7)		
		Prostate, incidence	Serum PFOS concentration (OR):				
			≤8.3 ng/mL (median for controls)	92	1		
			> 8.3 ng/mL	109	1.0 (0.6–1.5)		
		Prostate (Gleason score, 7–10), incidence	Serum PFOA concentration (OR):				
			≤1.9 ng/mL (median for controls)	56	1		
			> 1.9 ng/mL	67	1.2 (0.7–1.8)		
		Prostate (Gleason score, 7–10), incidence	Serum PFOS concentration (OR):				
			≤8.3 ng/mL (median for controls)	53	1		
			> 8.3 ng/mL	70	1.1 (0.7–1.9)		
		Prostate (PSA level ≥ 11), incidence	Serum PFOA concentration (OR):				
			≤1.9 ng/mL (median for controls)	39	1		
			> 1.9 ng/mL	52	1.3 (0.8–2.1)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description Exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hardell et al. (2014) Örebro County, Sweden 2007–2011 Case–control (cont.)	treatment, during hospitalization to receive treatment or at general practitioners	Prostate (PSA level ≥ 11), incidence	Serum PFOS concentration (OR):			Age, BMI, year of sampling	*Other strengths*: Availability of information on disease aggressiveness (Gleason score and PSA) and family history of prostate cancer. *Other limitations*: Lack of adjustment for other PFAS; relatively small sample size.
			≤8.3ng/mL(median for controls)	47	1		
			> 8.3 ng/mL	44	0.8 (0.4–1.3)		
		Prostate, incidence	Heredity and serum PFOA concentration (OR):				
			No heredity, ≤ 1.9 ng/mL	77	1		
			Heredity, ≤ 1.9 ng/mL	16	1.1 (0.5–2.6)		
			No heredity, > 1.9 ng/mL	84	1.0 (0.6–1.5)		
			Heredity, > 1.9 ng/mL	24	2.6 (1.2–6.0)		
		Prostate, incidence	Heredity and serum PFOS concentration (OR):			Age, BMI, year of sampling	
			No heredity, ≤ 8.3 ng/mL	72	1		
			Heredity, ≤ 8.3 ng/mL	20	1.2 (0.6–2.5)		
			No heredity, > 8.3 ng/mL	89	0.9 (0.5–1.4)		
			Heredity, > 8.3 ng/mL	20	2.7 (1.04–6.8)		

ADME, absorption, distribution, metabolism, and excretion; APFO, ammonium perfluorooctanoate; approx., approximately; BMI, body mass index; CI, confidence interval; CPS-II, Cancer Prevention Study II; *N*-EtFOSAA, 2-(*N*-ethyl-perfluorooctane sulfonamido) acetic acid; FOSA, perfluorooctane sulfonamide; HR, hazard ratio; IRR, incidence rate ratio; *N*-MeFOSAA, 2-(*N*-methyl-perfluorooctane sulfonamido) acetic acid; MN, Minnesota; mo, month(s); NC, not calculated; NDI, National Death Index; NR, not reported; OH, Ohio; OR, odds ratio; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptanesulfonic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFUnDA, perfluoroundecanoic acid; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; ppm, part per million; PSA, prostate-specific antigen; PTFE, polytetrafluoroethylene; RR, rate ratio; SIR, standardized incidence ratio; SMR, standardized mortality ratio; TGCT, testicular germ cell tumour; TFE, tetrafluoroethylene; UK, United Kingdom; US, United States; USA, United States of America; WV, West Virginia; yr, year(s).

[The Working Group considered this study to be highly informative for the relation between PFOA and testicular cancer. Its primary strengths included the detailed characterization of estimated serum PFOA levels over time, the high response rate among participants in the C8 Health Project, and the validation of diagnosed testicular cancers through state registries and medical chart review. However, the small number of confirmed cases of testicular cancer was a limitation.]

Li et al. (2022a) investigated the incidence of testicular cancer in the Ronneby community cohort in Sweden, which had high exposures to PFAS (primarily PFOS and PFHxS, but also PFOA to some extent) from contaminated drinking-water (Section 2.1.13). Based on 14 observed cases of incident testicular cancer among residents in areas with contaminated drinking-water, elevated but imprecise risks were observed relative to regional rates (SIR, 1.28; 95% CI, 0.70–2.15) and relative to those in the Ronneby municipality among people who had never resided in contaminated water districts (HR, 1.38; 95% CI, 0.73–2.61). In analyses of people residing in districts with contaminated water during the later years when levels of PFAS contamination were higher (versus those never residing in districts with contaminated water), the hazard ratio was 1.46 (95% CI, 0.55–3.83). For those with a longer duration of residence in contaminated districts, the hazard ratio was 1.51 (95% CI, 0.56–4.03). The risk estimates for exposure in later years and for longer duration of residence were both based on analyses with 5 exposed cases.

[The strengths of this study included the complete ascertainment of community members in the cohort and follow-up through register-based linkages, and the high contrast in PFAS exposures within the cohort. However, the PFAS exposure profile and exposure assessment approach in this investigation – which characterized the potential for PFAS exposure overall rather than for specific PFAS and did not account for individual-level factors such as water consumption or use of bottled water or water filtration – limited the ability to isolate potential effects of PFOS and PFOA individually and also probably resulted in non-differential exposure misclassification, which typically might be expected to attenuate the reported risk estimates. The Working Group also noted that the findings from this study may not be directly comparable to those for PFOA in Barry et al. (2013), given that PFAS exposure in this study was dominated by PFOS and PFHxS. PFOA serum levels (in samples collected 1–2 years after cessation of exposure) were lower in this population than in the C8 Science Panel study. Finally, with only 14 exposed cases of testicular cancer, the study had limited statistical power to evaluate associations with this malignancy.]

Purdue et al. (2023) conducted a nested case–control study of prediagnostic serum PFAS concentrations and risk of TGCT among US Air Force servicemen, using sera collected between 1988 and 2017 and stored in the DoD Serum Repository (Section 2.1.17). The study included 530 cases of TGCT among servicemen aged < 40 years and on active duty at diagnosis and 530 individually matched controls; of these, 187 cases and 187 matched controls also had measured PFAS concentrations from a second prediagnostic serum sample collected a median of 4 years after the first sample. In analyses conditioned on matching factors and adjusted for military grade and number of deployments, serum PFOA concentrations were not associated with TGCT risk on the basis of measurements in the first or only samples in the study population overall (fourth versus first quartile, OR, 0.8; 95% CI, 0.5–1.2; P for trend, 0.46) or the second samples from 187 case–control sets (OR, 0.7; 95% CI, 0.4–1.5; P for trend, 0.35); the results were similar after additionally adjusting for other PFAS. For PFOS, although no association with TGCT risk was seen in analyses of the

first or only samples (fourth versus first quartile, OR, 1.2; 95% CI, 0.7–2.0; *P* for trend, 0.64), the authors observed an exposure–response association with PFOS concentrations in the second samples (OR, 2.6; 95% CI, 1.1–6.4; *P* for trend, 0.02). After adjustment for other PFAS, the corresponding risk estimates for the fourth versus first quartiles of PFOS concentrations in the first/only and second serum samples were 1.8 (95% CI, 0.9–3.6; *P* for trend, 0.15) and 4.6 (95% CI, 1.4–15.1; *P* for trend, 0.009), respectively. Associations with seminomas (which are typically diagnosed at older ages than are nonseminomas) were observed for PFOS (e.g. above versus below median PFOS concentrations with adjustment for other PFAS: first or only sample, OR, 1.8; 95% CI, 1.0–3.3; second sample, OR, 2.8; 95% CI, 1.2–6.3).

[The Working Group considered this study to be highly informative because of a number of strengths, including its large sample size, the measurements of PFOA and PFOS in prediagnostic samples, the availability of repeated samples during potential etiologically relevant time periods from a subset of cases and controls, the ability to adjust for other PFAS, and the identification of cases in an age range during which most TGCTs are diagnosed. With respect to the timing of the repeated sample collections in this study, the Working Group noted that the first or only samples were often collected shortly after entering military service (a median of 0.3 and 0.4 years after enlistment for cases and controls, respectively) and probably reflected exposure patterns before military service. In contrast, the second samples (when available or selected) were typically collected after several years of service and may be more representative of PFAS levels during active duty, although PFOA and PFOS levels were still generally similar to those for comparably aged men in the US population overall. Participants with second samples also tended to be older and were more likely to be diagnosed with seminoma (which is consistent with the typical age distributions of TGCT subtypes). As such, it is possible that the association with PFOS observed in the analyses of second samples may reflect patterns of risk related to exposure during military service, during different etiological time windows, and/or for seminomas in particular. A limitation of this study was the lack of information provided on associations with PFOA for histological subtypes of TGCT, which precluded an assessment of potential differences in the relation between PFOA and seminoma and nonseminoma tumours in this population.]

A single non-nested case–control study evaluated testicular cancer risk in relation to exposure to PFOA. A case–control study by Vieira et al. (2013) using data from cancer registries in Ohio and West Virginia evaluated various malignancies, including testicular cancer, among Mid-Ohio Valley residents with exposure to PFOA-contaminated drinking-water (Section 2.1.22). In analyses based on ecological exposure assignment for residence in areas of West Virginia and Ohio with contaminated water (all participants in the study), the investigators found no association with testicular cancer overall (adjusted OR, 1.0; 95% CI, 0.6–1.8; 18 cases in districts with contaminated water). An elevated OR was observed in the water district with the highest levels of PFOA exposure (Little Hocking: adjusted OR, 5.1; 95% CI, 1.6–15.6; 8 exposed cases). In analyses based on estimated serum PFOA concentrations among Ohio participants (not available for West Virginia participants), an elevated risk of testicular cancer was observed among those in the highest category of exposure compared with unexposed individuals, although the confidence interval was wide and included the null value (adjusted OR, 2.8; 95% CI, 0.8–9.2; 6 exposed cases).

[The Working Group noted that the cancer cases included in Vieira et al. (2013) overlapped with those in the study by Barry et al. (2013), although the degree of overlap was unknown. It was also noted that in the analyses of more

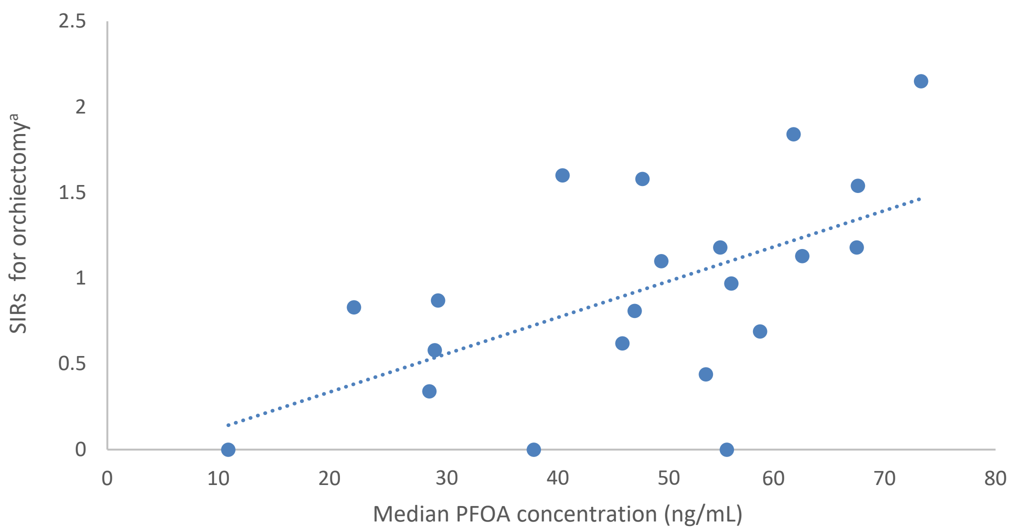
detailed estimates of serum PFOA concentrations (only available for Ohio participants), the testicular cancer cases from Little Hocking may have been overrepresented in the highest exposure category (as this district had the highest levels of PFOA contamination). The degree of overlap was not reported, but the risk estimates from the two analyses may not be independent.]

In the Veneto region of Italy, an area with water contaminated with PFAS (overwhelmingly PFOA) from a local manufacturing plant, residents were invited to participate in a surveillance programme (participation rate, 63.5%) to address public concern about their exposure. Some of the participants lived in areas of the region with less-contaminated water. Among adults aged 14–39 years at recruitment, more than 18 000 people (9230 men) participating in this programme provided serum (Pitter et al., 2020). The median serum PFOA concentration was 44.4 ng/mL. An epidemiological investigation evaluated the frequency of orchiectomies in this region between 1997 and 2014 (Sistema Epidemiologico Regionale, 2016). Orchiectomy was used as a proxy for a diagnosis of testicular cancer (sensitivity and positive predictive values of 91.7% (95% CI, 88.0–95.4%) and 92.8% (95% CI, 89.3–96.2%), respectively, in this region). Orchiectomies were ascertained using information in hospital discharge records, which included address of residence and the main medical procedures from hospital stays and were completed for the purpose of reimbursement from the Italian national health system. SIRs for orchiectomy were estimated for the 21 municipalities separately, comparing the observed orchiectomies (n = 70 overall) versus expected numbers based on regional rates in 5-year age groups (Sistema Epidemiologico Regionale, 2016). The Working Group combined the serum PFOA data and orchiectomy rates by municipality (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636); as shown in Fig. 2.1, a strong correlation (Spearman correlation, 0.57; P = 0.006) was observed between serum PFOA concentrations and rates of orchiectomy (standardized on age by 5-year age groups from 15–54 years to the overall regional rate) by municipality. The Working Group also conducted a Poisson regression of observed orchiectomies on median PFOA levels across 21 municipalities, using the log of expected events as an offset, and correcting for dispersion. The RR for each unit (ng/mL) increase of PFOA was 1.018 (95% CI, 1.006–1.031; P = 0.003).

[The Working Group considered the findings from these data from a region with high PFOA exposure to be informative because of the large number of serum measurements in the population, high PFOA levels, and good ascertainment of orchiectomy, which was shown to be an excellent surrogate for diagnosis of testicular cancer in this region. The ecological design and small numbers of orchiectomies by municipality and resulting imprecise SIR values were limitations.]

A review by Bartell and Vieira (2021) included a meta-analysis of associations between PFOA and testicular cancer from the Vieira et al. (2013) study and those reported in Barry et al. (2013); they found a 3% increase in the risk of testicular cancer for each 10 ng/mL increase in estimated serum PFOA concentration (random-effects meta-RR, 1.03; 95% CI, 1.02–1.04). Results from a fixed-effects meta-analysis were similar. [The Working Group considered the informativeness of this meta-analysis to be reduced because of the unknown degree of overlap between the studies by Vieira et al. (2013) and Barry et al. (2013), and because the study by Purdue et al. (2023) was not available that time. If there were substantial overlap between the studies by Vieira et al. (2013) and Barry et al. (2013), then the resulting meta-RRs could be overestimated. Another meta-analysis by Seyyedsalehi and Boffetta (2023) was not considered because it also did not include the Purdue et al. (2023) study and did not contribute any other information.]

Fig. 2.1 Serum PFOA concentrations and orchiectomy rates by municipality, in Veneto, Italy



PFOA, perfluorooctanoic acid; SIR, standardized incidence ratio.
[a] Age-standardized to the regional Veneto population.
Pearson correlation coefficient, 0.58; $P = 0.006$.
Note that SIRs were plotted because they followed approximately normal distribution.

2.3.2 Prostate cancer

As summarized in Table 2.3 and below, there have been six investigations of prostate cancer incidence, mortality, or both in cohorts with occupational (Steenland and Woskie, 2012; Consonni et al., 2013; Raleigh et al., 2014; Steenland et al., 2015) or high environmental (Barry et al., 2013; Li et al., 2022a) exposure to PFOA and/or PFOS, and three investigations of serum PFOA and PFOS concentrations and risk of prostate cancer nested within general population cohorts (Eriksen et al., 2009; Rhee et al., 2023a; Winquist et al., 2023). Prostate cancer was also evaluated in two case–control studies (Vieira et al., 2013; Hardell et al., 2014). As described above and in Section 2.1.22, the study by Vieira et al. (2013) included individuals in the Mid-Ohio Valley with high exposure to PFOA, and the population overlapped with that of the cohort study by Barry et al. (2013). Hardell et al. (2014) conducted a population-based case–control study of serum PFAS concentrations and prostate cancer in a population with background levels of exposure in Sweden. Beyond these studies, an occupational cohort of 652 PFOS-exposed employees at a fluorochemical-production facility evaluated prostate cancer (5 exposed cases) identified from health claims data (Olsen et al., 2004). [The Working Group considered the study by Olsen et al. (2004) to be uninformative, given the small number of exposed cases and the focus on prevalent (rather than incident) cancers as the outcome; as such, it was not included in Table 2.3 or in the summary below.]

Raleigh et al. (2014) examined prostate cancer incidence and mortality among 3716 male workers at an APFO-production facility in Cottage Grove, Minnesota, USA (Section 2.1.1). They found no evidence of excess prostate cancer mortality on the basis of 24 deaths among PFOA-exposed workers (relative to Minnesota state rates: SMR, 0.83; 95% CI, 0.53–1.23), and no associations were observed in exposure–response analyses with unexposed workers as the reference group. Exposure–response analyses of

prostate cancer incidence (on the basis of 188 cases among PFOA-exposed workers) were similarly null.

Steenland and Woskie (2012) evaluated prostate cancer mortality among 5791 workers exposed to PFOA at a fluoropolymer-production plant in Parkersburg, West Virginia, USA, in the Mid-Ohio Valley (Section 2.1.3). In analyses based on 21 deaths among PFOA-exposed workers, the authors found no evidence of excess prostate cancer mortality relative either to workers from other plants within the same company in the same region (SMR, 0.76; 95% CI, 0.47–1.16) or to the general US population (SMR, 0.72; 95% CI, 0.45–1.10), and no associations were observed in exposure–response analyses of estimated cumulative PFOA exposure. In a subsequent analysis of prostate cancer incidence that included 129 cases among a subset of 2955 male workers from this cohort, Steenland et al. (2015) observed elevated but imprecise estimates of prostate cancer rates among those with higher estimated cumulative PFOA exposure. Relative to those in the lowest quartile, the rate ratios in the second, third, and fourth quartiles were 1.81 (95% CI, 0.69–4.78), 2.45 (95% CI, 0.96–6.25), and 1.88 (95% CI, 0.72–4.88), respectively, and the P for trend was 0.11. Similar patterns were observed in analyses lagged by 10 years.

[The Working Group noted that the small numbers of deaths from prostate cancer in Raleigh et al. (2014) and Steenland and Woskie (2012) limited the ability to evaluate associations with prostate cancer mortality in both studies. Analyses of prostate cancer incidence by Steenland et al. (2015) and Raleigh et al. (2014) – which included 129 and 188 cases, respectively – had somewhat better statistical power.]

Eriksen et al. (2009) conducted a prospective case–cohort study nested within a cohort of older Danish adults with background levels of exposure to PFOA and PFOS. Samples were collected at cohort enrolment between 1993 and 1997, and prediagnostic plasma concentrations of PFOA and PFOS were measured for 713 cases of prostate cancer and 680 non-cases (Section 2.1.4). For PFOS, the authors observed about 30–40% increased risks of prostate cancer in the three upper quartiles compared with the lowest quartile (e.g. fourth versus first quartile, incidence rate ratio, IRR, 1.38; 95% CI, 0.99–1.93); in regression analyses in which plasma PFOS concentration was included as a continuous variable, the IRR corresponding to a 10 ng/mL increase in plasma PFOS levels was 1.05 (95% CI, 0.97–1.14). For PFOA, an exposure–response pattern was not apparent in analyses based on quartiles of measured levels; a modest increase in risk was observed when PFOA was modelled continuously (per 1 ng/mL increase, IRR, 1.03; 95% CI, 0.99–1.07).

[The Working Group noted that the main strengths of this study were the large number of cases of prostate cancer and non-cases, a well-defined national cohort with complete ascertainment of incident cancer cases, data on a wide range of potential confounding factors, and a reasonable exposure contrast in a population with plasma PFOA and PFOS concentrations consistent with background levels of exposure. The measurements of PFOA and PFOS were conducted using samples collected a median of 7 years before cancer diagnosis; however, the authors did not report results for different time intervals between serum collection and diagnosis of prostate cancer to assess potential etiologically relevant periods of exposure. Also, the investigators observed a strong correlation between plasma PFOA and PFOS concentrations (Spearman correlation, 0.70) but did not evaluate prostate cancer risk in analyses with mutual adjustment for both chemicals, limiting the ability to assess the associations with each of these exposures independently.]

Barry et al. (2013) evaluated the risk of incident prostate cancer among community members and workers in the C8 Science Panel study (Section 2.1.5). A total of 446 validated prostate

cancer cases were included in the analyses; the investigators found no evidence of an exposure–response association with estimated cumulative PFOA serum concentrations (unlagged analysis: adjusted HR corresponding to a unit increase in natural log-transformed levels, 0.99; 95% CI, 0.93–1.04). The corresponding risk estimate from an analysis with a 10-year lag period was similar (HR, 0.99; 95% CI, 0.94–1.05).

[The Working Group noted that the strengths of the study by Barry et al. (2013) included the detailed enumeration of the cohort, ascertainment or confirmation of cancer diagnoses, and relatively large numbers of incident prostate cancer cases.]

Consonni et al. (2013) evaluated prostate cancer mortality in a pooled international cohort of 4773 male workers who had ever been exposed to TFE (Section 2.1.6). Among those who had ever been exposed to APFO (n = 4205), the investigators observed reduced mortality from prostate cancer in analyses based on 3 observed deaths (using national reference rates: SMR, 0.24; 95% CI, 0.05–0.70).

[The Working Group noted that the small numbers of deaths from prostate cancer in this occupational cohort limited the ability to evaluate associations with prostate cancer mortality.]

Rhee et al. (2023a) conducted a nested case–control study of aggressive prostate cancer (750 cases, 750 matched controls) in relation to prediagnostic serum PFAS concentrations (including PFOA and PFOS) within the PLCO Cancer Screening Trial cohort (Section 2.1.11). Aggressive prostate cancer was defined as having stage III or IV disease, Gleason score ≥ 8, or Gleason score 7 and death from prostate cancer. The study included cases with serum samples collected > 300 days before prostate cancer diagnosis (a median of 9 years from blood collection to diagnosis). Controls were selected from among participants who were alive and cancer-free as of the case diagnosis date and were individually matched to cases on age at baseline, race or ethnicity, study centre, calendar year and study year of blood collection, and previous freeze–thaw cycles. For a subset of 60 controls, the investigators measured PFAS concentrations in sera collected at three time points up to 6 years apart. In overall logistic regression analyses of PFOA conditioned on matching factors, the investigators observed an inverse association with aggressive prostate cancer (per doubling in serum PFOA concentration, OR for a 1-unit increase in PFOA serum concentration on the $\log_2$ scale, 0.82; 95% CI, 0.71–0.96). This association remained apparent in analyses adjusted for prostate cancer risk factors (BMI, smoking status, family history of prostate cancer, history of diabetes) and other PFAS – PFOS, PFHxS, PFNA, *N*-EtFOSAA, perfluorooctane sulfonamide (FOSA), 2-*N*-methyl-perfluorooctane sulfonamido acetate (*N*-MeFOSAA), and perfluoroheptanesulfonic acid (PFHpS). However, in analyses restricted to cases diagnosed > 3 years after blood collection, the association was less apparent (OR_{log2}, 0.90; 95% CI, 0.79–1.03). For PFOS, a modest inverse association was observed in analyses conditioned on matching factors but not adjusted for other covariates (OR_{log2}, 0.93; 95% CI, 0.83–1.05), whereas no association was observed after adjustment for prostate cancer risk factors and other PFAS (OR_{log2}, 0.99; 95% CI, 0.79–1.23). Analyses of serial samples collected up to 6 years apart from a subset of controls demonstrated good within-subject agreement in measurements of PFOA and PFOS over time, with overall intraclass correlation coefficients of 0.73 (95% CI, 0.62–0.81) and 0.85 (95% CI, 0.78–0.90) for PFOA and PFOS, respectively (Rhee et al., 2023a).

[The Working Group identified several strengths of this study that contributed to its informativeness, including its large sample size, measurements of serum PFOA and PFOS concentrations in prediagnostic samples, and the ability to adjust for measured concentrations of other PFAS and other potential confounding factors.]

In the Ronneby community cohort (Section 2.1.13), Li et al. (2022a) identified 181 cases of prostate cancer among residents in areas with PFAS-contaminated drinking-water. The investigators found that prostate cancer incidence rates among men who resided in exposed areas were similar to regional rates (SIR, 0.96; 95% CI, 0.82–1.11) and that risk for these men was lower than that for men in the Ronneby municipality who had never resided in contaminated water districts (HR, 0.83; 95% CI, 0.71–0.98). Inverse associations with prostate cancer risk were also observed among those residing in contaminated districts during the later years (HR, 0.76; 95% CI, 0.59–0.98; 67 exposed cases) and those with longer duration of residence in contaminated districts (HR, 0.72; 95% CI, 0.58–0.91; 86 exposed cases).

[The Working Group noted that the strengths of the study (Li et al., 2022a) included the detailed enumeration of the cohort, ascertainment and confirmation of cancer diagnoses, and relatively large numbers of incident prostate cancer cases. However, a limitation of the exposure assessment in the study by Li et al. (2022a) was the inability of their analysis to distinguish between the potential effects of PFOS and PFHxS.]

Winquist et al. (2023) evaluated prostate cancer in their case–cohort investigation in the ACS CPS-II LifeLink Cohort (see Section 2.1.21). In analyses with 1599 selected prostate cancer cases, they found no associations with prostate cancer risk for either PFOA or PFOS; when $\log_2$-transformed levels were modelled continuously, the observed hazard ratios were 0.93 (95% CI, 0.79–1.08) and 1.00 (95% CI, 0.88–1.14) for PFOA and PFOS, respectively. Analyses based on quartiles of PFOA and PFOS were similarly null. [The Working Group noted several strengths of this study, including its large sample size, measurements of serum PFOA and PFOS concentrations in prediagnostic samples, and the ability to adjust for other potential confounding factors. Limitations included the relatively low exposure contrast in the study population.]

In addition to the cohort-based studies summarized above, the Working Group also reviewed two case–control studies of PFOA and/or PFOS exposure and prostate cancer (Vieira et al., 2013; Hardell et al., 2014).

Vieira et al. (2013) evaluated prostate cancer in their case–control studies on multiple cancer types among Mid-Ohio Valley residents exposed to PFOA from contaminated drinking-water. In analyses with 434 PFOA-exposed cases in West Virginia and Ohio, they found no association with prostate cancer (adjusted OR, 0.9; 95% CI, 0.8–1.1); a modest increased risk was observed in the water district with the highest levels of PFOA exposure (adjusted OR, 1.4; 95% CI, 0.9–2.3; 36 exposed cases). Among Ohio participants, an elevated but imprecise OR was also observed among those in the highest category of PFOA exposure compared with unexposed individuals (adjusted OR, 1.5; 95% CI, 0.9–2.5; 31 exposed cases).

[The Working Group noted that the degree of overlap in the cancer cases included in the studies by Vieira et al. (2013) and Barry et al. (2013) was unknown, and as such the results of the two studies cannot necessarily be interpreted independently.]

Hardell et al. (2014) conducted a case–control study of serum PFAS concentrations (including PFOA and PFOS) and prostate cancer in Örebro County, Sweden. The study included 201 cases and 186 population-based controls, with blood samples collected in the period 2007–2011. Samples were collected from cases after diagnosis of prostate cancer but before initiating treatment. The investigators found no associations with PFOA or PFOS in overall analyses and among those with markers indicative of more advanced disease (Gleason score 7–10, or prostate-specific antigen, PSA ≥ 11). However, for participants who reported a family history of disease (prostate cancer in a first-degree relative)

and had serum concentrations above the median (24 cases), increased risks were observed for both PFOA (OR, 2.6; 95% CI, 1.2–6.0) and PFOS (OR, 2.7; 95% CI, 1.04–6.8) relative to participants with no family history and serum concentrations at or below the median for the respective chemicals.

[The Working Group noted several limitations of this study. Samples were collected from cases after diagnosis of prostate cancer, and it was possible that the measurements of serum PFOA and PFOS concentrations may have been influenced by disease status, which could have resulted in differential exposure misclassification between cases and controls, possibly biasing risk estimates either towards or away from the null value. It was also possible that PFAS concentrations at the time of diagnosis may not have reflected exposure levels during an etiologically relevant time period. However, the sample collections occurred before cases were treated, so any potential treatment-related effects on PFAS levels were not a concern in this study. Other limitations included the non-participation of some selected cases and matched controls (response rates were 79% and 54%, respectively), the lack of adjustment for other PFAS in the statistical analyses, and the relatively small sample size, particularly for analyses stratified by family history of prostate cancer.]

2.4 Cancers of the breast and thyroid gland

2.4.1 *Cancer of the breast*

See Table 2.4.

(a) Cohort and nested case–control studies

There were 12 cohort or nested case–control studies that contributed evidence on PFOA and PFOS exposure and the risk of breast cancer in women. Three of these studies were occupational cohorts (Alexander et al., 2003; Steenland and Woskie, 2012; Raleigh et al., 2014). [The Working Group noted that these occupational cohorts included few women and thus had extremely limited power with which to consider associations with breast cancer, resulting in limited inference from those studies.] Two studies (Barry et al., 2013; Li et al., 2022a) considered how environmental exposure to high levels of PFAS in the contaminated environment from nearby industrial or occupational sources was related to breast cancer risk using modelled exposure assessment. The remaining studies included two nested case–control studies focused on PFAS measurements during pregnancy in relation to subsequent risk of breast cancer in the individual (Ghisari et al., 2017) and the offspring (Cohn et al., 2020), as well as blood measurements in nested substudies within larger population-based cohorts (Hurley et al., 2018; Mancini et al., 2020a; Feng et al., 2022; Chang et al., 2023; Winquist et al., 2023).

Raleigh et al. (2014) evaluated cancer incidence and mortality in an occupational cohort that included 4668 workers (of whom 952 were women) who had worked for ≥ 1 year and were exposed to APFO at a factory in Cottage Grove, Minneapolis, USA, between 1947 and 2002, and a comparison group of 4359 employees (of whom 526 were women) who were unexposed workers at a factory in Saint Paul (see Section 2.1.1). Individual inhalation exposure was estimated using a JEM, and information on cancer incidence and mortality was obtained via linkages to registries, with follow-up until 2008. Women represented only 21% of the workers at the Cottage Grove facility and 12% at the Saint Paul facility. There were 26 deaths (11 exposed, 15 unexposed) from breast cancer (25 among women) and 62 cases (34 exposed, 28 unexposed) of incident breast cancer. There was little evidence to suggest that increased APFO exposure was associated with a higher SMR for breast cancer relative to population mortality rates or with an increased hazard ratio for incident breast cancer cases, although this was based on few cases. [The Working Group noted that very

Table 2.4 Epidemiological studies on exposure to PFOA or PFOS and cancers of the breast and thyroid gland

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Raleigh et al. (2014) MN, USA Enrolment, 1947–2002/follow-up, 1947–2008 (mortality), 1988–2008 (incidence) Cohort	9027 (952 exposed women, 526 reference women); Cottage Grove (MN) PFOA cohort; workers employed for ≥ 1 yr in 1947–2002 at an APFO facility (Cottage Grove; n = 4668); reference workers without any exposure to APFO employed at a tape and abrasives production facility located in the same suburban geographical area and managed by the same company (Saint Paul; n = 4359) Exposure assessment method: see Table 2.1	Breast, mortality	Exposed to APFO (SMR, MN referent):			Age, sex, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. Unlikely TFE co-exposure; Reference population sharing similar socioeconomic characteristics. *Other limitations*: See Table 2.1. Lacking data on workers that left Minnesota or Wisconsin. Small numbers especially for women (12% of the cohort).
			Unexposed (Saint Paul plant)	15	1.39 (0.78–2.29)		
			Exposed (Cottage Grove plant)	11	0.82 (0.41–1.47)		
		Breast, mortality	Estimated cumulative airborne APFO exposure quartile (SMR, MN referent):				
			1st quartile (< 2.6×10^{-5} µg/m³-years)	5	0.80 (0.26–1.86)		
			2nd quartile (2.6×10^{-5} to < 1.4×10^{-4} µg/m³-years)	3	0.88 (0.18–2.56)		
			3rd quartile (1.4×10^{-4} to < 7.3×10^{-4} µg/m³-years)	2	0.73 (0.09–2.62)		
			4th quartile (≥ 7.3×10^{-4} µg/m³-years)	1	1.02 (0.03–5.69)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Raleigh et al. (2014) MN, USA Enrolment, 1947–2002/ follow-up, 1947–2008 (mortality), 1988–2008 (incidence) Cohort (cont.)		Breast, incidence	Estimated cumulative airborne APFO exposure quartile (HR):			Age, [sex], year of birth	
			Unexposed (Saint Paul plant)	28	1		
			1st quartile (< 2.9×10^{-5} µg/ m^3-years)	8	0.36 (0.16–0.79)		
			2nd quartile (2.9×10^{-5} to < 1.5×10^{-4} µg/ m^3-years)	8	0.65 (0.29–1.42)		
			3rd quartile (1.5×10^{-4} to < 7.9×10^{-4} µg/ m^3-years)	14	1.47 (0.77–2.80)		
			4th quartile (≥ 7.9×10^{-4} µg/ m^3-years)	4	0.85 (0.29–2.46)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lundin et al. (2009) MN, USA Enrolment, 1947–1997/ follow-up, 1947–2002 (mortality) Cohort	3993 employees; Cottage Grove (MN) PFOA cohort; workers employed at a PFOA-production plant for ≥ 365 days before 31 December 1997 Exposure assessment method: see Table 2.1	Thyroid, mortality	Employed in APFO-exposed job (SMR, MN referent):			Age, sex, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths*: Occupational cohort with relatively high exposures. *Other limitations*: Small cohort with limited number of deaths; potential healthy-worker effect due to external comparison of rates with general population; limited information on covariates.
			Never	1	2.16 (0.05–12.00)		
			Ever probable/ never definite	0	0 (0.00–8.45)		
			Ever definite	0	0 (0.00–42.96)		
Alexander et al. (2003) Decatur (AL), USA Enrolment, 1961–1997/ follow-up, 1961–1998 (mortality) Cohort	2083 (241 exposed and 112 unexposed women); Decatur (AL) PFOS cohort; production workers (men, 83%) who worked ≥ 365 days in a plant producing speciality films and fluorochemicals, one of the main ones being perfluorooctane-sulfonyl (POSF). Exposure assessment method: See Table 2.1	Breast, mortality	PFOS exposure group (SMR, AL referent):			Sex, age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1 *Other limitations*: Occupational cohort with few breast cancer deaths ($n = 2$); outcome assessment limited to mortality; mostly men (83%).
			All jobs	2	1.57 (0.19–5.66)		
			Only non-exposed	2	5.11 (0.62–18.45)		
			Ever low, never high	0	0		
			Ever high	0	0		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Steenland and Woskie (2012) Parkersburg (WV), USA Enrolment, 1948–2002/ follow-up, 1952–2008 Cohort	5791 (women, 19%); Parkersburg (WV), polymer-production PFOA cohort; workers (men, 81%) at a polymer-manufacturing facility who had potential exposure to fluoropolymers and had sufficiently detailed work histories Exposure assessment method: see Table 2.1	Breast, mortality	PFOA-exposed workers (SMR):			Age, sex, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. *Other limitations*: Occupational cohort with few breast cancer deaths ($n = 4$); outcome assessment limited to mortality; mostly men (81%).
			Other workers referent (same region and company)	4	0.65 (0.13–1.90)		
			US referent	4	0.79 (0.21–2.02)		
		Breast, mortality	Cumulative serum exposure, no lag (SMR, other workers referent, same region and company), women only:				
			1st quartile (0 to < 904 ppm-years)	2	1.49 (0.18–5.39)		
			2nd quartile (904 to < 1520 ppm-years)	0	0.00 (0.00–3.56)		
			3rd quartile (1520 to < 2700 ppm-years)	1	0.87 (0.02–4.83)		
			4th quartile (≥ 2700 ppm-years)	0	0.00 (0.00–3.42)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Leonard et al. (2008) Parkersburg (WV), USA Enrolment, 1948–2002/ follow-up, 1948–2002 (mortality) Cohort	6027 workers; Parkersburg (WV), polymer-production occupational PFOA cohort; workers (men, 81%) at a polymer-manufacturing facility who had potential exposure to fluoropolymers and had sufficiently detailed work histories; most recent follow-up for some cancer sites Exposure assessment method: records	Thyroid, mortality	Workers in the Parkersburg (WV), polymer-production plant (SMR):			Sex, age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths*: Occupational cohort with relatively high exposures; complete cohort ascertainment and follow-up; use of local reference groups increased comparability with respect to socioeconomic factors and health behaviours. *Other limitations*: Small numbers. *Other comments*: The Parkersburg (WV, USA), facility manufactured a broad range of commercial products including fluoropolymers, nylon filaments, and acrylic polymers; all study participants, regardless of work area, had detectable levels of serum PFOA.
			Referent US population	3	[3.120 (0.644–9.119)]		
			Referent WV population	3	[2.856 (0.589–8.347)]		
			Referent other workers (same region and company)	3	[6.286 (1.297–18.369)]		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Barry et al. (2013) Mid-Ohio Valley (OH and WV) Enrolment, August 2005-August 2006/follow-up, 1952 to 2011 (incidence) Cohort	32 254 (women, 17 360; community, 16 602; and occupational, 758); C8 Science Panel Study included people enrolled in the C8 Health Project who lived, worked, or attended school for ≥ 1 yr between 1950 and 3 December 2004 in a contaminated-water district in the vicinity of a chemical plant (Parkersburg (WV), polymer production) using PFOA in manufacturing, as well as a subset of those from the original Parkersburg (WV), polymer-production occupational cohort who worked at the plant between 1948 and 2002 Exposure assessment method: see Table 2.1	Breast, incidence	Estimated cumulative PFOA serum concentration (ng/mL), no lag (HR):			Age, time-varying smoking, time-varying alcohol consumption, sex, education, birth year (5-yr calendar intervals)	*Exposure assessment critique*: See Table 2.1. *Other strengths*: Wide range of PFOA exposure levels; availability of detailed information on potential confounding factors; relatively high participation rates; validation of cancer diagnoses through medical chart review. *Other limitations*: Mostly retrospective with relatively few validated cases for prospective analyses.
			Continuous (per unit on natural log scale)	559	0.94 (0.89–1.00)		
		Breast, incidence	Estimated cumulative PFOA serum concentration (ng/mL), 10-yr lag (HR):				
			Continuous (per unit on natural log scale)	559	0.93 (0.88–0.99)		
		Thyroid, incidence	Estimated cumulative PFOA serum concentration (ng/mL), no lag (HR):				
			1st quartile	NR	1		
			2nd quartile	NR	1.54 (0.77–3.12)		
			3rd quartile	NR	1.48 (0.74–2.93)		
			4th quartile	NR	1.73 (0.85–3.54)		
			Continuous (per unit on natural log scale)	86	1.10 (0.95–1.26)		
			Trend-test *P*-value, 0.25				
		Thyroid, incidence	Excluding person-time before estimated date first known to have lived or worked in the contaminated-water districts: estimated cumulative PFOA serum concentration (ng/mL), no lag (HR):				
			Continuous (per unit on natural log scale)	NR	1.06 (0.92–1.23)		

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Barry et al. (2013) Mid-Ohio Valley (OH and WV) Enrolment, August 2005-August 2006/follow-up, 1952 to 2011 (incidence) Cohort (cont.)		Thyroid, incidence	Estimated cumulative PFOA serum concentration (ng/mL), 10-yr lag (HR):			Age, time-varying smoking, time-varying alcohol consumption, sex, education, birth year (5-yr calendar intervals)	
			1st quartile	NR	1		
			2nd quartile	NR	2.06 (0.93–4.56)		
			3rd quartile	NR	2.02 (0.90–4.52)		
			4th quartile	NR	1.51 (0.67–3.39)		
			Continuous (per unit on natural log scale)	86	1.04 (0.89–1.20)		
			Trend-test *P*-value, 0.57				
		Thyroid, incidence	Excluding person-time before estimated date first known to have lived or worked in the contaminated-water districts: estimated cumulative PFOA serum concentration (ng/mL), 10-yr lag (HR):				
			Continuous (per unit on natural log scale)	NR	1.02 (0.87–1.19)		
		Thyroid, incidence	Community residents: estimated cumulative PFOA serum concentration (ng/mL), no lag (HR):				
			1st quartile	NR	1		
			2nd quartile	NR	1.54 (0.73–3.26)		
			3rd quartile	NR	1.71 (0.81–3.59)		
			4th quartile	NR	1.40 (0.66–2.97)		
			Continuous (per unit on natural log scale)	78	1.04 (0.89–1.23)		
			Trend-test *P*-value, 0.46				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Barry et al. (2013) Mid-Ohio Valley (OH and WV) Enrolment, August 2005-August 2006/follow-up, 1952 to 2011 (incidence) Cohort (cont.)		Thyroid, incidence	Community residents: estimated cumulative PFOA serum concentration (ng/mL), 10-yr lag (HR):			Age, time-varying smoking, time-varying alcohol consumption, sex, education, birth year (5-yr calendar intervals)	
			1st quartile	NR	1		
			2nd quartile	NR	2.09 (0.91–4.82)		
			3rd quartile	NR	1.92 (0.82–4.50)		
			4th quartile	NR	1.42 (0.60–3.37)		
			Continuous (per unit on natural log scale)	78	1.00 (0.84–1.20)		
			Trend-test *P*-value, 0.56				
		Thyroid, incidence	Workers: estimated cumulative PFOA serum concentration (ng/mL), no lag (HR):				
			1st quartile	NR	1		
			2nd quartile	NR	4.64 (0.42–50.8)		
			3rd quartile	NR	9.70 (0.67–141.2)		
			4th quartile	NR	14.72 (0.85–253.9)		
			Continuous (per unit on natural log scale)	8	1.93 (1.00–3.71)		
			Trend-test *P*-value, 0.04				
		Thyroid, incidence	Workers: estimated cumulative PFOA serum concentration (ng/mL), 10-yr lag (HR):				
			1st quartile	NR	1		
			2nd quartile	NR	1.65 (0.09–31.5)		
			3rd quartile	NR	4.52 (0.10–198.4)		
			4th quartile	NR	5.85 (0.13–257.1)		
			Continuous (per unit on natural log scale)	8	1.12 (0.61–2.05)		
			Trend-test *P*-value, 0.01				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ghisari et al. (2017) Denmark Enrolment, 1996–2002/ follow-up, through 2010 Nested case–control	Nested within the Danish National Birth Cohort (see Table 2.1) Cases: 178; nulliparous women at the time of blood draw during pregnancy followed for breast cancer, selected from ~100 000 pregnant women Controls: 233; nulliparous women at the time of blood draw during pregnancy frequency-matched on age Exposure assessment method: see Table 2.1	Breast (pre-menopausal), incidence	Serum PFOA (ng/mL) (RR):			Age at blood draw, BMI before pregnancy, total gravidities, oral contraceptive use, age at menarche, smoking status during pregnancy, alcohol intake during pregnancy, physical activity, education	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. *Other limitations* See Table 2.1.
			Continuous (per unit on natural log scale)	158	1.17 (0.63–2.17)		
		Breast (pre-menopausal), incidence	Serum PFOS (ng/mL) (RR):				
			Continuous (per unit on natural log scale)	158	1.15 (0.64–2.08)		
		Breast (pre-menopausal), incidence	CYP19 (C > T) genotype (RR per unit natural log transformed PFOA, ng/mL):				
			CC	35	7.24 (1.00–52)		
			CT	59	0.79 (0.26–2.38)		
			TT	34	0.55 (0.14–2.24)		
		Breast (pre-menopausal), incidence	CYP19 (C > T) genotype (RR per unit natural log transformed PFOS, ng/mL):				
			CC	35	6.42 (1.08–38.3)		
			CT	59	1.16 (0.44–3.10)		
			TT	34	0.45 (0.10–1.97)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hurley et al. (2018) CA, USA Enrolment, 1995–1996/ follow-up, 1 January 2006 to 1 August 2014 (incidence) Nested case–control	Nested within the California Teachers Study (See Table 2.1) Cases: 902; California Teachers Study; female public-school teachers and other professionals with a diagnosis of invasive breast cancer, age < 80 yr at diagnosis with no prior history of breast cancer, who provided a blood specimen and answered a questionnaire, who were continuous residents of CA; participation rate, 65% Controls: 858; women drawn from probability sample of at-risk cohort members, frequency-matched on age, race/ethnicity, and residence; participation rate, 55% Exposure assessment method: see Table 2.1	Breast, incidence	Serum PFOA (ng/mL) (OR):			Age at baseline, race/ethnicity, region of residence, date of blood draw, (date of blood draw)[2], season of blood draw, total smoking pack-years, BMI, family history of breast cancer, age at first full-term pregnancy, pork consumption[a], and menopausal status at blood draw	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. [a] This is the standard covariate set used for all analyses. Additional covariates are indicated as required.
			1st tertile	331	1		
			2nd tertile	298	0.901 (0.705–1.152)		
			3rd tertile	273	0.925 (0.715–1.197)		
			Continuous (per unit $\log_{10}$ scale)	902	0.733 (0.496–1.081)		
			Trend-test *P*-value, 0.54				
		Breast, incidence	Serum PFOS (ng/mL) (OR):				
			1st tertile	318	1		
			2nd tertile	297	0.883 (0.691–1.129)		
			3rd tertile	287	0.898 (0.695–1.161)		
			Continuous (per unit $\log_{10}$ scale)	902	0.934 (0.683–1.277)		
			Trend-test *P*-value, 0.41				
		Breast, post-menopausal at blood draw, incidence	Serum PFOA (ng/mL) (OR):			Standard covariates[a]	
			1st tertile	306	1		
			2nd tertile	287	0.889 (0.689–1.147)		
			3rd tertile	266	0.912 (0.699–1.189)		
			Continuous (per unit $\log_{10}$ scale)	859	0.715 (0.476–1.073)		
			Trend-test *P*-value, 0.49				
		Breast (post-menopausal at blood draw), incidence	Serum PFOS (ng/mL) (OR):			Standard covariates[a]	
			1st tertile	293	1		
			2nd tertile	284	0.843 (0.653–1.088)		
			3rd tertile	282	0.860 (0.661–1.118)		
			Continuous (per unit $\log_{10}$ scale)	859	0.885 (0.641–1.223)		
			Trend-test *P*-value, 0.26				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hurley et al. (2018) CA, USA Enrolment, 1995–1996/ follow-up, 1 January 2006 to 1 August 2014 (incidence) Nested case–control (cont.)		Breast (pre-menopausal at blood draw), incidence	Serum PFOA (ng/mL) (OR):			Age at baseline, race/ethnicity, region of residence, date of blood draw, (date of blood draw)^2, season of blood draw, dietary fat, total red meat consumption	
			1st tertile	25	1		
			2nd tertile	11	0.888 (0.239–3.302)		
			3rd tertile	7	0.669 (0.143–3.119)		
			Continuous (per unit $\log_{10}$ scale)	43	0.177 (0.023–1.342)		
			Trend-test *P*-value, 0.62				
		Breast (pre-menopausal at blood draw), incidence	Serum PFOS (ng/mL) (OR):			Age at baseline, race/ethnicity, region of residence, season of blood draw, total red meat consumption	
			1st tertile	25	1		
			2nd tertile	13	1.796 (0.493–6.546)		
			3rd tertile	5	1.208 (0.163–8.944)		
			Continuous (per unit $\log_{10}$ scale)	43	0.900 (0.166–4.876)		
			Trend-test *P*-value, 0.57				
		Breast (ER+ or PR+), incidence	Serum PFOA (ng/mL) (OR):			Age at baseline, race/ethnicity, region of residence, date of blood draw, (date of blood draw)^2, season of blood draw, total smoking pack-years, BMI, family history of breast cancer, age at first full-term pregnancy, menopausal status at blood draw, pork consumption	
			1st tertile	266	1		
			2nd tertile	247	0.918 (0.707–1.191)		
			3rd tertile	230	0.952 (0.725–1.251)		
			Continuous (per unit on $\log_{10}$ scale)	743	0.779 (0.513–1.183)		
			Trend-test *P*-value, 0.71				
		Breast (ER+ or PR+), incidence	Serum PFOS (ng/mL) (OR):				
			1st tertile	250	1		
			2nd tertile	247	0.937 (0.721–1.218)		
			3rd tertile	246	0.967 (0.737–1.270)		
			Continuous (per unit on $\log_{10}$ scale)	743	1.054 (0.744–1.493)		
			Trend-test *P*-value, 0.81				

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hurley et al. (2018) CA, USA Enrolment, 1995–1996/follow-up, 1 January 2006 to 1 August 2014 (incidence) Nested case–control (cont.)		Breast (ER– and PR–), incidence	Serum PFOA (ng/mL) (OR):			Age at baseline, race/ethnicity, region of residence, date of blood draw, (date of blood draw)[2], season of blood draw, physical activity	
			1st tertile	43	1		
			2nd tertile	35	0.846 (0.510–1.403)		
			3rd tertile	29	0.792 (0.460–1.365)		
			Continuous (per unit on $\log_{10}$ scale)	107	0.528 (0.239–1.165)		
			Trend-test *P*-value, 0.39				
		Breast (ER– and PR–), incidence	Serum PFOS (ng/mL) (OR):				
			1st tertile	47	1		
			2nd tertile	32	0.628 (0.378–1.041)		
			3rd tertile	28	0.615 (0.357–1.059)		
			Continuous (per unit on $\log_{10}$ scale)	107	0.573 (0.323–1.016)		
			Trend-test *P*-value, 0.06				

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mancini et al. (2020a) France Enrolment, 1990/follow-up, through 2013 (incidence) Nested case–control	Nested within E3N cohort (see Table 2.1) Cases: 194; incident postmenopausal breast cancers among women with serum (≥ 3 aliquots) collected before diagnosis, a completed dietary questionnaire in 1993, and randomly selected from 240 eligible breast cancers Controls: 194; density-sampled at time of case occurrence and matched by age within 2 yr, menopausal status at blood collection, BMI at blood collection, and year of blood collection Exposure assessment method: see Table 2.1	Breast (post-menopausal), incidence	Serum PFOA (OR):			Age at blood draw, BMI at blood draw, menopausal status at blood draw, year of blood draw, total serum lipids, BMI, smoking status, physical activity (MET-h/week), education level, history of benign breast disease, family history of breast cancer, parity/age at first full-term pregnancy, total breastfeeding duration, age at menarche, age at menopause, use of oral contraceptives, current use of MHT, adherence to Western diet, adherence to Mediterranean diet	*Exposure assessment critique*: See Table 2.1. *Other strengths*: Adjustment for breast cancer risk factors; pathology reports for > 93% of cases. *Other limitations*: Limited statistical power, especially to explore differences by subtype.
			1st quartile (1.3–4.8 ng/mL)	85	1		
			2nd quartile (4.8–6.8 ng/mL)	118	1.69 (0.89–3.21)		
			3rd quartile (6.8–8.8 ng/mL)	91	0.88 (0.43–1.80)		
			4th quartile (8.8–21.4 ng/mL)	94	0.92 (0.43–1.98)		
			Trend-test *P*-value, 0.43				
		Breast (post-menopausal), incidence	Serum PFOS (OR):				
			1st quartile (5.8–13.6 ng/mL)	80	1		
			2nd quartile (13.6–17.3 ng/mL)	109	1.94 (1.00–3.78)		
			3rd quartile (17.3–22.5 ng/mL)	99	2.03 (1.02–4.04)		
			4th quartile (22.5–85.3 ng/mL)	100	1.72 (0.88–3.36)		
			Trend-test *P*-value, 0.25				

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mancini et al. (2020a) France Enrolment, 1990/follow-up, through 2013 (incidence) Nested case–control (cont.)		Breast (post-menopausal, ER+), incidence	Serum PFOA (OR):			Age at blood draw, BMI at blood draw, menopausal status at blood draw, year of blood draw, total serum lipids, BMI, smoking status, physical activity (MET-h/week), education level, history of benign breast disease, family history of breast cancer, parity/age at first full-term pregnancy, total breastfeeding duration, age at menarche, age at menopause, use of oral contraceptives, current use of MHT, adherence to Western diet, adherence to Mediterranean diet	
			1st quartile (1.3–4.8 ng/mL)	NR	1		
			2nd quartile (4.8–6.8 ng/mL)	NR	1.72 (0.88–3.36)		
			3rd quartile (6.8–8.8 ng/mL)	NR	1.34 (0.66–2.73)		
			4th quartile (8.8–21.4 ng/mL)	NR	1.42 (0.68–2.95)		
			Trend-test *P*-value, 0.64				
		Breast (post-menopausal, ER+), incidence	Serum PFOS (OR):				
			1st quartile (5.8–13.6 ng/mL)	NR	1		
			2nd quartile (13.6–17.3 ng/mL)	NR	1.85 (0.90–3.82)		
			3rd quartile (17.3–22.5 ng/mL)	NR	2.22 (1.05–4.69)		
			4th quartile (22.5–85.3 ng/mL)	NR	2.33 (1.11–4.90)		
			Trend-test *P*-value, 0.04				
		Breast (post-menopausal, ER–), incidence	Serum PFOA (OR):				
			1st quartile (1.3–4.8 ng/mL)	NR	1		
			2nd quartile (4.8–6.8 ng/mL)	NR	7.73 (1.46–41.08)		
			3rd quartile (6.8–8.8 ng/mL)	NR	3.18 (0.55–18.47)		
			4th quartile (8.8–21.4 ng/mL)	NR	3.98 (0.67–23.52)		
			Trend-test *P*-value, 0.59				

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mancini et al. (2020a) France Enrolment, 1990/follow-up, through 2013 (incidence) Nested case–control (cont.)		Breast (post-menopausal, ER–), incidence	Serum PFOS (OR):			Age at blood draw, BMI at blood draw, menopausal status at blood draw, year of blood draw, total serum lipids, BMI, smoking status, physical activity (MET-h/week), education level, history of benign breast disease, family history of breast cancer, parity/age at first full-term pregnancy, total breastfeeding duration, age at menarche, age at menopause, use of oral contraceptives, current use of MHT, adherence to Western diet, adherence to Mediterranean diet	
			1st quartile (5.8–13.6 ng/mL)	NR	1		
			2nd quartile (13.6–17.3 ng/mL)	NR	15.40 (1.84–129.19)		
			3rd quartile (17.3–22.5 ng/mL)	NR	4.74 (0.45–49.62)		
			4th quartile (22.5–85.3 ng/mL)	NR	7.07 (0.73–68.03)		
			Trend-test *P*-value, 0.72				
		Breast (post-menopausal), PR+), incidence	Serum PFOA (OR):				
			1st quartile (1.3–4.8 ng/mL)	NR	1		
			2nd quartile (4.8–6.8 ng/mL)	NR	1.40 (0.67–2.93)		
			3rd quartile (6.8–8.8 ng/mL)	NR	1.28 (0.59–2.77)		
			4th quartile (8.8–21.4 ng/mL)	NR	1.54 (0.70–3.69)		
			Trend-test *P*-value, 0.37				
		Breast (post-menopausal, PR+), incidence	Serum PFOS (OR):				
			1st quartile (5.8–13.6 ng/mL)	NR	1		
			2nd quartile (13.6–17.3 ng/mL)	NR	1.84 (0.82–4.14)		
			3rd quartile (17.3–22.5 ng/mL)	NR	2.47 (1.07–5.65)		
			4th quartile (22.5–85.3 ng/mL)	NR	2.76 (1.21–6.30)		
			Trend-test *P*-value, 0.02				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mancini et al. (2020a) France Enrolment, 1990/follow-up, through 2013 (incidence) Nested case–control (cont.)		Breast (post-menopausal, PR–), incidence	Serum PFOA (OR):			Age at blood draw, BMI at blood draw, menopausal status at blood draw, year of blood draw, total serum lipids, BMI, smoking status, physical activity (MET-h/week), education level, history of benign breast disease, family history of breast cancer, parity/age at first full-term pregnancy, total breastfeeding duration, age at menarche, age at menopause, use of oral contraceptives, current use of MHT, adherence to Western diet, adherence to Mediterranean diet	
			1st quartile (1.3–4.8 ng/mL)	NR	1		
			2nd quartile (4.8–6.8 ng/mL)	NR	3.44 (1.30–9.10)		
			3rd quartile (6.8–8.8 ng/mL)	NR	1.80 (0.62–5.19)		
			4th quartile (8.8–21.4 ng/mL)	NR	1.69 (0.56–3.12)		
			Trend-test *P*-value, 0.90				
		Breast (post-menopausal, PR–), incidence	Serum PFOS (OR)				
			1st quartile (5.8–13.6 ng/mL)	NR	1		
			2nd quartile (13.6–17.3 ng/mL)	NR	3.47 (1.29–9.15)		
			3rd quartile (17.3–22.5 ng/mL)	NR	1.82 (0.61–5.45)		
			4th quartile (22.5–85.3 ng/mL)	NR	1.71 (0.57–5.10)		
			Trend-test *P*-value, 0.93				

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[Chang et al. (2023)](#) USA Enrolment, 1993–2001/follow-up, through November 2013 Nested case–control	Nested within PLCO cohort Cases: 621; all incident invasive breast cancer cases diagnosed up to and including November 2013 among women who were postmenopausal and not using hormone therapy at baseline (unless they were hormone receptor-negative cases) Controls: 621; controls were selected using incidence density sampling, all were postmenopausal, still alive and cancer-free at the time of case diagnosis with matching by age at baseline, date of blood draw and baseline MHT use Exposure assessment method: see [Table 2.1](#)	Breast (post-menopausal), incidence	Serum PFOA (OR):			Age at baseline, date of blood draw, MHT use at baseline, age at blood draw, study centre, race/ethnicity, education, age at menarche, age at first live birth and number of births, age at menopause, duration of MHT use, first degree family history of female breast cancer, personal history of benign breast disease, BMI, smoking status, vigorous physical activity[a], PFOS (natural log transformed)	*Exposure assessment critique*: See [Table 2.1](#). *Other strengths*: See [Table 2.1](#). *Limitations*: See [Table 2.1](#). [a] This is the standard covariate set used for all analyses. Additional covariates are indicated as required.
			1st quartile	147	1		
			2nd quartile	148	0.91 (0.64–1.30)		
			3rd quartile	162	1.07 (0.73–1.55)		
			4th quartile	164	1.01 (0.66–1.55)		
			Trend-test *P*-value, 0.83				
		Breast (post-menopausal), incidence	Serum PFOS (OR):			Standard covariates[a] and PFOA (natural log transformed)	
			1st quartile	145	1		
			2nd quartile	158	1.21 (0.84–1.74)		
			3rd quartile	167	1.39 (0.96–1.99)		
			4th quartile	151	1.17 (0.77–1.79)		
			Trend-test *P*-value, 0.58				
		Breast (post-menopausal, ER+/PR+), incidence	Serum PFOA (OR):			Standard covariates[a] and PFOS (natural log transformed)	
			1st quartile	NR	1		
			2nd quartile	NR	1.14 (0.66–1.97)		
			3rd quartile	NR	0.99 (0.55–1.80)		
			4th quartile	NR	0.81 (0.40–1.62)		
			Trend-test *P*-value, 0.41				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Chang et al. (2023) USA Enrolment, 1993–2001/ follow-up, through November 2013 Nested case–control (cont.)		Breast (post-menopausal, ER+/PR+), incidence	Serum PFOS (OR):			Standard covariates[a] and PFOA (natural log transformed)	
			1st quartile	NR	1		
			2nd quartile	NR	1.46 (0.84–2.54)		
			3rd quartile	NR	2.19 (1.21–3.98)		
			4th quartile	NR	1.89 (0.97–3.69)		
			Trend-test *P*-value, 0.08				
		Breast (post-menopausal, ER–/PR–), incidence	Serum PFOA (OR)			Standard covariates[a] and PFOS (natural log transformed)	
			1st quartile	NR	1		
			2nd quartile	NR	0.90 (0.38–2.10)		
			3rd quartile	NR	2.23 (0.90–5.54)		
			4th quartile	NR	1.62 (0.62–4.23)		
			Trend-test *P*-value, 0.21				
		Breast (post-menopausal, ER–/PR–), incidence	Serum PFOS (OR):			Standard covariates[a] and PFOA (natural log transformed)	
			1st quartile	NR	1		
			2nd quartile	NR	1.01 (0.38–2.63)		
			3rd quartile	NR	1.12 (0.48–2.62)		
			4th quartile	NR	0.60 (0.19–1.83)		
			Trend-test *P*-value, 0.34				
		Breast (post-menopausal, ER+), incidence	Serum PFOA (OR):			Standard covariates[a] and PFOS (natural log transformed)	
			1st quartile	NR	1		
			2nd quartile	NR	1.07 (0.68–1.66)		
			3rd quartile	NR	1.01 (0.64–1.61)		
			4th quartile	NR	1.03 (0.61–1.75)		
			Trend-test *P*-value, 0.96				
		Breast (post-menopausal, ER–), incidence	Serum PFOA (OR):			Standard covariates[a] and PFOS (natural log transformed)	
			1st quartile	NR	1		
			2nd quartile	NR	0.84 (0.36–1.95)		
			3rd quartile	NR	2.08 (0.85–5.07)		
			4th quartile	NR	1.63 (0.63–4.20)		
			Trend-test *P*-value, 0.19				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Chang et al. (2023) USA Enrolment, 1993–2001/ follow-up, through November 2013 Nested case–control (cont.)		Breast (post-menopausal, ER+), incidence	Serum PFOS (OR):			Standard covariates[a] and PFOA (natural log transformed)	
			1st quartile	NR	1		
			2nd quartile	NR	1.26 (0.81–1.95)		
			3rd quartile	NR	1.59 (1.01–2.50)		
			4th quartile	NR	1.29 (0.77–2.15)		
			Trend-test *P*-value, 0.39				
		Breast (post-menopausal, ER–), incidence	Serum PFOS (OR):			Standard covariates[a] and PFOA (natural log transformed)	
			1st quartile	NR	1		
			2nd quartile	NR	0.98 (0.39–2.47)		
			3rd quartile	NR	1.13 (0.49–2.62)		
			4th quartile	NR	0.52 (0.18–1.55)		
			Trend-test *P*-value, 0.20				
		Breast (post-menopausal, PR+), incidence	Serum PFOA (OR):			Standard covariates[a] and PFOS (natural log transformed)	
			1st quartile	NR	1		
			2nd quartile	NR	1.14 (0.66–1.96)		
			3rd quartile	NR	1.02 (0.57–1.83)		
			4th quartile	NR	0.77 (0.39–1.52)		
			Trend-test *P*-value, 0.31				
		Breast (post-menopausal, PR–), incidence	Serum PFOA (OR):			Standard covariates[a] and PFOS (natural log transformed)	
			1st quartile	NR	1		
			2nd quartile	NR	0.90 (0.47–1.70)		
			3rd quartile	NR	2.05 (1.06–3.94)		
			4th quartile	NR	1.48 (0.75–2.93)		
			Trend-test *P*-value, 0.15				
		Breast (post-menopausal, PR+), incidence	Serum PFOS (OR):			Standard covariates[a] and PFOA (natural log transformed)	
			1st quartile	NR	1		
			2nd quartile	NR	1.55 (0.90–2.67)		
			3rd quartile	NR	2.34 (1.29–4.23)		
			4th quartile	NR	1.79 (0.92–3.48)		
			Trend-test *P*-value, 0.14				

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Chang et al. (2023) USA Enrolment, 1993–2001/follow-up, through November 2013 Nested case–control (cont.)		Breast (post-menopausal, PR–), incidence	Serum PFOS (OR):			Standard covariates[a] and PFOA (natural log transformed)	
			1st quartile	NR	1		
			2nd quartile	NR	1.00 (0.52–1.92)		
			3rd quartile	NR	0.91 (0.50–1.64)		
			4th quartile	NR	0.61 (0.29–1.31)		
			Trend-test P-value, 0.15				
Cohn et al. (2020) Oakland (CA), USA Enrolment, at birth between 1959 and 1967/follow-up, birth to March 2013 (incidence) Nested case–control	Nested within the CHDS cohort (see Table 2.1) Cases: 102; offspring in the Child Health and Development Studies pregnancy cohort who had incident invasive or non-invasive breast cancer diagnosed by age 52 yr and who had a maternal perinatal blood sample and complete information on potential confounders and effect modifiers Controls: 310; 3 per case, density-sampled on case age and matched on birth year and trimester of maternal blood draw Exposure assessment method: see Table 2.1	Breast, incidence	Log_2 maternal serum PFOS (OR):			Age, birth year, trimester of maternal blood draw, maternal age at pregnancy, maternal history of breast cancer, African-American, primipara, maternal overweight at first prenatal visit, maternal serum log_2(p,p'-DDE), maternal serum log_2(o,p'-DDT), daughter breastfed, log_2(N-EtFOSAA), log_2(total cholesterol), log_2(N-EtFOSAA) × log_2 (total cholesterol)	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. *Other limitations*: See Table 2.1.
			Continuous (for 4th quartile median vs 1st quartile median (difference of 3.15 ng/mL))	102	0.3 (0.1–0.9)		

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Li et al. (2022a) Ronneby, southern Sweden Enrolment, 1985–2016/follow-up, 1985–2016 (incidence) Cohort	60 507 (28 569 women: 20 933 never high, 7636 ever high exposure); the Ronneby Register Cohort included all individuals who ever lived in Ronneby municipality 1985–2013; one third of the households received PFAS-contaminated drinking-water from a waterworks situated near a military airfield where PFAS-containing firefighting foam was used in 1985–2013 (15 811 individuals considered "ever-high"); subsets with long-term exposure (≥ 11 yr) in the latest part of the follow-up period (2005–2013) were considered more highly exposed Exposure assessment method: see Table 2.1	Breast, incidence	Women, residential exposure to highly PFAS-contaminated drinking-water (SIR, Blekinge county excluding Ronneby referent):			Age, calendar year	*Exposure assessment critique*: See Table 2.1. *Other strengths*: Large study population, strong exposure contrast; unbiased inclusion; complete follow-up; long follow-up for part of the population; reference group from same municipality. *Other limitations*: Mixed exposure profile without possibility to single out effects due to specific compounds; limited information on potential confounders.
			Never	525	0.80 (0.73–0.87)		
			Ever	156	0.75 (0.64–0.88)		
		Breast, incidence	Women, residential exposure to highly PFAS-contaminated drinking-water (HR):				
			Never	525	1		
			Ever	156	0.95 (0.79–1.13)		
		Breast, incidence	Women, time period of residential exposure to highly PFAS-contaminated drinking-water (HR):				
			Never	525	1		
			Early (1985–2004)	102	0.94 (0.76–1.16)		
			Late (2005–2013)	54	0.96 (0.72–1.29)		
		Breast, incidence	Women, duration of residential exposure to highly PFAS-contaminated drinking-water (HR):				
			Never	525	1		
			Short (1–10 yr)	89	1.01 (0.80–1.26)		
			Long (≥ 11 yr)	67	0.87 (0.67–1.13)		
		Thyroid, incidence	Men, residential exposure to highly PFAS-contaminated drinking-water (SIR, Blekinge county excluding Ronneby referent):			Calendar year, age, sex	
			Never	14	1.33 (0.73–2.23)		
			Ever	3	0.89 (0.18–2.61)		
		Thyroid, incidence	Women, residential exposure to highly PFAS-contaminated drinking-water (SIR, Blekinge county excluding Ronneby referent):				
			Never	32	1.38 (0.94–1.95)		
			Ever	16	2.08 (1.19–3.38)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Li et al. (2022a) Ronneby, southern Sweden Enrolment, 1985–2016/ follow-up, 1985–2016 (incidence) Cohort (cont.)		Thyroid, incidence	Residential exposure to highly PFAS-contaminated drinking-water (HR):				
			Never	46	1		
			Ever	19	1.36 (0.79–2.33)		
		Thyroid, incidence	Time period of residential exposure to highly PFAS-contaminated drinking-water (HR):				
			Never	46	1		
			Early (1985–2004)	11	1.20 (0.62–2.33)		
			Late (2005–2013)	8	1.69 (0.77–3.73)		
		Thyroid, incidence	Duration of residential exposure to highly PFAS-contaminated drinking-water (HR):				
			Never	46	1		
			Short (1–10 yr)	12	1.35 (0.71–2.56)		
			Long (≥ 11 yr)	7	1.38 (0.60–3.18)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Feng et al. (2022) Shiyan, China Enrolment, September 2008 to June 2010 and April to October 2013/follow-up, 2008 to 2018 (incidence) Case–cohort	Nested within the Dongfeng-Tongji cohort (see Table 2.1) Cases: 226; incident breast cancer drawn from 18 387 female retirees of an auto facility who provided a specimen; total of 226 breast cancer diagnoses included 13 diagnoses among women in the subcohort Comparison cohort: 990 (including 13 cases); subcohort of women randomly selected according to age strata. The 13 cases included among the 990 in the comparison cohort served as controls until time of cancer diagnosis Exposure assessment method: see Table 2.1	Breast, incidence	Plasma PFOA concentration (HR):			Calendar time, age, BMI, smoking, drinking, marital status, education level, occupation, batch to enter cohort, parity, menopausal status, history of mastitis, use of HRT, family history of cancer	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. *Other limitations*: See Table 2.1.
			1st quartile (< 0.84 ng/mL)	53	1		
			2nd quartile (0.84–1.18 ng/mL)	48	0.88 (0.56–1.39)		
			3rd quartile (1.19–1.79 ng/mL)	58	1.28 (0.80–2.04)		
			4th quartile (≥ 1.80 ng/mL)	67	1.69 (1.05–2.70)		
			Continuous (per unit on natural log scale)	226	1.35 (1.03–1.78)		
		Breast, incidence	Plasma PFOS concentration (HR):				
			1st quartile (< 6.39 ng/mL)	53	1		
			2nd quartile (6.39–10.35 ng/mL)	48	0.75 (0.47–1.19)		
			3rd quartile (10.36–15.66 ng/mL)	67	1.05 (0.66–1.67)		
			4th quartile (≥ 15.67 ng/mL)	58	0.87 (0.54–1.39)		
			Continuous (per unit on natural log scale)	226	0.88 (0.66–1.16)		
		Breast (post-menopausal), incidence	Plasma PFOA concentration (HR):				
			Low (< 1.19 ng/mL)	90	1		
			High (≥ 1.19 ng/mL)	115	1.53 (1.06–2.20)		
			Continuous (per unit on natural log scale)	205	1.34 (1.01–1.77)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Feng et al. (2022) Shiyan, China Enrolment, September 2008 to June 2010 and April to October 2013/follow-up, 2008 to 2018 (incidence) Case–cohort (cont.)		Breast (post-menopausal), incidence	Plasma PFOS concentration (HR):			Calendar time, age, BMI, smoking, drinking, marital status, education level, occupation, batch to enter cohort, parity, history of mastitis, age at menopause, use of HRT, family history of cancer	
			Low (< 10.36 ng/mL)	84	1		
			High (≥ 10.36 ng/mL)	121	1.13 (0.80–1.58)		
			Continuous (per unit on natural log scale)	205	0.91 (0.71–1.17)		
Madrigal et al. (2024) Finland Enrolment, 1986–2010/ follow-up, through 2016 Nested case–control	Nested within the Finnish Maternity Cohort (see Table 2.1) Cases: 400; National registry of nulliparous women who donated serum during the first trimester of pregnancy. 400 cases were randomly selected from cases diagnosed among women who donated serum for their first pregnancy and had a live, full-term birth delivered between 1987–2010, and who had no prior diagnosis of cancer at enrolment	Thyroid (papillary), incidence	PFOA serum concentration (OR):			Calendar year of delivery, age at first birth, PFOS, *N*-EtFOSAA, PFHpS detected	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1; data available in the Medical Birth Registry included many host factors and potential confounders. *Other limitations*: See Table 2.1; data on host factors and potential confounders were collected during the pregnancy only.
			≤ 2.82 ng/mL	94	1		
			> 2.82 to 3.77 ng/mL	105	1.10 (0.73–1.64)		
			> 3.77 to 4.85 ng/mL	98	0.99 (0.65–1.50)		
			> 4.85 to 6.75 ng/mL	78	1.30 (0.80–2.11)		
			> 6.75 ng/mL	25	0.54 (0.27–1.08)		
			Continuous (per unit on $\log_2$ scale)	400	0.90 (0.68–1.19)		
			Trend-test *P*-value, 0.31				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Madrigal et al. (2024) Finland Enrolment, 1986–2010/ follow-up, through 2016 Nested case–control (cont.)	Controls: 400; individually matched on year of delivery (4–5-yr increments) and age at first birth (3-yr increments) Exposure assessment method: see Table 2.1	Thyroid (papillary), incidence	PFOA serum concentration (OR):			Calendar year of delivery, age at first birth, smoking status at the time of pregnancy	
			≤ 2.82 ng/mL	94	1		
			> 2.82 to 3.77 ng/mL	105	1.13 (0.76–1.69)		
			> 3.77 to 4.85 ng/mL	98	1.05 (0.70–1.57)		
			> 4.85 to 6.75 ng/mL	78	1.40 (0.89–2.21)		
			> 6.75 ng/mL	25	0.63 (0.34–1.14)		
			Continuous (per unit on $\log_2$ scale)	400	0.95 (0.75–1.20)		
			Trend-test *P*-value, 0.48				
		Thyroid (papillary), incidence	Age < 40 yr, PFOA serum concentration (OR):			Calendar year of delivery, age at first birth	
			Continuous (per unit on $\log_2$ scale)	185	1.37 (0.92–2.03)		
		Thyroid (papillary), incidence	Age < 40 yr, PFOA serum concentration (OR):			Calendar year of delivery, age at first birth, PFOS, *N*-EtFOSAA, PFHpS	
			Continuous (per unit on $\log_2$ scale)	185	1.20 (0.71–2.01)		
		Thyroid (papillary), incidence	Age ≥ 40 yr, PFOA serum concentration (OR):			Calendar year of delivery, age at first birth	
			Continuous (per unit on $\log_2$ scale)	215	0.77 (0.57–1.04)		
		Thyroid (papillary), incidence	Age ≥ 40 yr, PFOA serum concentration (OR):			Calendar year of delivery, age at first birth, PFOS, *N*-EtFOSAA, PFHpS detected, *N*-EtFOSAA detected	
			Continuous (per unit on $\log_2$ scale)	215	0.70 (0.45–1.08)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Madrigal et al. (2024) Finland Enrolment, 1986–2010/ follow-up, through 2016 Nested case–control (cont.)		Thyroid (papillary), incidence	PFOS serum concentration (OR):			Calendar year of delivery, age at first birth, PFOA, *N*-EtFOSAA, PFHpS detected, total PCBs, hexachlorobenzene, β-HCH, chlordane metabolites, DDT metabolites	
			≤ 11.49 ng/mL	98	1		
			> 11.49 to 15.76 ng/mL	94	0.98 (0.61–1.57)		
			> 15.76 to 22.63 ng/mL	119	1.28 (0.76–2.18)		
			> 22.63 to 27.95 ng/mL	54	0.95 (0.50–1.82)		
			> 27.95 ng/mL	35	0.86 (0.38–1.95)		
			Continuous (per unit on log_2 scale)	400	1.14 (0.81–1.59)		
			Trend-test *P*-value, 0.74				
		Thyroid (papillary), incidence	PFOS serum concentration (OR):			Calendar year of delivery, age at first birth, smoking status at the time of pregnancy	
			≤ 11.49 ng/mL	98	1		
			> 11.49 to 15.76 ng/mL	94	0.98 (0.62–1.54)		
			> 15.76 to 22.63 ng/mL	119	1.23 (0.75–2.00)		
			> 22.63 to 27.95 ng/mL	54	0.92 (0.52–1.62)		
			> 27.95 ng/mL	35	0.87 (0.45–1.65)		
			Continuous (per unit on log_2 scale)	400	1.04 (0.81–1.33)		
			Trend-test *P*-value, 0.61				
		Thyroid (papillary), incidence	Age < 40 yr, PFOS serum concentration (OR):			Calendar year of delivery, age at first birth	
			Continuous (per unit on log_2 scale)	185	1.34 (0.92–1.96)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Madrigal et al. (2024) Finland Enrolment, 1986–2010/ follow-up, through 2016 Nested case–control (cont.)		Thyroid (papillary), incidence	Age < 40 yr, PFOS serum concentration (OR):			Calendar year of delivery, age at first birth, PFOA, *N*-EtFOSAA, total PCBs, hexachlorobenzene, β-HCH, chlordane metabolites, DDT metabolites	
			Continuous (per unit on $\log_2$ scale)	185	1.14 (0.68–1.93)		
		Thyroid (papillary), incidence	Age ≥ 40 yr, PFOS serum concentration (OR):			Calendar year of delivery, age at first birth	
			Continuous (per unit on $\log_2$ scale)	215	0.86 (0.61–1.20)		
		Thyroid (papillary), incidence	Age ≥ 40 yr, PFOS serum concentration (OR):			Calendar year of delivery, age at first birth, PFOA, *N*-EtFOSAA, total PCBs, hexachlorobenzene, β-HCH, chlordane metabolites, DDT metabolites	
			Continuous (per unit on $\log_2$ scale)	215	1.01 (0.60–1.71)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
van Gerwen et al. (2023) Mount Sinai, New York, USA Enrolment 2008–2021 Nested case–control	Nested within Bio*Me* cohort (see Table 2.1). Cases: 88 adult patients diagnosed with thyroid cancer using ICD codes 193 (9th Revision) and C73 (10th Revision) within Bio*Me*, a medical record-linked biobank within the Institute for Personalized Medicine at the Icahn School of Medicine at Mount Sinai Controls: 88 healthy (non-cancer) participants, pair-matched on sex, age (± 5 yr), race/ethnicity, BMI, smoking status (“Have you ever smoked ≥ 100 cigarettes in your entire life”, yes/no), and calendar year of sample collection Exposure assessment method: see Table 2.1	Thyroid, incidence and prevalence	Plasma PFOA concentration (OR):			Age, BMI, sex, race, storage time of plasma sample	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. *Other limitations*: See Table 2.1. *Other comments*: Analyses were repeated for the time between plasma sample collection and thyroid cancer diagnosis: < 1 yr (cross-sectional group) and ≥ 1 yr (longitudinal group).
			Continuous (per unit on $\log_2$ scale)	88	0.99 (0.63–1.56)		
			Continuous (per IQR increase)	88	0.99 (0.53–1.83)		
		Thyroid, incidence and prevalence	Plasma PFOA concentration (OR):			Age, BMI, sex, race, storage time of plasma sample, other PFAS	
			Continuous (per unit on $\log_2$ scale)	88	0.74 (0.31–1.72)		
			Continuous (per IQR increase)	88	0.66 (0.20–2.08)		
		Thyroid, incidence	Longitudinal study population (diagnosed ≥ 1 yr after sample collection), plasma PFOA concentration (OR):			Age, BMI, sex, race, storage, time of plasma sample	
			Continuous (per unit on $\log_2$ scale)	31	1.52 (0.77–2.98)		
		Thyroid, prevalence	Cross-sectional study population (diagnosed < 1 yr after sample collection), plasma PFOA concentration (OR):				
			Continuous (per unit on $\log_2$ scale)	57	0.84 (0.49–1.40)		
		Thyroid, incidence and prevalence	Plasma sb-PFOS (branched PFOS) concentration (OR):			Age, BMI, sex, race, storage time of plasma sample	
			Continuous (per unit on $\log_2$ scale)	88	1.32 (0.99–1.81)		
			Continuous (per IQR increase)	88	1.73 (0.97–3.24)		
		Thyroid, incidence and prevalence	Plasma sb-PFOS (branched PFOS) concentration (OR):			Age, BMI, sex, race, storage time of plasma sample, other PFAS	
			Continuous (per unit on $\log_2$ scale)	88	1.21 (0.43–3.55)		
			Continuous (per IQR increase)	88	1.47 (0.18–12.26)		

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
van Gerwen et al. (2023) Mount Sinai, New York, USA Enrolment 2008–2021 Nested case–control (cont.)		Thyroid, incidence	Longitudinal study population (diagnosed ≥ 1 yr after sample collection), plasma sb-PFOS (branched PFOS) concentration (OR):			Age, BMI, sex, race, storage time of plasma sample	
			Continuous (per unit on log_2 scale)	31	3.09 (1.73–6.13)		
		Thyroid, prevalence	Cross-sectional study population (diagnosed < 1 yr after sample collection), plasma sb-PFOS (branched PFOS) concentration (OR):				
			Continuous (per unit on log_2 scale)	57	1.13 (0.83–1.56)		
		Thyroid, incidence and prevalence	Plasma *n*-PFOS (linear PFOS) concentration (OR):				
			Continuous (per unit on log_2 scale)	88	1.56 (1.17–2.15)		
			Continuous (per IQR increase)	88	2.32 (1.34–4.26)		
		Thyroid, incidence and prevalence	Plasma *n*-PFOS (linear PFOS) concentration (OR):			Age, BMI, sex, race, storage time of plasma sample, other PFAS	
			Continuous (per unit on log_2 scale)	88	2.80 (1.32–6.45)		
			Continuous (per IQR increase)	88	7.09 (1.69–34.54)		
		Thyroid, incidence	Longitudinal study population (diagnosed ≥ 1 yr after sample collection), plasma *n*-PFOS (linear PFOS) concentration (OR):			Age, BMI, sex, race, storage time of plasma sample	
			Continuous (per unit on log_2 scale)	31	2.67 (1.59–4.88)		
		Thyroid, prevalence	Cross-sectional study population (diagnosed < 1 yr after sample collection), plasma *n*-PFOS (linear PFOS) concentration (OR):				
			Continuous (per unit on log_2 scale)	57	1.45 (1.07–2.01)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
van Gerwen et al. (2023) Mount Sinai, New York, USA Enrolment 2008–2021 Nested case–control (cont.)		Thyroid (papillary), incidence and prevalence	Plasma PFOA concentration (OR):				
			Continuous (per unit on $\log_2$ scale)	74	1.03 (0.63–1.68)		
			Continuous (per IQR increase)	74	1.03 (0.55–1.96)		
		Thyroid (papillary), incidence and prevalence	Plasma sb-PFOS (branched PFOS) concentration (OR):				
			Continuous (per unit on $\log_2$ scale)	74	1.30 (0.95–1.83)		
			Continuous (per IQR increase)	74	1.61 (0.91–2.97)		
		Thyroid (papillary), incidence and prevalence	Plasma *n*-PFOS (linear PFOS) concentration (OR):				
			Continuous (per unit on $\log_2$ scale)	74	1.56 (1.13–2.21)		
			Continuous (per IQR increase)	74	2.22 (1.24–4.20)		

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Winquist et al. (2023) 20 states, USA Enrolment, 1998–200/follow-up, through 30 June 2015 Case–cohort	Case–cohort within the CPS-II Lifelink Cohort Cases: 3762 overall (786 female breast); incidence cases from the CPS-II Lifelink Cohort (surviving CPS-II Nutrition cohort participants) with first cancer diagnosis of kidney, bladder, breast (females only), prostate (males only), or pancreatic cancer, leukaemia, or lymphoma, detected through self-report or NDI linkage and verified through medical records review or cancer registry; all participants with incident cancers	Breast (post-menopausal), incidence	Women, serum PFOA concentration (HR):			Year of serum sample collection, age at serum collection, race, education, smoking status, alcohol consumption	*Exposure assessment critique*: See Table 2.1. *Strengths*: See Table 2.1. *Limitations*: See Table 2.1.
			1st quartile (< 3.700 ng/mL)	193	1		
			2nd quartile (3.700 to < 5.000 ng/mL)	196	0.80 (0.56–1.15)		
			3rd quartile (5.000 to < 6.900 ng/mL)	189	0.75 (0.52–1.09)		
			4th quartile (≥ 6.900 ng/mL)	202	0.82 (0.57–1.17)		
			Continuous (per unit on log_2 scale)	780	0.96 (0.82–1.12)		
		Breast (post-menopausal), incidence	Women, serum PFOS concentration (HR):				
			1st quartile (< 12.000 ng/mL)	160	1		
			2nd quartile (12.000 to < 17.000 ng/mL)	195	0.66 (0.45–0.97)		
			3rd quartile (17.000 to < 24.000 ng/mL)	211	0.84 (0.57–1.23)		
			4th quartile (≥ 24.000 ng/mL)	214	0.70 (0.48–1.01)		
			Continuous (per unit on log_2 scale)	780	0.87 (0.75–1.01)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Winquist et al. (2023) 20 states, USA Enrolment, 1998–200/ follow-up, through 30 June 2015 Case–cohort (cont.)	Comparison cohort: 999; a sex-stratified simple random sample of 499 women and 500 men (~3% of the eligible cohort); stratification sampling was to ensure an adequate number of subcohort participants in sex-specific analyses (for breast and prostate cancers) Exposure assessment method: see Table 2.1						
Vieira et al. (2013) OH and WV, USA 1996–2005 (incidence) Case–control	Cases: study 1: 4057 female breast, 343 thyroid cancer; study 2: 1260 female breast, 94 thyroid; cancer cases were retrieved from cancer registries covering a community sample with relatively high exposure to PFOA because of contamination of drinking-water from the Parkersburg (WV), polymer-production plant	Breast, incidence	Analysis 1. Residence in a PFOA-contaminated water district (OH and WV) (OR)			Age, diagnosis year, insurance provider, smoking status	*Exposure assessment critique*: See Table 2.1. *Other strengths*: ascertainment of cases from cancer registries; large exposure contrast. *Other limitations*: use of other types of cancer as controls; lack of adjustment for several potential confounding variables; lack of information concerning tumour hormone-receptor status.
			Females: Unexposed	3621	1		
			Any exposed water district	436	1.0 (0.9–1.1)		
		Breast, incidence	Analysis 2. Individual-level annual PFOA serum exposure, assuming 10-yr residency and latency (OH only) (OR)			Age, race, diagnosis year, insurance provider smoking status	
			Females: Unexposed	1037	1		
			Low (3.7–12.8 µg/L)	72	0.9 (0.7–1.2)		
			Medium (12.9–30.7 µg/L)	77	1.1 (0.8–1.5)		
			High (30.8–109 µg/L)	45	0.7 (0.5–1.0)		
			Very high (110–655 µg/L)	29	1.4 (0.9–2.3)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Vieira et al. (2013) OH and WV, USA 1996–2005 (incidence) Case–control (cont.)	Controls: NR; for each cancer site evaluated, controls were cases of cancer at all other sites among women, with the exclusion of four cancers of a priori interest (kidney, testis, pancreas, and liver) that have been associated with PFOA in studies in experimental animals or humans Exposure assessment method: see Table 2.1	Thyroid, incidence	Analysis 1: residence in a PFOA-contaminated water district (OH and WV) (OR):			Age, sex, diagnosis year, insurance provider, smoking status	
			Unexposed	303	1		
			Any exposed water district	40	1.1 (0.7–1.5)		
			Little Hocking	3	0.8 (0.3–2.7)		
			Lubeck	7	1.2 (0.6–2.6)		
			Tuppers Plains	2	0.3 (0.1–1.4)		
			Belpre	5	0.9 (0.4–2.2)		
			Pomeroy	0	NC		
			Mason	23	1.4 (0.9–2.2)		
		Other (specify), thyroid, incidence	Analysis 2: individual-level annual PFOA serum exposure, assuming 10-yr residency and latency (OH only) (OR):				
			Unexposed	79	1		
			Low (3.7–12.8 µg/L)	5	0.9 (0.4–2.3)		
			Medium (12.9–30.7 µg/L)	5	0.9 (0.4–2.3)		
			High (30.8–109 µg/L)	3	0.7 (0.2–2.1)		
			Very high (110–655 µg/L)	2	0.8 (0.2–3.5)		

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Wielsøe et al. (2017) Greenland Enrolment, 2000–2003/ follow-up, 2011–2014 Case–control	Cases: 77 cases of breast cancer; recruited at diagnosis at Dronning Ingrids Hospital in Nuuk (where all breast cancer cases in Greenland are registered) during two time periods: 2000–2003 and 2011–2014; all cases were among women of Greenland Inuit descent	Breast, incidence	Serum PFOA concentration (OR):			Age, BMI, cotinine levels, parity, breastfeeding	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; measurement error low. Key limitations were that if breast cancer alters ADME of PFAS there could be possible differential exposure misclassification, as blood was collected after diagnosis (also see Bonefeld-Jorgensen et al., 2011); single samples at time of case hospitalization may not reflect exposure at crucial windows in cancer development.
			1st tertile	14	1		
			2nd tertile	26	1.86 (0.80–4.31)		
			3rd tertile	37	2.64 (1.17–5.97)		
			Continuous (per unit increase)	77	1.26 (1.01–1.58)		
		Breast, incidence	Serum PFOS concentration (OR):				
			1st tertile	8	1		
			2nd tertile	25	3.13 (1.20–8.15)		
			3rd tertile	44	5.50 (2.19–13.84)		
			Continuous (per unit increase)	77	1.02 (1.01–1.03)		

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Wielsøe et al. (2017) Greenland Enrolment, 2000–2003/follow-up, 2011–2014 Case–control (cont.)	Controls: 81 controls for the participants recruited during 2000–2003 were selected from two cross-sectional studies on healthy persons with POPs serum measurements in the same period; the controls recruited during 2011–2014 were patients with nonmalignant diagnoses at the Dronning Ingrids hospital; controls were frequency-matched on age and geographical living area to cases; all controls were in people of Greenland Inuit descent Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; a single blood sample was collected; blood was collected at the hospital before treatment						*Other strengths*: cases confirmed by a positive histological sample. *Other limitations*: exclusion of cases and controls from the final analyses not clearly explained; some of the controls were hospital patients with nonmalignant abnormalities in the uterus, ovaries and breasts; small sample size and limited statistical power; cross-sectional design; no information about the delay between diagnosis and the collection of blood or if treatment occurred before blood collection; unexplained elevation in median PFOS level for cases recruited in early time period.

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Tsai et al. (2020) Taiwan, China 2014–2016 Case–control	Cases: 120 patients aged 25–80 yr at diagnosis, recruited at NTUH Controls: 119 controls aged 25–80 yr and without any history of malignancy; recruited through advertisements on posters and flyers at NTUH and in the community; controls received a small financial compensation (~US$ 6.30) after completing the study Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; a single blood sample was collected during approx. the same time period for cases and matched controls; blood was collected at the hospital before treatment	Breast, incidence	Serum PFOA concentration (OR):			Age, history of pregnancy, oral contraception use, abortion, BMI, menopause, education level	*Exposure assessment critique*: Key strengths were that plasma levels represent the combined exposure through all exposure pathways; although blood samples were collected after diagnosis, a strength was that they were collected before treatment; measurement error low. Key limitations were that if breast cancer alters ADME of PFAS there could be possible differential exposure misclassification as plasma collected after diagnosis; single samples at time of case hospitalization may not reflect exposure at crucial windows in cancer development.
			Continuous (per unit increase on natural log scale)	120	0.89 (0.59–1.34)		
		Breast, incidence	Serum PFOS concentration (OR):				
			Continuous (per unit increase on natural log scale)	120	1.07 (0.64–1.79)		
		Breast, incidence	Serum PFOA concentration (OR per unit increase on natural log scale):			History of pregnancy, oral contraception use, abortion, BMI, menopause, education level	
			Age ≤ 50 yr	60	1.14 (0.66–1.96)		
			Age > 50 yr	60	0.78 (0.40–1.51)		
		Breast, incidence	Serum PFOS concentration (OR per unit increase on natural log scale):				
			Age ≤ 50 yr	60	2.34 (1.02–5.38)		
			Age > 50 yr	60	0.62 (0.29–1.29)		
		Breast (ER–), incidence	Serum PFOA concentration (OR per unit increase on natural log scale):				
			Age ≤ 50 yr	11	0.42 (0.17–1.06)		
			Age > 50 yr	12	1.08 (0.33–3.59)		

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Tsai et al. (2020) Taiwan, China 2014–2016 Case–control (cont.)		Breast (ER –), incidence	Serum PFOS concentration (OR per unit increase on natural log scale):			History of pregnancy, oral contraception use, abortion, BMI, menopause, education level	*Other strengths*: cases confirmed by positive histological samples; controls included participants without any history of malignancy; models adjusted for important confounding variables; available information on tumour hormone-receptor status. *Other limitations*: small sample size and limited statistical power; cross sectional design; strategy for recruiting controls could have induced a control selection bias if people positively responding to advertisement had a healthier lifestyle and a higher medical awareness compared with the source population for cases.
			Age ≤ 50 yr	11	0.23 (0.05–1.15)		
			Age > 50 yr	12	0.66 (0.20–2.22)		
		Breast (ER+), incidence	Serum PFOA concentration (OR per unit increase on natural log scale):				
			Age ≤ 50 yr	49	1.41 (0.77–2.56)		
			Age > 50 yr	48	0.70 (0.35–1.42)		
		Breast (ER+), incidence	Serum PFOS concentration (OR per unit increase on natural log scale):				
			Age ≤ 50 yr	49	3.25 (1.29–8.23)		
			Age > 50 yr	48	0.53 (0.24–1.18)		

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Itoh et al. (2021) Japan Enrolment, May 2001 to September 2005 Case–control	Cases: 401 women aged 20–74 yr with new invasive breast cancer, admitted to any of the four hospitals included in the study; of 412 eligible patients, 405 (98%) agreed to participate Controls: 401 selected among individuals attending two of the hospitals for medical check-ups during the study period; they were confirmed to not have cancer and were matched with cases by age (within 3 yr) and residential area (urban or rural); two of the control participants refused to provide blood specimens and two refused to allow their samples to be used in the present analysis	Breast, incidence	Serum PFOA concentration (OR):			Age and residential area (urban or rural), BMI, height, menopausal status and age at menopause, age at first childbirth, family history of breast cancer, smoking status, physical activity, age at menarche, number of births, breastfeeding duration, alcohol intake, isoflavone intake, and education level	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways. Measurement error low. Key limitations were that if breast cancer alters ADME of PFAS there could be possible differential exposure misclassification; no information available concerning the delay between diagnosis and blood sample used for PFAS measurements and if cases had received cancer treatment before blood sample; single samples at time of case hospitalization may not reflect exposure at crucial windows in cancer development; minimal information on potential carcinogenic co-exposures.
			1st quartile (0.72–3.98 ng/mL)	167	1		
			2nd quartile (4.00–5.57 ng/mL)	100	0.45 (0.25–0.80)		
			3rd quartile (5.57–7.62 ng/mL)	82	0.39 (0.20–0.73)		
			4th quartile (7.64–62.98 ng/mL)	52	0.21 (0.10–0.45)		
			Trend-test *P*-value, 0.0001				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Itoh et al. (2021) Japan Enrolment, May 2001 to September 2005 Case–control (cont.)	Exposure assessment method: quantitative serum measurements; analytical method state-of-the-art; a single serum sample was collected during hospitalization for cases of invasive cancer and matched non-cancer controls in the hospital for medical check-up	Breast, incidence	Serum PFOA concentration (OR):			Age and residential area (urban or rural), BMI, height, menopausal status and age at menopause, age at first childbirth, family history of breast cancer, smoking status, physical activity, age at menarche, number of births, breastfeeding duration, alcohol intake, isoflavone intake, and education level, serum total concentrations of PCBs (lipid-adjusted), fish and shellfish intake, vegetable intake, and calendar year of blood sampling	*Other strengths*: cases were histologically confirmed invasive breast cancer; high response rate reduced the possibility of selection bias; large sample size; detailed information on diet, available information on tumour hormone-receptor status; analysis examined impact of individual isomers and combinations of isomers, including the sum of 6 PFOS isomers and the sum of 2 PFOA isomers as well as combinations of PFSAs and PFCAs.
			1st quartile (0.72–3.98 ng/mL)	167	1		
			2nd quartile (4.00–5.57 ng/mL)	100	0.37 (0.19–0.73)		
			3rd quartile (5.57–7.62 ng/mL)	82	0.39 (0.18–0.84)		
			4th quartile (7.64–62.98 ng/mL)	52	0.20 (0.08–0.51)		
			Trend-test *P*-value, 0.001				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Itoh et al. (2021) Japan Enrolment, May 2001 to September 2005 Case–control (cont.)		Breast, incidence	Serum PFOS concentration (OR):			Age and residential area (urban or rural), BMI, height, menopausal status and age at menopause, age at first childbirth, family history of breast cancer, smoking status, physical activity, age at menarche, number of births, breastfeeding duration, alcohol intake, isoflavone intake, and education level	*Other limitations*: potential selection bias for controls; lack of information and adjustment for socioeconomic status; cross sectional design; no adjustment for education; use of medical check-up examinees as controls may have caused selection bis due to a higher medical awareness and different socioeconomic status compared to the source population for cases.
			1st quartile (1.13–10.25 ng/mL)	183	1		
			2nd quartile (10.29–14.27 ng/mL)	85	0.41 (0.22–0.77)		
			3rd quartile (14.27–19.24 ng/mL)	86	0.37 (0.19–0.71)		
			4th quartile (19.28–377.33 ng/mL)	47	0.14 (0.07–0.31)		
			Trend-test *P*-value, < 0.0001				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Itoh et al. (2021) Japan Enrolment, May 2001 to September 2005 Case–control (cont.)		Breast, incidence	Serum PFOS concentration (OR)			Age and residential area (urban or rural), BMI, height, menopausal status and age at menopause, age at first childbirth, family history of breast cancer, smoking status, physical activity, age at menarche, number of births, breastfeeding duration, alcohol intake, isoflavone intake, and education level, serum total concentrations of PCBs (lipid-adjusted), fish and shellfish intake, vegetable intake, and calendar year of blood sampling	
			1st quartile (1.13–10.25 ng/mL)	183	1		
			2nd quartile (10.29–14.27 ng/mL)	85	0.38 (0.18–0.82)		
			3rd quartile (14.27–19.24 ng/mL)	86	0.31 (0.14–0.69)		
			4th quartile (19.28–377.33 ng/mL)	47	0.15 (0.06–0.39)		
			Trend-test *P*-value, 0.0001				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Itoh et al. (2021) Japan Enrolment, May 2001 to September 2005 Case–control (cont.)		Breast (pre-menopausal), incidence	Serum PFOA concentration (OR):			Age and residential area (urban or rural), BMI, height, age at first childbirth, family history of breast cancer, smoking status, physical activity, age at menarche, number of births, breastfeeding duration, alcohol intake, isoflavone intake, education level	
			Low (0.7–4.5 ng/mL)	NR	1		
			Middle (4.5–6.7 ng/mL)	NR	0.72 (0.38–1.37)		
			High (6.73–62.98 ng/mL)	NR	0.66 (0.28–1.54)		
			Trend-test *P*-value, 0.26				
		Breast (pre-menopausal), incidence	Serum PFOS concentration (OR):				
			Low (1.1–11.5 ng/mL)	NR	1		
			Middle (11.5–17.0 ng/mL)	NR	0.52 (0.27–1.01)		
			High (17.1–377.33 ng/mL)	NR	0.28 (0.09–0.85)		
			Trend-test *P*-value, 0.007				
		Breast (post-menopausal), incidence	Serum PFOA concentration (OR):			Age and residential area (urban or rural), BMI, height, age at menopause, age at first childbirth, family history of breast cancer, smoking status, physical activity, age at menarche, number of births, breastfeeding duration, alcohol intake, isoflavone intake, education level, years after menopause	
			Low (0.7–4.5 ng/mL)	NR	1		
			Middle (4.5–6.7 ng/mL)	NR	0.61 (0.34–1.07)		
			High (6.73–62.98 ng/mL)	NR	0.41 (0.23–0.75)		
			Trend-test *P*-value, 0.005				

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Itoh et al. (2021) Japan Enrolment, May 2001 to September 2005 Case–control (cont.)		Breast (post-menopausal), incidence	Serum PFOS concentration (OR):			Age and residential area (urban or rural), BMI, height, age at menopause, age at first childbirth, family history of breast cancer, smoking status, physical activity, age at menarche, number of births, breastfeeding duration, alcohol intake, isoflavone intake, education level, years after menopause	
			Low (1.1–11.5 ng/mL)	NR	1		
			Middle (11.5–17.0 ng/mL)	NR	0.60 (0.33–1.09)		
			High (17.1–377.33 ng/mL)	NR	0.35 (0.19–0.66)		
			Trend-test *P*-value, 0.001				
		Breast (ER– and PR–), incidence	Serum PFOA concentration (OR):			Age and residential area (urban or rural), BMI, height, menopausal status and age at menopause, age at first childbirth, family history of breast cancer, smoking status, physical activity, age at menarche, number of births, breastfeeding duration, alcohol intake, isoflavone intake, education level	
			Low (0.7–4.5 ng/mL)	NR	1		
			Middle (4.5–6.7 ng/mL)	NR	0.78 (0.40–1.49)		
			High (6.73–62.98 ng/mL)	NR	0.62 (0.30–1.32)		
			Trend-test *P*-value, 0.23				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Itoh et al. (2021) Japan Enrolment, May 2001 to September 2005 Case–control (cont.)		Breast (ER+ and PR–), incidence	Serum PFOA concentration (OR):			Age and residential area (urban or rural), BMI, height, menopausal status and age at menopause, age at first childbirth, family history of breast cancer, smoking status, physical activity, age at menarche, number of births, breastfeeding duration, alcohol intake, isoflavone intake, education level	
			Low (0.7–4.5 ng/mL)	NR	1		
			Middle (4.5–6.7 ng/mL)	NR	0.86 (0.44–1.68)		
			High (6.73–62.98 ng/mL)	NR	0.27 (0.11–0.69)		
			Trend-test *P*-value, 0.007				
		Breast (ER+ and PR+), incidence	Serum PFOA concentration (OR):				
			Low (0.7–4.5 ng/mL)	NR	1		
			Middle (4.5–6.7 ng/mL)	NR	0.63 (0.39–1.01)		
			High (6.73–62.98 ng/mL)	NR	0.57 (0.33–0.97)		
			Trend-test *P*-value, 0.035				
		Breast (ER – and PR–), incidence	Serum PFOS concentration (OR):				
			Low (1.10–11.5 ng/mL)	NR	1		
			Middle (11.5–17.0 ng/mL)	NR	0.61 (0.31–1.20)		
			High (17.1–377.33 ng/mL)	NR	0.44 (0.20–0.96)		
			Trend-test *P*-value, 0.037				
		Breast (ER+ and PR–), incidence	Serum PFOS concentration (OR):				
			Low (1.10–11.5 ng/mL)	NR	1		
			Middle (11.5–17.0 ng/mL)	NR	1.07 (0.52–2.20)		
			High (17.1–377.33 ng/mL)	NR	0.33 (0.13–0.83)		
			Trend-test *P*-value, 0.016				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Itoh et al. (2021) Japan Enrolment, May 2001 to September 2005 Case–control (cont.)		Breast (ER+ and PR+), incidence	Serum PFOS concentration (OR):			Age and residential area (urban or rural), BMI, height, menopausal status and age at menopause, age at first childbirth, family history of breast cancer, smoking status, physical activity, age at menarche, number of births, breastfeeding duration, alcohol intake, isoflavone intake, education level	
			Low (1.10–11.5 ng/mL)	NR	1		
			Middle (11.5–17.0 ng/mL)	NR	0.56 (0.34–0.90)		
			High (17.1–377.33 ng/mL)	NR	0.33 (0.18–0.59)		
			Trend-test *P*-value, 0.0001				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Liu et al. (2022) Jinan City, Shandong Province, east China 2016–2017 Case–control	Cases: 134 cases were diagnosed with thyroid cancer by pathological examination at the Shandong Provincial Qianfoshan Hospital; participants in the case group stopped taking thyroid medication for 2 weeks Controls: 185 controls were randomly selected from patients undergoing routine medical visits at the hospital with normal thyroid B-ultrasound examination and no history of thyroid disease or taking iodine or thyroid hormone drugs during the blood collection, and frequency-matched to the case group on age (± 5 yr) and sex	Thyroid, incidence	Serum PFOA (OR):			Age, sex, diabetes status	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; measurement error low. Key limitations were that if thyroid cancer alters ADME of PFAS there could be possible differential exposure misclassification as serum collected between treatment periods for cases; single samples at time of case hospitalization may not reflect exposure at crucial windows in cancer development.
			1st quartile (< 7.9 ng/mL)	69	1		
			2nd quartile (7.9 to < 10.9 ng/mL)	23	0.24 (0.12–0.50)		
			3rd quartile (10.9 to < 16.1 ng/mL)	21	0.24 (0.11–0.49)		
			4th quartile (≥ 16.1 ng/mL)	21	0.20 (0.09–0.44)		
			Trend-test *P*-value, < 0.001				
		Thyroid, incidence	Serum PFOS (OR):			Age, sex, diabetes status	
			1st quartile (< 4.7 ng/mL)	49	1		
			2nd quartile (4.7 to < 7.5 ng/mL)	48	0.81 (0.42–1.53)		
			3rd quartile (7.5 to < 10.8 ng/mL)	17	0.26 (0.12–0.57)		
			4th quartile (≥ 10.8 ng/mL)	20	0.28 (0.12–0.66)		
			Trend-test *P*-value, 0.001				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Liu et al. (2022) Jinan City, Shandong Province, east China 2016–2017 Case–control (cont.)	Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; a single blood sample was collected during the same time period for cases and matched controls; blood was collected between treatment periods; control samples collected during routine visits to the hospital						*Other strengths*: use of novel statistical methods to evaluate the impact of PFAS on thyroid function and thyroid hormones using a WQS model. *Other limitations*: limited exposure contrast; small sample size; limited confounding adjustment; potential for reverse causation.
Velarde et al. (2022) Philippines 2018 Case–control	Cases: 75 cases recruited through the Philippine General Hospital Breast Cancer Center, including Filipino women aged 18–60 yr, with no comorbidity Controls: 75 women aged 18–59 yr, without prior diagnosis of cancer and without family history of breast, ovarian, and endometrial cancer in first-degree relatives; controls were recruited through posters, social media advertisements, and by word of mouth	Breast, incidence	Serum PFOA concentration (OR):			Age, region of residence	*Exposure assessment critique*: Key strengths were that serum levels represent the combined exposure through all exposure pathways; measurement error low. Key limitations were that if breast cancer alters ADME of PFAS there could be possible differential exposure misclassification as serum collected at case identification
			1st quartile (0.56–1.47 ng/mL)	18	1		
			2nd quartile (1.50–1.77 ng/mL)	14	0.64 (0.21–1.90)		
			3rd quartile (1.77–2.30 ng/mL)	21	1.05 (0.38–2.93)		
			4th quartile (2.31–8.46 ng/mL)	13	0.44 (0.14–1.36)		
			Trend-test *P*-value, 0.380				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Velarde et al. (2022) Philippines 2018 Case–control (cont.)	Exposure assessment method: quantitative serum measurements; analytical method was state-of-the-art; a single serum sample was collected from cases and non-cancer community controls; measured 12 PFAS but did not measure isomers of PFOA or PFOS	Breast, incidence	Serum PFOS concentration (OR):			Age, region of residence	(however, cases had not received neoadjuvant chemotherapy before blood sample used for PFAS measurements); single samples at time of case and control identification may not reflect exposure at crucial windows in cancer development; no info on other carcinogens (e.g. alcohol and smoking). *Other strengths*: histologically confirmed malignant breast cancer. *Other limitations*: lack of adjustment for important confounders; small sample size and limited statistical power; no information concerning hormone-receptor status; cross sectional design; strategy for recruiting controls could have induced a control selection bias if people positively
			1st quartile (0.17–2.15 ng/mL)	9	1		
			2nd quartile (2.20–3.02 ng/mL)	11	1.36 (0.42–4.52)		
			3rd quartile (3.05–3.82 ng/mL)	11	1.25 (0.38–4.17)		
			4th quartile (3.90–23.03 ng/mL)	35	2.38 (0.81–7.31)		
			Trend-test *P*-value, 0.400				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Velarde et al. (2022) Philippines 2018 Case–control (cont.)							responding to advertisement had healthier lifestyle and a higher medical awareness compared with the source population for cases; analysis by each PFAS separately did not account for isomers of PFOA or PFOS; measured a variety of other exposures but analysed separately from PFAS relative to outcome. *Other comments*: All participants had no prior use of hormonal contraceptives or HRT within 1 mo from the last day of use of an oral agent, or within 6 mo from the last day of use of an intramuscular agent.

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Li et al. (2022b) China 2012–2016 Case–control	Cases: 373 cases recruited at diagnosis from the Tianjin Medical University Cancer Institute and Hospital Controls: 657 controls were randomly selected from the participants in the Chinese National Breast Cancer Screening Program; cohort from a time period similar to that of the cases Exposure assessment method: quantitative plasma measurements; analytical method was state-of-the-art; a single blood sample was collected; blood was collected at the hospital before treatment	Breast, incidence	Plasma PFOA concentration (OR):			Age, BMI, smoking history, age at menarche, age of menopause, parity, breastfeeding duration, use of estrogen or estrogen replacement therapy, family history of breast cancer, education, monthly household income per capita, red meat consumption, pickled, fried, smoked, and barbecued food consumption.	*Exposure assessment critique*: Key strengths were that plasma levels represent the combined exposure through all exposure pathways; blood samples of cases were collected within a week after breast cancer diagnosis and before treatment; measurement error low. Key limitations were that if breast cancer alters ADME of PFAS there could be possible differential exposure misclassification, as plasma was collected after diagnosis in cases; single samples at time of case hospitalization may not reflect exposure at crucial windows in cancer development.
			1st quartile (< 2.4 ng/mL)	96	1		
			2nd quartile (2.24–3.35 ng/mL)	67	0.66 (0.41–1.08)		
			3rd quartile (3.35–5.11 ng/mL)	83	1.19 (0.75–1.90)		
			4th quartile (≥ 5.11 ng/mL)	127	2.83 (1.79–4.49)		
			Continuous (per standard deviation on natural log scale)	373	1.57 (1.31–1.89)		
			Trend-test *P*-value, 0.000				
		Breast, incidence	Plasma PFOS concentration (OR):				
			1st quartile (< 7.45 ng/mL)	119	1		
			2nd quartile (7.45–12.18 ng/mL)	85	0.61 (0.40–0.95)		
			3rd quartile (12.18–17.72 ng/mL)	83	0.58 (0.37–0.91)		
			4th quartile (≥ 17.72 ng/mL)	86	0.64 (0.41–1.00)		
			Continuous (per standard deviation on natural log scale)	373	0.81 (0.68–0.96)		
			Trend-test *P*-value, 0.002				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Li et al. (2022b) China 2012–2016 Case–control (cont.)		Breast, incidence	Plasma PFOA concentration (OR for one standard deviation increase on natural log scale):			Age, BMI, smoking history, age at menarche, age of menopause, parity, breastfeeding duration, use of estrogen or estrogen replacement therapy, family history of breast cancer, education, monthly household income per capita, red meat consumption, pickled, fried, smoked, and barbecued food consumption.	*Other strengths*: histologically confirmed malignant breast cancer; adjustment for important confounding variables; detailed information on diet; available information on the ER/PR status of breast cancer; large number of cases and controls allowed for stratified analyses with good statistical power. *Other limitations*: cross-sectional design.
			ER–	96	1.08 (0.82–1.41)		
			ER+	218	1.47 (1.19–1.80)		
			PR–	131	1.03 (0.81–1.30)		
			PR+	183	1.36 (1.09–1.69)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Li et al. (2023) Shijiazhuang, Hebei Province, China January to May 2022 Case–control	Cases: 150 recent hospital-based diagnoses of thyroid cancer, histologically confirmed by the hospital pathology unit, among adults aged 20–78 yr residing in Shijiazhuang for 10 yr or longer Controls: 150 healthy individuals, aged 26–83 yr, receiving routine physical examinations and residing in Shijiazhuang for 10 yr or longer and without thyroid nodules or thyroid disease; controls were individually matched to cases on sex and age (± 5 yr) Exposure assessment method: plasma measurements of all participants	Thyroid, incidence	Plasma PFOA concentration (OR):			Age, sex, BMI, smoking status, drinking status, education, household income	*Exposure assessment critique*: Key strengths were that plasma levels represent the combined exposure through all exposure pathways; measurement error low. Key limitations were that if thyroid cancer alters ADME of PFAS there could be possible differential exposure misclassification; single samples at time of case hospitalization may not reflect exposure at crucial windows in cancer development. *Other limitations*: Selection bias due to selection of controls among participants who were undergoing routine physical examination; potential for reverse causation.
			1st tertile	NR	1		
			2nd tertile	NR	0.14 (0.05–0.39)		
			3rd tertile	NR	0.32 (0.15–0.69)		
			Continuous (per unit on natural log scale)	150	0.78 (0.52–1.17)		
			Trend-test *P*-value, 0.006				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Li et al. (2023) Shijiazhuang, Hebei Province, China January to May 2022 Case–control (cont.)		Thyroid, incidence	Plasma PFOS concentration (OR):			Age, sex, BMI, smoking status, drinking status, education, household income	
			1st tertile	NR	1		
			2nd tertile	NR	0.68 (0.33–1.41)		
			3rd tertile	NR	1.21 (0.60–2.45)		
			Continuous (per unit on natural log scale)	150	1.02 (0.77–1.36)		
			Trend-test *P*-value, 0.655				

ADME, absorption, distribution, metabolism, and excretion; AL, Alabama; APFO, ammonium perfluorooctanoate; approx., approximately; BMI, body mass index; CA, California; CI, confidence interval; CPS-II, Cancer Prevention Study II; CYP, cytochrome P450; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; E3N, Etude épidémiologique auprès de femmes de la Mutuelle générale de l'Education nationale; ER, estrogen receptor; *N*-EtFOSAA, 2-(*N*-ethyl-perfluorooctane sulfonamido) acetic acid; β-HCH, β-hexachlorocyclohexane; HR, hazard ratio; HRT, hormone replacement therapy; ICD, International Classification of Diseases; IQR, interquartile range; MET-h, metabolic equivalent of task per hour; MHT, menopausal hormone therapy; MN, Minnesota; mo, month(s); NC, not calculated; NHL, non-Hodgkin lymphoma; NR, not reported; NTUH, National Taiwan University Hospital; OH, Ohio; OR, odds ratio; PCB, polychlorinated biphenyl; PFAS, per- and polyfluoroalkyl substances; PFCA, perfluoroalkyl carboxylic acid; PFHpS, perfluoroheptanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; POP, persistent organic pollutant; POSF, perfluorooctanesulfonyl fluoride; ppm, parts per million; PR, progesterone receptor; RR, relative risk; SIR, standardized incidence ratio; SMR, standardized mortality ratio; TFE, tetrafluoroethylene; US, United States; USA, United States of America; vs, versus; WQS, weighted quantile sum; WV, West Virginia; yr, year(s).

few women were included in this occupationally exposed cohort and there were few cases of breast cancer, limiting the ability to draw conclusions. The low number of cases may have been further affected by residential migration out of Minnesota and Wisconsin. Linkage to a cancer registry was a strength here when considering breast cancer, since it is not a cancer with a high rate of fatality and relying on NDI linkage alone would underestimate cases.]

Alexander et al. (2003) investigated a cohort of 2083 (353 women) PFOS-exposed production workers (Section 2.1.2). Mortality follow-up was conducted using linkage to the NDI until 1998. PFOS exposure was estimated based on a JEM that was validated from a subset of workers from whom blood samples had been collected. Only 17% of the cohort were women. There were two breast cancer-specific deaths identified (both among workers who only held non-exposed jobs); the resulting SMR was very imprecise. [The Working Group noted that there were very few women included in this occupationally exposed cohort and only 2 cases of breast cancer, both among workers holding non-exposed jobs, limiting the ability to draw conclusions. Further, there was little information available for confounding adjustment. The study relied on NDI linkage to identify cases, which would have underestimated the number of breast cancer cases, given the favourable survival after diagnosis, resulting in non-differential outcome misclassification that probably caused bias towards the null.]

Steenland and Woskie (2012) conducted a study of PFOA-exposed workers at the polymer-production plant in Parkersburg, West Virginia, USA (see Section 2.1.3). There were 5791 workers (19% women) who were employed for ≥ 1 day between 1948 and 2002 and for whom there were sufficient work history details to estimate PFOA exposure using a JEM informed by a subset with measured PFAS levels. A total of 4 deaths related to breast cancer were observed during follow-up from 1952 to 2008; mortality was not elevated overall, nor did it increase with quartile of estimated PFOA exposure. [The Working Group noted that although this study was in a highly exposed cohort, it was not well powered for breast cancer evaluation because of the small proportion of women, the few breast cancer-related deaths identified, and the lack of incidence data.]

Barry et al. (2013) conducted an investigation of community residents and workers who were exposed to PFOA from a polymer-production plant in the West Virginia and Ohio region, USA (Section 2.1.5). The study included 32 254 community residents and workers (17 360 women) who had a measurement of serum PFOA between 2005–2006, had participated in at least one survey between 2008 and 2011, and for whom either environmental or occupational modelled cumulative PFOA estimates were available. There was a modest inverse association between estimated cumulative PFOA exposure levels (natural log-transformed) and validated breast cancer (559 cases) with a hazard ratio of 0.94 (95% CI, 0.89–1.00), and results remained similar with a 10-year lag. [The Working Group noted that this study was informative because of its large size and consideration of cancer risk in highly exposed community members and in people exposed occupationally. It also considered confounders including education and alcohol intake, although it did not report information on other established breast cancer risk factors such as reproductive history. However, these results were based on estimated PFOA serum levels using data from 2005–2006, which may not include the most etiologically relevant time window. Finally, this study did not include information on breast cancer characteristics, including hormone receptor-related tumour subtypes, and presented results for breast cancer overall, which could mask any subtype-specific associations.]

Ghisari et al. (2017) evaluated the association between serum PFAS concentrations and breast cancer risk in a nested case–control study of pregnant nulliparous women in Denmark (see Section 2.1.7). PFAS, including PFOA and PFOS, were measured in blood samples collected during the first trimester of pregnancy (1996–2002), and breast cancer cases in the mothers were ascertained using linkage to a nationwide cancer registry, with follow-up until 2010. The study included 158 cases of breast cancer and 215 randomly selected controls. After adjusting for confounders, no association was observed between serum PFOA or PFOS concentrations and breast cancer incidence. However, when considering interactions with cytochrome P450 (CYP) family member 19 (CYP19, aromatase), which acts on the aromatization of androgens to estrogens, increases in levels of both PFOA and PFOS were associated with a notably higher incidence of breast cancer among women who had the CC genotype (relative risk for a 1-unit increase in natural log-transformed PFOA, 7.24; 95% CI, 1.00–52; and relative risk for a 1-unit increase in natural log-transformed PFOS, 6.42; 95% CI, 1.08–38.3), with significant *P* values for interaction (for PFOA, $P = 0.047$; for PFOS, $P = 0.055$). [The Working Group noted that this study had a number of important strengths, including serum PFAS levels that were measured at baseline and adjustment for several relevant breast cancer risk factors. Pregnancy may be an important window of susceptibility during which exposures may be particularly relevant for subsequent risk of breast (Terry et al., 2019). The study was also somewhat underpowered to investigate interactions with genotype and had very few years of follow-up after pregnancy, therefore focusing on premenopausal breast cancer. This study did not include information on diagnoses of postmenopausal breast cancer or other characteristics, including hormone-receptor tumour subtypes, and presented results for breast cancer overall, which could mask any subtype-specific associations.]

Hurley et al. (2018) analysed serum PFAS levels in relation to breast cancer risk in a nested case–control study within the prospective CTS cohort in the USA (see Section 2.1.8). Breast cancer cases were identified by linkage to cancer registries and were analysed in relation to blood samples collected on average 35 months after a cancer diagnosis and any treatment (range, 9 months to 8.5 years). Average serum PFOA and PFOS levels in this cohort (median in controls, PFOA, 2.48 ng/mL, and PFOS, 6.95 ng/mL) were generally lower than those measured in previous studies (e.g. Ghisari et al., 2017), with the exception of PFOA in the study by Wielsøe et al. (2017). Among the 902 cases and 858 controls with serum PFAS concentrations, there was little evidence for an association between breast cancer and either PFOA or PFOS. There was also no association observed when the analyses were limited to either premenopausal or postmenopausal breast cancers or when considering the combined ER/PR status of the tumour. [The Working Group noted that this study included several established breast cancer risk factors and was able to consider stratification by menopausal status and hormone-receptor status, which are important factors to consider. However, the collection of blood samples on average 35 months after a case diagnosis was a major limitation as it was unclear whether these measurements reflect the relevant etiological window for breast cancer or whether they may have been influenced by breast cancer or any treatment.]

Mancini et al. (2020a) investigated the association between serum PFAS measures and breast cancer risk in a nested case–control study in the E3N cohort of women in France (Section 2.1.10). Blood samples were collected in the period 1994–1999, and women were followed for breast cancer until 2013. There were 194 cases of postmenopausal breast cancer and 194 matched controls. For PFOA, the association for all breast

cancers was elevated in the second quartile but not in the third and fourth quartiles. When stratifying by ER and PR status, this increase in risk for the second quartile was driven by ER– or PR– tumours (e.g. quartile 2 versus quartile 1 for ER–, OR, 7.73; 95% CI, 1.46–41.08), although estimates were imprecise. A non-monotonic association was also observed for increasing serum levels of PFOS, with higher ORs in the second and third quartile, and associations that were elevated but with wide confidence intervals for the fourth quartile. However, a monotonic trend with increasing PFOS levels was observed for ER+ and, separately, PR+ tumours (e.g. quartile 4 versus quartile 1 for ER+, OR, 2.33; 95% CI, 1.11–4.90). [The Working Group noted that this study was particularly informative since serum samples were collected prospectively, there was a long follow-up period, and the authors were able to evaluate how associations varied by ER or PR status of the tumour, although the confidence intervals were wide.]

Chang et al. (2023) conducted a nested case–control study within the PLCO Cancer Screening Trial (Section 2.1.11). This study included 621 cases of invasive postmenopausal breast cancer diagnosed until November 2013 and 621 controls in postmenopausal women who were selected with matching on age at baseline, date of blood draw, and baseline use of hormone replacement therapy. There was no association between PFOA, by quartiles of exposure, and overall breast cancer risk. The ORs for serum PFOS, categorized in quartiles, were elevated but mainly with wide confidence intervals in relation to overall breast cancer risk. However, for PFOS, associations were evident for hormone receptor-positive breast cancer (ER+/PR+ quartile 3 versus quartile 1, OR, 2.19; 95% CI, 1.21–3.98 and quartile 4 versus quartile 1, OR, 1.89; 95% CI, 0.97–3.69). For PFOA, there was a non-monotonic positive exposure–response relation observed for ER–/PR– tumours, with wide confidence intervals (ER–/PR–: quartile 3 versus quartile 1, OR, 2.23; 95% CI, 0.90–5.54; and quartile 4 versus quartile 1, OR, 1.62; 95% CI, 0.62–4.23). [The Working Group noted that this study was very informative because it was the largest prospective study evaluating prediagnostic serum PFAS levels in relation to breast cancer risk. The findings from this report were strengthened by the evaluation of differences in joint ER/PR status of the tumour. However, the assessment of PFAS levels using untargeted measurement methods limited direct comparisons with other studies. Finally, these results were generalizable only to postmenopausal women, because premenopausal breast cancer cases were not included.]

In a case–control study nested in the CHDS cohort in California, USA (see Section 2.1.12), Cohn et al. (2020) estimated the relation between maternal serum PFAS levels during pregnancy and the daughter's risk of breast cancer by age 52 years. There were 102 cases identified using validated self-report and registry linkage and they were matched to 310 controls. No association was observed for maternal PFOA exposure in utero in relation to breast cancer risk in the daughters, although the specific results were not reported. Maternal PFOS exposure in utero was inversely associated with breast cancer risk in daughters in a model that included terms for $\log_2$-transformed *N*-ethyl-perfluorooctane sulfonamido acetic acid (*N*-EtFOSAA), which is a precursor of PFOS, $\log_2$-transformed total cholesterol, and their interaction. The OR for the fourth quartile median versus the first quartile median (an increase of 3.15 ng/mL) in $\log_2$-transformed maternal PFOS was 0.3 (95% CI, 0.1–0.9). [The Working Group noted that although this study was unique in its focus on maternal serum PFAS levels in relation to daughter's breast cancer risk, it did not incorporate other measures of PFAS during childhood, adolescence, or adulthood, which may also be relevant. Additionally, the case counts were small, especially with stratification, which meant that interpretation of these findings was challenging and that the findings were not

easily comparable to those of other studies. This study mainly focused on premenopausal breast cancer diagnoses or did not include other breast cancer characteristics, including hormone-receptor tumour subtypes, and presented results for breast cancer overall, which could mask any subtype-specific associations.]

Li et al. (2022a) followed more than 60 000 individuals (more than 28 000 of whom were women) who lived in Ronneby municipality in Sweden between 1985 and 2013; approximately one third of the participants were exposed to water contaminated with PFAS, primarily with PFOS and PFHxS and, to a lesser extent, PFOA (Section 2.1.13). Exposure assessment was based on annual residential addresses and information on drinking-water supply, and cases were identified on the basis of linkage to the cancer registry until 2016. With 681 cases of female breast cancer identified, there was no evidence of an excess risk of breast cancer; SIRs were below the null and were similar for women both with "never-high" or "ever-high" exposure living at an address supplied with PFAS-contaminated water compared with an external reference group. In the internal cohort comparison analysis, there was no difference in the hazard ratios for breast cancer across categories based on estimated duration or timing of exposure. [The Working Group noted that although this study included a large general population sample with a strong exposure contrast and a near-complete registry-based case identification, there was limited control for confounding, particularly for established breast cancer risk factors such as education and reproductive history, and the mixed exposure to multiple PFAS did not allow for the identification of associations with individual compounds. This study did not incorporate information on other breast cancer characteristics, including hormone-receptor status, and it presented results for breast cancer overall, which could mask any subtype-specific associations.]

Feng et al. (2022) evaluated the association between plasma PFAS and breast cancer risk in an ongoing prospective study in Shiyan, China, of retired workers from an automotive company (Section 2.1.14). Incident breast cancer cases were identified by medical record review or death certificates. The nested case–cohort sample included a random subcohort of 990 participants and all non-subcohort participants identified with incident breast cancer (n = 213). The random subcohort included 13 cases of breast cancer, thus there was a total of 226 incident breast cancer cases. Plasma PFAS levels were quantified for the entire case–cohort sample. Increasing levels of serum PFOA were associated with a higher risk of breast cancer (natural log-transformed PFOA levels, HR, 1.35; 95% CI, 1.03–1.78; quartile 4 versus quartile 1, HR, 1.69; 95% CI, 1.05–2.70), but no increase in risk was observed for PFOS. The association for PFOA was similar when the analysis was restricted to postmenopausal women. [The Working Group noted that this study provided compelling evidence, using prospective sample collection and a case–cohort design with adjustment for many potential confounders. However, it was unable to explore how this association may vary by hormone-receptor tumour subtype and, by presenting results for breast cancer overall, could mask any subtype-specific associations.]

Winquist et al. (2023) evaluated the concentrations of several PFAS compounds in serum samples in relation to breast cancer incidence as part of a nested case–cohort study in the ACS prospective CPS-II LifeLink Cohort. Between 1998 and 2001, participants were selected if they had no previous cancer diagnosis and donated blood samples at a median age of 70 years (69 years for women) (Section 2.1.21). Cancer cases were identified by self-report and using NDI linkage. There were 786 cases of postmenopausal breast cancer and 499 women in the subcohort, and the median follow-up time was 14 years. Higher serum PFOA and PFOS concentrations were

not related to higher incidence of breast cancer (PFOA, quartile 4 versus quartile 1, HR, 0.82; 95% CI, 0.57–1.17; PFOS, quartile 4 versus quartile 1, HR, 0.70; 95% CI, 0.48–1.01). [The Working Group noted that this study included prospective sample collection and a case–cohort design with adjustment for many potential confounders. However, the blood sample used to measure PFAS was collected at a median age of 69 years, which is after the peak age at diagnosis for breast cancer. Further, it did not consider variability in the associations by hormone-receptor tumour subtype and by presenting results for breast cancer overall could mask any subtype-specific associations.]

(b) Case–control studies and meta-analyses

The eight case–control studies contributing evidence on PFOA and PFOS exposure and risk of breast cancer in women are described below. [The Working Group noted that nearly all the case–control studies listed below had a design in which exposure was measured after disease diagnosis, thus reverse causation bias cannot be excluded. Indeed, the disease could potentially affect PFOA and PFOS internal levels as a consequence of physiological changes associated with tumour development, such as altered albumin levels or altered glomerular filtration rate. Despite this concern, the Working Group considered it unlikely that such alterations would be observed in patients with breast cancer at diagnosis and that there was too little information available concerning the toxicokinetics of PFOA and PFOS in patients with cancer to reach conclusions on the presence of reverse causation bias in case–control studies. In all the case–control studies, except for that by Vieira et al. (2013) in which PFOA serum levels were inferred from geocoded addresses, environmental exposure, and toxicokinetic models, exposure classification was based on PFOA and PFOS measurements in blood samples collected only once when entering the study. The Working Group also questioned whether blood PFOA and PFOS levels measured at the time of diagnosis reflect exposure during the most relevant windows of exposure with regard to breast cancer risk. Indeed, for cancer (and in particular for breast cancer) the relevant time windows of exposure are several years before diagnosis, so that the levels of exposure at the time of diagnosis may not be pertinent to the disease. Nevertheless, since there is some evidence that single samples may represent long-term average levels of exposure to PFOA over a 5–8-year period (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636), and since there was limited information concerning the most relevant window of exposure to PFOA and PFOS with regard to breast cancer, the Working Group could not come to a conclusion on the informativeness of these studies.]

Vieira et al. (2013) investigated the relation between exposure to PFOA and breast cancer risk among residents living near the polymer-production plant in Parkersburg, West Virginia, USA, in two case–control studies (Vieira et al., 2013) (Section 2.1.22). In the case–control study in West Virginia and Ohio, 4057 cases of female breast cancer diagnosed from 1996 through 2005 in five Ohio counties and eight West Virginia counties were included in the study, whereas controls comprised all other cancers registered during the same study period (excluding kidney, pancreatic, testicular, and liver cancers). Of the 13 counties included in this study, 6 areas were classified as contaminated public water districts, and living within a contaminated water district was the exposure of interest for the main analyses. In the other case–control study, additional analyses were conducted only for Ohio counties for which it was possible to geolocalize the street addresses for all cancer cases and then to estimate serum PFOA concentration as an exposure metric assuming 10 years residence and latency. All analyses were restricted to women and

adjusted for age, race (White or non-White, only for Ohio), smoking status, and health insurance provider. No evidence of associations with breast cancer risk was observed in the two studies. [The Working Group noted that other types of cancer were used as controls and that the presence of exposure misclassification bias cannot be excluded because the exposure was estimated on the basis of the address of residence at diagnosis, although participants could have moved between water districts. Potential exposure misclassification was probably non-differential, so the bias would be expected to be towards the null. Finally, the Working Group noted that several important confounding variables, mainly related to reproductive history (e.g. age at menarche, number of pregnancies, age at menopause) and exogenous hormone exposure (e.g. use of contraceptive pill, use of hormone replacement therapy), were not included in the analyses.]

Bonefeld-Jørgensen et al. (2011) conducted a case–control study among women of Inuit descent in Greenland. Between 2000 and 2003, 31 women with breast cancer were recruited at the Dronning Ingrids Hospital in Nuuk, where all breast cancer cases of Greenland are registered. Women acting as controls (*n* = 115) were selected by frequency-matching on age and district of residence from a cross-sectional study conducted in 2000 (Côté et al., 2006) and from the Arctic Monitoring and Assessment Programme study conducted between 1999 and 2005 (Deutch et al., 2007). This study was extended by Wielsøe et al. (2017), who enrolled 66 cases and 62 controls during 2011–2014. Although cases were always recruited at the Dronning Ingrids Hospital in Nuuk, controls enrolled during 2011–2014 were selected by frequency-matching on age and district of residence among patients admitted to the hospital in the department of orthopaedic surgery, or to the department of gynaecology and obstetrics because of the diagnosis of non-malignant abnormities in the uterus, ovaries, or breast. Controls recruited from 2000 to 2003 were then reduced to 1 control per case, so that the final study population included 77 cases of breast cancer and 81 controls. [The Working Group noted that it was unclear how the controls for the period 2000–2003 were selected from the two surveys. Moreover, the authors did not explain on which criteria the final study population was selected, so that selection bias could not be excluded. The Working Group also noted that controls enrolled between 2011 and 2014 were hospital patients attending the orthopaedic surgery department or with non-malignant abnormalities in the uterus, ovary, or breast. If there were an association between PFOA or PFOS exposure and the health conditions affecting the patients recruited as controls, selecting controls from among women admitted at the hospital could have introduced a bias.] Blood samples were collected at diagnosis for the cases and when enrolled in the study for the controls, and PFOA and PFOS serum levels were measured for both cases and controls. After adjusting for age, BMI, serum cotinine levels, number of pregnancies, and breastfeeding, the authors reported a positive association between serum levels of PFOA (OR per unit increase of PFOA, 1.26; 95% CI, 1.01–1.58) and of PFOS (OR per unit increase of PFOS, 1.02; 95% CI, 1.01–1.03) and breast cancer risk. [The Working Group noted that no information was available concerning the delay between diagnosis and the collection of blood samples used for PFAS measurements, thus it could not be excluded that patients with breast cancer changed their behaviours after diagnosis and that this change could have an impact on circulating levels of PFAS. Moreover, the authors did not specify whether the women enrolled as cases had received cancer treatment before blood samples were collected. This could potentially affect PFOA and PFOS internal levels because of physiological changes associated with the treatment. The lack of evidence on the impact of cancer treatment on PFOA and PFOS internal levels did not permit the Working Group to

reach a conclusion on the possible presence of bias. Finally, the Working Group noted that the median serum level of PFOS (45.60 ng/mL) for breast cancer cases among women recruited between 2000 and 2003 was more than double that measured in controls (18.06 ng/mL) selected in the same time period but also those measured in cases (19.35 ng/mL) and controls (18.20 ng/mL) recruited between 2011 and 2014. The authors did not provide an explanation or interpretation of this important variation in PFOS levels that would be expected to have had an impact on the results.] Additional analyses to explore interactions between gene polymorphisms and PFOA and PFOS serum levels with regard to breast cancer risk were conducted using the same study population as Bonefeld-Jorgensen et al. (2011), including 31 cases and 115 controls (Ghisari et al., 2014). [The Working Group noted that the interaction between genotype and exposure was not formally tested by Ghisari et al. (2014), so that the results were considered to be not informative. Moreover, the limited number of cases included had a strong impact on the statistical power of the analyses, preventing correct interpretation of the results.]

Between 2014 and 2015, 120 cases of histologically confirmed breast cancer were consecutively recruited from women attending the National Taiwan University Hospital, China (Tsai et al., 2020). A total of 119 women without any history of malignancy were recruited as controls between 2014 and 2016 through advertisements of posters and flyers at the hospital and in the community. All participants answered a questionnaire and donated a blood sample at enrolment. For the cases, blood samples were collected before receiving any treatment for breast cancer. Plasma PFOA and PFOS levels were measured for both cases and controls. Adjusted ORs of 0.89 (95% CI, 0.59–1.34) and 1.07 (95% CI, 0.64–1.79) were calculated for a natural log 1-unit increase in PFOA and PFOS, respectively. When the analyses were stratified on the basis of age of participants (> 50 years versus ≤ 50 years), an adjusted OR of 2.34 (95% CI, 1.02–5.38) for PFOS exposure was observed for women aged ≤ 50 years, whereas an adjusted OR of 0.62 (95% CI, 0.29–1.29) was observed for women aged > 50 years. When also considering the tumour ER status, PFOS exposure was significantly associated only with risk of ER+ breast cancer in women aged ≤ 50 years (OR per unit increase in natural log-transformed PFOS levels, 3.25 (95% CI, 1.29–8.23). The other results were generally not positive. [The Working Group noted that the small number of cases and controls included in the study could have limited the statistical power of the analyses, especially when stratifying on the basis of age and tumour hormone-receptor status. Moreover, the Working Group noticed that the recruitment strategy for the controls could have induced a selection bias, because people positively responding to advertisement through posters and flyers could have had healthier lifestyles and a higher medical awareness compared with the source population for cases.]

A multicentric hospital-based case–control study conducted in Japan between 2001 and 2005 included 401 cases of histologically confirmed invasive breast cancer (Itoh et al., 2021). Controls were selected from among individuals attending hospital medical check-ups during the study period who had not been diagnosed with cancer. The controls were matched individually to cases on age and residential area (urban or rural). At recruitment, all participants completed a self-administered questionnaire, and a blood sample was collected. Among participants serving as cases, blood samples were collected before any cancer treatment. Multivariable analysis showed a precise inverse association between risk of breast cancer and serum concentrations of PFOA (OR for fourth quartile versus first quartile, 0.21 (95% CI, 0.10–0.45) and PFOS (OR for fourth quartile versus first quartile, 0.14; 95% CI, 0.07–0.31). Results from models that additionally adjusted for vegetable intake, fish and shellfish

intake, calendar year of blood sampling, and quartiles of serum lipid-adjusted total concentration of PCBs remained virtually unchanged. The association between PFOA or PFOS and risk of breast cancer did not differ accordingly to menopausal status or hormone-receptor status. [The Working Group noted that the use of medical check-up examinees as controls may have caused selection bias because of their higher medical awareness and possibly different socioeconomic status compared with the source population for cases. Moreover, educational and socioeconomic status were not included as adjustment variables in the main analyses.]

Velarde et al. (2022) recruited 75 cases of histologically confirmed breast cancer in women aged 18–60 years in the Philippines, with no comorbidity, who visited the Philippine General Hospital between January and December 2018. Patients who underwent neoadjuvant chemotherapy were excluded from the study. Controls were randomly recruited through posters, social media advertisements, and by word of mouth. The control group included 75 women within the age range of 18–59 years, without a previous diagnosis of cancer and without a family history of breast, ovarian, or endometrial cancer in first-degree relatives. This study did not observe any associations between serum PFOA and PFOS levels and breast cancer risk. [The Working Group noted that the study did not adjust for important confounding variables, such as anthropometric characteristics, reproductive history, and hormone exposure. Indeed, the final model included only age and region of residence as covariables. Moreover, the small number of included cases and controls limited the statistical power of the analyses and thus the informativeness of the results. Finally, the Working Group noted that the recruitment strategy for the controls may have caused selection bias, because people positively responding to advertisements through posters and social media could have a higher medical awareness or possibly a different socioeconomic status compared with the source population for cases.]

Li et al. (2022b) conducted a case–control study that included 373 cases of breast cancer and 657 controls, all participants having available blood samples. Cases were recruited at the Tianjin Medical University Cancer Institute and Hospital, China, between January 2012 and December 2016. Diagnosis of malignant breast cancer was confirmed histologically, and a blood sample was collected within 1 week after diagnosis and before receiving any treatment. Controls were randomly selected among women participating in the Chinese National Breast Cancer Screening Program (CNBCSP) cohort. The CNBCSP was launched in 2012 and included women without a history of cancer who lived in four cities (Shijiazhuang, Tangshan, Xingtai, and Handan) in Hebei Province for ≥ 3 years, and were aged 40–74 years (Wu et al., 2023). Both case and control participants answered a questionnaire (including dietary information) at recruitment. Li et al. (2022b) found that plasma concentrations of PFOA were positively associated with breast cancer risk. The authors estimated an adjusted OR for an increase of 1 standard deviation (SD) in natural log-transformed PFOA plasma levels of 1.57 (95% CI, 1.31–1.89). PFOA was more strongly associated with the ER+ (OR, 1.47; 95% CI, 1.19–1.80) and PR+ (OR, 1.36; 95% CI, 1.09–1.69) breast cancer than with receptor-negative tumours. An inverse association was observed between PFOS plasma levels and breast cancer risk, with an OR for one SD increase in natural log-transformed PFOS plasma levels of 0.81 (95% CI, 0.68–0.96). [The Working Group considered as strengths of this study that all cases were histologically confirmed malignant breast cancer, and blood samples were collected within 1 week after diagnosis and before any cancer treatment. Moreover, the Working Group noted that the large number of cases and controls permitted stratified analyses while still ensuring a good statistical power.

The Working Group noted as a limitation the fact that the controls were selected from women participating in the breast cancer screening programme, who may have had a higher medical awareness and possibly different socioeconomic status compared with the source population for cases.]

Three meta-analyses have been conducted on the association between exposure to PFOA and PFOS and breast cancer risk. The first meta-analysis (Jiang et al., 2022) included eight studies: seven case–control studies, among which three were case–control studies nested in prospective cohort studies, and one cross-sectional study. The exposure assessment for all included studies was based on PFOA and PFOS blood measurements. The overall results showed that PFOA was positively associated with breast cancer risk, and the pooled OR was 1.32 (95% CI, 1.19–1.46), whereas PFOS was not associated with breast cancer risk (pooled OR, 1.01; 95% CI, 0.87–1.17). [The Working Group noted that the present meta-analyses included studies with different designs (case–control studies, nested case–control studies, cross-sectional study) which could have caused heterogeneity and instability of the pooled OR. Moreover, the Working Group noted that the results were mainly driven by the only cross-sectional study included in the meta-analyses (Omoike et al., 2021) and that this study was identified as the main source of heterogeneity by the authors. Finally, the Working Group noted that the authors counted studies multiple times when performing comparisons between exposure categories.]

In the second meta-analysis, Cong et al. (2023) included eleven studies: nine case–control studies, of which three were nested in prospective cohort studies, one cohort study, and one case–cohort study. PFOA and PFOS blood levels were used as the main exposure variable in all studies except for one study in which individual cumulative PFOA serum concentration estimates were calculated retrospectively from 1952 through 2011. The results of the meta-analyses found little evidence of a positive association between PFOA and PFOS and breast cancer risk (pooled OR, 1.07; 95% CI, 0.84–1.38; and pooled OR, 1.01; 95% CI, 0.95–1.08, respectively). The authors observed significant heterogeneity among the included studies for both PFOA (I^2 = 85.9%; P < 0.001) and PFOS (I^2 = 65.7%; P = 0.003). When omitting one study at a time from the pooled analyses, a weakly positive OR was observed for PFOS in relation to breast cancer when excluding Itoh et al. (2021) (pooled OR, 1.02; 95% CI, 1.01–1.03, I^2 = 2.6%; P = 0.41). Results remained unchanged for PFOA. [The Working Group noted that studies having different designs (case–control studies, nested case–control studies, case–cohort study and cohort study) and applying different methods to estimate the exposure were included in this meta-analysis and that this could explain the high observed heterogeneity. Moreover, the results of the meta-analyses seemed to be strongly influenced by the only study that highlighted an inverse association between PFOA and PFOS and breast cancer risk.]

The third meta-analysis on the association between exposure to PFOA and PFOS and breast cancer risk was conducted by Chang et al. and included 11 case–control studies, 5 of which were nested in prospective cohort studies (Chang et al., 2024). For all studies included in the meta-analyses PFOA and PFOS levels were measured in blood samples (serum or plasma). The results of the meta-analyses were not consistent with an association between PFOA and PFOS blood levels and the risk of breast cancer overall, but they noted substantial heterogeneity across studies. Indeed, the authors estimated a rate ratio for a natural log-unit increase of PFOA of 0.95 (95% CI, 0.77–1.18; I^2 = 67%; P for heterogeneity, < 0.01) and for a natural log-unit increase of PFOS of 0.98 (95% CI, 0.87–1.11; I^2 = 54%; P for heterogeneity, 0.02). In subanalyses, when limiting to studies with prospectively collected blood samples, there was a positive association

with PFOA (RR, 1.16; 95% CI, 0.96–1.40). [The Working Group noted that this meta-analysis incorporated important subgroup analyses, including by timing of sample collection and tumour subtype. However, there was substantial heterogeneity across the published studies, limiting the informativeness of the results.]

2.4.2 Cancer of the thyroid gland

See Table 2.4.

The Working Group identified six cohort studies and three case–control studies investigating the risk of thyroid cancer associated with PFOA or PFOS exposure. Among the cohort studies, two were occupational cohorts (Leonard et al., 2008; Lundin et al., 2009), one was a combination of general population members and workers (Barry et al., 2013), one was composed of residents in area with highly contaminated drinking-water (Ronneby Register cohort; Li et al., 2022a), one nested case–control study was within the FMC (Madrigal et al., 2024), and one nested case–control study was within the Bio*Me* biobank (van Gerwen et al., 2023). One of the case–control studies was population-based (Vieira et al., 2013) and two were hospital-based (Liu et al., 2022; Li et al., 2023).

(a) Cohort studies

Lundin et al. (2009) conducted a mortality study in a cohort of 3993 employees of an APFO-manufacturing facility in Cottage Grove, Minnesota, USA (see Section 2.1.1). The cohort was followed until 31 December 2002, and 807 decedents were identified. Using rates for the state of Minnesota as the referent, SMRs were calculated for different jobs classified by exposure to APFO (the ammonium salt of PFOA). There was only 1 observed death from thyroid cancer, which was assigned to the "never" exposure group. The SMR for the "never" exposure group was 2.16 (95% CI, 0.05–12.00). [The Working Group noted that the important limitations of the study included the small occupational cohort with only 1 death from thyroid cancer and crude exposure assessment by job classification, which made this study uninformative for the evaluation of an association with thyroid cancer.]

Leonard et al. (2008) conducted a retrospective cohort mortality study for the PFOA cohort in a polymer-production plant in Parkersburg, West Virginia, USA, which included 6027 participants who had worked at the facility between 1948 and 2002 (Section 2.1.3). SMRs were calculated by comparing the observed number of deaths to expected numbers derived from mortality rates for three reference populations (the US population, the West Virginia state population, and an eight-state regional employee population from the same company). There were only 3 observed deaths for thyroid cancer. The SMRs for the cohort from the Parkersburg plant were [3.120] (95% CI, [0.644–9.119]), [2.856] (95% CI, [0.589–8.347]), and [6.286] (95% CI, [1.297–18.369]), respectively, for the three reference populations (the US population, the West Virginia population, and the workers in the same company and region). [The Working Group noted that the major limitation of the study was the limited statistical power to evaluate mortality rates for thyroid cancer because of the small numbers of observed deaths, which made this study uninformative for the evaluation of an association with thyroid cancer.]

Barry et al. (2013) examined PFOA exposures and incident cancers among community residents and workers who were exposed to PFOA from a chemical plant, using the C8 Health Project cohort combined with the worker cohort from the polymer-production plant in Parkersburg, West Virginia, USA (Section 2.1.5). There were 32 254 participants in the entire cohort, with 28 541 participants classified as the community group and 3713 as the occupational group. There were 98 cases of primary thyroid cancer reported. The analysis included 86 cases of validated primary thyroid cancer with complete

covariate information. In the total cohort, the hazard ratios for a 1-unit increase in natural log-transformed estimated cumulative PFOA exposure in relation to thyroid cancer were 1.10 (95% CI, 0.95–1.26) for unlagged exposures and 1.04 (95% CI, 0.89–1.20) for exposures lagged by 10 years. When stratified by community residents and workers, the hazard ratios for cumulative PFOA exposure in relation to thyroid cancer were 1.04 (95% CI, 0.89–1.23) and 1.93 (95% CI, 1.00–3.71), respectively, for unlagged exposures, and 1.00 (95% CI, 0.84–1.20) and 1.12 (95% CI, 0.61–2.05), respectively, for exposures lagged by 10 years. In sensitivity analyses, when excluding years before each participant began living or working in the contaminated water districts in the survival models, results were similar to the reported results above. When calculating hazard ratios by PFOA quartile in the total cohort, there was no indication of an exposure–response relation between PFOA exposure and thyroid cancer. However, an exposure–response relation was indicated when calculating hazard ratios by PFOA quartile among the occupational group (8 cases) but not the community group (78 cases). [The Working Group noted as strengths the large cohort, strong exposure contrast, assessment of individual cumulative PFOA exposure, and lagged analyses. Limitations included self-reported cancer cases, the low sample size for thyroid cancer, and lack of evaluation of residents' co-exposure to other PFAS.]

Li et al. (2022a) studied cancer incidence in the Ronneby Register cohort, which included a community of residents in Sweden with high-level environmental exposure to PFAS, dominated by PFOS and PFHxS, in drinking-water (Section 2.1.13). SIRs were calculated by comparing with a regional external reference population (the population of Blekinge County excluding Ronneby municipality) and the national reference population (the whole population of Sweden). By the end of the follow-up on 31 December 2016, there were 17 cases of incident thyroid cancer in men and 48 cases in women. External comparisons and internal comparisons were both performed within the Ronneby Register cohort. To facilitate comparison, Ronneby residents were assigned to mutually exclusive groups: "never-high" and "ever-high" based on the source of drinking-water at their residence. When compared with the regional external reference population, women in the ever-high group had nominally higher estimates (defined as > 25% difference) for cancers of the thyroid than did women in the never-high group, with an SIR of 2.08 (95% CI, 1.19–3.38) in the ever-high group, and 1.38 (95% CI, 0.94–1.95) in the never-high group. However, that relation was not observed among men. In internal comparisons, the never-high group was used as the referent, and the thyroid cancer hazard ratio for different groups was calculated. The authors observed modestly increased point estimates but with wide confidence intervals, which showed limited indications of an exposure–response relation between PFAS exposure and the incidence of thyroid cancer for time period of high exposure ("early-high" in 2004 or earlier versus "late-high" in 2005 or later) or for duration of time in a high-exposure area ("short-high" for ≤ 10 years versus "long-high" for ≥ 11 years) compared with "never-high" group. [The Working Group noted as strengths the large study population, strong exposure contrast, and unbiased inclusion. The group-based exposure assessment can be assumed to provide unbiased risk estimates but less exposure contrast and broader confidence intervals than would be expected with individual-level estimates. However, even at the group level, there was a large exposure contrast, which was one of the strengths of this study. The main limitations were the crude exposure assessment, not including individual water intake or sources of exposure other than drinking-water, the mixed exposure profile, and the limited information on potential confounders.]

Madrigal et al. (2024) conducted a nested case–control study of papillary thyroid cancer in the FMC, restricting eligibility to women for whom serum samples were collected in their first pregnancy and whose pregnancy resulted in a full-term live birth, with delivery dates from 1987 to 2010 (see Section 2.1.18). Thyroid cancer cases and controls were identified by the nationwide Finnish Cancer Registry and the population registry until 2016. All cases were randomly selected women with primary papillary thyroid cancer diagnosed ≥ 3 years after the delivery date, without a history of other cancers. Controls were individually matched to cases on year of delivery (increments of 4–5 years) and age at first birth (increments of 3 years). A total of 800 participants (400 cases of thyroid cancer and 400 controls) were included in the nested case–control analysis. No clear pattern was observed in the association between papillary thyroid cancer risk and serum concentrations of PFOA (OR per log_2, 0.90; 95% CI, 0.68–1.19) or PFOS (OR per log_2, 1.14; 95% CI, 0.81–1.95). When stratified by age at diagnosis (< 40 years, ≥ 40 years), the associations per each doubling in concentrations of PFOA and PFOS were elevated but imprecise (OR per log_2, 1.20; 95% CI, 0.71–2.01; and OR per log_2, 1.14; 95% CI, 0.68–1.93; respectively) among women diagnosed before age 40 years. However, among women diagnosed at age ≥ 40 years, the associations were inverse or were close to 1.0 (OR per log_2, 0.70; 95% CI, 0.45–1.08; and OR per log_2, 1.01; 95% CI, 0.60–1.71; for PFOA and PFOS, respectively). [The Working Group noted that use of a single prediagnostic sample would result in only minor misclassification of long-term exposure over a period of 5–8 years, on the basis of a simulation study (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). The lack of information on pre-pregnancy BMI (a risk factor for thyroid cancer) and the population's low-level exposure with small exposure contrast were noted as limitations.]

van Gerwen et al. (2023) conducted a case–control study nested within the Bio*Me* biobank of Mount Sinai hospital in New York (see Section 2.1.20). Among 88 cases (57 with < 1 year between sample collection and thyroid cancer diagnosis) and 88 controls, plasma PFOA concentration was not associated with thyroid cancer risk, whereas plasma concentrations of branched PFOS and linear-PFOS were associated with increased thyroid cancer risk (ORs for increment of log_2-plasma concentration of branched PFOS and linear-PFOS were 1.32; 95% CI, 0.99–1.81; and 1.56; 95% CI, 0.99–1.81; respectively). In the sensitivity analysis restricted to 31 cases with > 1 year between sample collection and incident thyroid cancer diagnosis (median time, 3.7 years), log_2-plasma concentrations of branched PFOS and linear-PFOS were also associated with increased risk of thyroid cancer (OR, 3.09; 95% CI, 1.73–6.13; and OR, 2.67; 95% CI, 1.59–4.88; respectively). [The Working Group noted the limited follow-up time in this study and that use of a single prediagnostic sample would result in only minor misclassification of long-term exposure over a period of 5–8 years, on the basis of a simulation study carried out by the Working Group (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636).]

(b) Case–control studies

One population-based case–control study was conducted by Vieira et al. (2013) among residents of 13 counties in Ohio and West Virginia surrounding the Parkersburg polymer-production facility (see Section 2.1.22). The final data set included 343 cases of thyroid cancer. Controls were defined as all other cancers in the study data set, except for cancers of the kidney, pancreas, testis, liver, and thyroid. All cancer diagnoses were classified as exposed (living within a

contaminated water district) or unexposed (not living in a contaminated water district) using geocoding. The AORs varied among the water districts exposed to contaminated water, with the AOR of the overall exposure risk being 1.1 (95% CI, 0.7–1.5). Furthermore, for each case in Ohio, annual PFOA serum levels were calculated by linking environmental, exposure, and toxicokinetics models. The AORs were calculated using individual-level exposure categorized on the basis of the distribution of annual PFOA serum concentrations among the exposed study population. Using the unexposed group as the reference category, the AORs for very high, high, medium, and low individual-level exposures were 0.8 (95% CI, 0.2–3.5), 0.7 (95% CI, 0.2–2.1), 0.9 (95% CI, 0.4–2.3), and 0.9 (95% CI, 0.4–2.3), respectively. [The Working Group noted that the strengths of the study included its focus on a population with high PFOA exposure, the strong contrast in exposure levels, and the estimation of individual-level exposure for a subset of the people. Limitations included the use of other cancers as the referent, the lack of geocoded residence information among participants from West Virginia, and the risk of exposure misclassification (reliance on the address at the time of diagnosis rather than a complete residential history in analyses among Ohio participants).]

Liu et al. (2022) conducted a hospital-based case–control study in the Shandong Provincial Qianfoshan Hospital in Jinan City, Shandong Province, China, from 2016 to 2017. A total of 319 participants (134 cases of thyroid cancer and 185 controls) were included in the case–control analysis. The control group was randomly selected from patients undergoing routine medical visits at the hospital, with normal thyroid B-ultrasound examination, without a history of thyroid disease, and without taking iodine or thyroid hormone drugs during the blood collection. Serum samples of the participants were used to assess exposure to individual PFAS compounds. Serum samples for the case group were collected after the patients had stopped taking thyroid medication for 2 weeks under the guidance of their doctors. Serum samples for the control group were collected when they underwent routine medical visits at the hospital. The associations between serum levels of PFAS (including PFOA and PFOS) and thyroid cancer were examined using logistic regression models. Concentrations of PFAS compounds were categorized into quartiles according to the distribution in the control group. Compared with the first quartile of PFOA concentration, the ORs for the second, third, and last quartiles were 0.24 (95% CI, 0.12–0.50), 0.24 (95% CI, 0.11–0.49), and 0.20 (95% CI, 0.09–0.44), respectively, with a P for trend of < 0.001. Compared with the first quartile of PFOS concentration, the ORs for the second, third, and last quartiles of PFOS concentration were 0.81 (95% CI, 0.42–1.53), 0.26 (95% CI, 0.12–0.57), and 0.28 (95% CI, 0.12–0.66), respectively, with a P for trend of 0.001. [The Working Group noted that the limitations of the study included the sampling of serum after diagnosis and treatment, limited exposure contrast, small sample size, and the likelihood of potential reverse causation.]

Li et al. (2023) conducted a hospital-based case–control study in the Fourth Hospital of Hebei Medical University in Shijiazhuang, Hebei Province, from January to May 2022. All cases were newly arising thyroid cancer cases in the hospital, confirmed histologically by the hospital pathology unit, among patients who had resided in Shijiazhuang for ≥ 10 years. Controls were healthy individuals attending routine physical examinations in the health examination centre who had resided in Shijiazhuang for ≥ 10 years without thyroid cancer or other malignancies and were individually matched to cases on age (± 5 years) and sex. A total of 300 participants (150 cases of thyroid cancer and 150 healthy controls) were included in the case–control analysis. Plasma samples were collected before the start of thyroid cancer therapy for the cases

and during the physical examination for the controls. The associations between plasma levels of PFAS compounds (including PFOA and PFOS) and thyroid cancer were examined using conditional logistic regression and restricted cubic spline models. Plasma PFAS concentrations were analysed as continuous variables and categorized variables (classified into tertiles according to the distribution among controls). The results showed no consistent indication of a positive exposure–response relation between plasma PFOA or PFOS and thyroid cancer, with the ORs associated with a 1-unit increase in natural log-transformed levels being 0.78 (95% CI, 0.52–1.17) and 1.02 (95% CI, 0.77–1.36), respectively. Further, compared with the first tertile of PFOA concentration, the OR for the highest tertile of PFOA concentration was 0.32 (95% CI, 0.15–0.69), indicating an inverse association between PFOA and thyroid cancer risk (*P* for trend, 0.006). However, the restricted cubic spline model did not show this inverse dose–response relation. [The Working Group noted that the study relied on postdiagnostic serum samples, which might have been affected by reverse causation. The Working Group noted that only minor misclassification of long-term exposure because of reliance on a single prediagnostic sample would be expected, according to a simulation study (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636).]

2.5 Cancers of the digestive tract

2.5.1 Liver cancer

See Table S2.5 (Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636).

There were 11 epidemiological studies with information on liver cancer. Most were cohort studies, but three were case–control studies (one nested within a cohort), and one was a case–cohort study. Six studies were conducted in the USA (Alexander et al., 2003; Steenland and Woskie, 2012; Barry et al., 2013; Vieira et al., 2013; Raleigh et al., 2014; Goodrich et al., 2022), and one each in Denmark (Eriksen et al., 2009), China (Cao et al., 2022), Italy (Girardi and Merler, 2019), and Sweden (Li et al., 2022a). One included cohorts from multiple countries (Consonni et al., 2013). Five were occupational cohort mortality studies (Alexander et al., 2003; Steenland and Woskie, 2012; Consonni et al., 2013; Raleigh et al., 2014; Girardi and Merler, 2019); for four of these PFOA was the exposure of interest, whereas for one (Alexander et al., 2003) the exposure of interest was PFOS. The three community cohort studies (Eriksen et al., 2009; Barry et al., 2014; Li et al., 2022a) had incident cancer data, as did all three of the case–control studies (Vieira et al., 2013; Cao et al., 2022; Goodrich et al., 2022). Eriksen et al. (2009) reported results for PFOA and PFOS, Barry et al. (2014) reported on PFOA, and Li et al. (2022a) was not able to identify a specific PFAS of interest, but levels of PFOS were the highest in the studied population. Among the case–control studies, Vieira et al. (2013) focused on PFOA, whereas Cao et al. (2022) and Goodrich et al. (2022) reported results for both PFOA and PFOS. One additional study (Olsen et al., 2004) had some cross-sectional data on liver cancer among active and some inactive employees, but it was not considered informative regarding liver cancer incidence or mortality and therefore is not discussed further.

(a) Cohort, case–cohort, and nested case–control studies

Raleigh et al. (2014) studied liver cancer mortality among 4668 PFOA-exposed workers and 4359 unexposed workers at a different plant, all working for ≥ 1 year (Section 2.1.1 for more details). Using Minnesota rates as the referent, the SMR for exposed workers was 0.81 (95% CI, 0.35–1.59; 8 deaths from liver cancer). When

estimated PFOA air exposure was divided into four quartiles, the SMRs for exposed workers versus the Minnesota population were 1.40, 0.86, 0.75, and 0.00, based on only 4, 2, 2, and 0 deaths from liver cancer, respectively. When exposed workers were compared with non-exposed workers, combined quartiles 1 and 2 of cumulative PFOA exposure showed a hazard ratio of 2.09 (95% CI, 0.69–6.31), whereas combined quartiles 3 and 4 had a hazard ratio of 0.67 (95% CI, 0.14–3.27).

Alexander et al. (2003) studied mortality in a cohort of 2083 production workers (145 deaths) who were exposed to PFOS at a plant in Decatur, Alabama, USA, that produced speciality films and fluorochemicals, and who had worked ≥ 1 year at the plant between 1961 and 1997 (Section 2.1.2). On the basis of only 2 deaths from biliary and liver cancer, these authors estimated an SMR for the entire cohort (using an Alabama referent) of 1.61 (95% CI, 0.20–5.82).

Steenland and Woskie (2012) studied mortality from liver and gall bladder cancer among 5791 workers exposed to PFOA at a polymer-production plant in Parkersburg, West Virginia, USA (Section 2.1.3). Compared with non-exposed workers at other plants within the same company, the authors found an SMR of 1.07 (95% CI, 0.51–1.96) based on 10 deaths from liver and gall bladder cancer. By quartile of estimated cumulative exposure, SMRs were 2.39 (95% CI, 0.65–6.13; 4 deaths), 0 (95% CI, 0–1.81; 0 deaths;), 2.01 (95% CI, 0.65–4.68; 5 deaths), and 0.32 (95% CI, 0.01–1.76; 1 death).

[The Working Group noted that, for all these occupational cohort mortality studies (Alexander et al., 2003; Steenland and Woskie, 2012; Raleigh et al., 2014), the numbers of deaths from liver cancer were too small to be informative.]

Eriksen et al. (2009) conducted a case–cohort study (67 patients with liver cancer and 782 cancer-free participants selected randomly from the full cohort) in a general population national cohort of 57 053 people in Denmark. Analyses of liver cancer incidence were done using baseline-measured plasma levels of both PFOA and PFOS (Section 2.1.4). All participants had no previous diagnoses of cancer at the beginning of follow-up. Follow-up for cancer patients ranged from 0 to 12 years (median, 7 years). Analyses of IRRs for liver cancer by quartile of PFOA, using quartile 1 as referent, were 1.00 (95% CI, 0.44–2.23), 0.49 (95% CI, 0.22–1.09), and 0.60 (95% CI, 0.26–1.37), respectively, based on 17, 17, and 16 cases, respectively. Corresponding IRRs for PFOS were 0.62 (95% CI, 0.29–1.33), 0.72 (95% CI, 0.33–1.56), and 0.59 (95% CI, 0.27–1.27). [The Working Group noted that the number of cases was larger in the case–cohort study by Eriksen et al. (2009) (67 cases) compared with other studies reporting on liver cancer after PFOA or PFOS exposure; however, this study was limited to some degree by low exposure contrasts for both PFOA and PFOS.]

Barry et al. (2013) analysed liver cancer incidence in a cohort of 32 254 participants with both low and high exposure to PFOA from drinking-water (with high exposure being similar to the high levels in occupational cohorts), who were living near the Parkersburg polymer-production plant in West Virginia, USA (Section 2.1.5). The median PFOA level measured in all cohort members in 2005–2006 was 26 µg/L [ng/mL], and the mean was 87 µg/L [ng/mL] (whereas in the USA the general population levels were about 4 µg/L [ng/mL] at the time). Approximately 12% of participants in this study had worked in the Parkersburg plant that was the source of the PFOA contamination. Cancer incidence was determined via interview with confirmation from medical records, or by matching to Ohio and West Virginia cancer registries. Liver cancer hazard ratios per unit natural log-transformed cumulative serum level were 0.73 (95% CI, 0.43–1.23) and 0.74 (95% CI, 0.43–1.26), based on 9 cases, for unlagged and 10-year lagged estimates, respectively. [The Working Group noted

that the exposure–response analysis was based on a continuous variable, with serum levels over time estimated by a model with good correlation (ρ = 0.71) to observed serum levels, which were available in 2005 or 2006 for all cohort members. However, the number of deaths from or number of cases of incident liver cancer in Barry et al. (2014) (8 cases) was too small to draw conclusions.]

Consonni et al. (2013) conducted an international cohort mortality study of male workers at six TFE-production sites, who were concomitantly exposed to APFO (or equivalently PFOA) (Spearman correlation, 0.72). [The Working Group noted that the high correlation between TFE and PFOA exposure precluded evaluation of the effects of the individual compounds (Section 2.1.6). At two plants there was also possible exposure to vinyl chloride, a liver carcinogen, but no details were given.] Restricting the cohort to workers who ever had exposure to APFO, the authors reported an SMR for liver and bile duct cancer (versus national rates) of 1.43 (95% CI, 0.57–2.94), based on 7 deaths from liver cancer. The authors reported a trend with increasing cumulative APFO exposure that was estimated on the basis of a JEM, with liver cancer SMRs for low-, medium-, and high-exposure groups of 0.70 (95% CI, 0.02–3.87; 1 death), 1.25 (95% CI, 0.15–4.52; 2 deaths), and 2.14 (95% CI, 0.58–5.49; 4 deaths) (*P* for trend, 0.24).

Girardi and Merler (2019) studied mortality among industrial workers who were exposed to very high levels of PFOA and, to a somewhat lesser extent, PFOS (Section 2.1.9). In a subsample of 120 workers for whom 696 serum samples were available, the geometric mean concentration of PFOA was 4048 ng/mL (a geometric mean of 8862 ng/mL was found in the subgroup of PFOA operators), whereas for PFOS it was 148.8 ng/mL. SMRs and risk ratios for liver cancer mortality in exposed workers (7 deaths) versus the general population (SMR, 2.32; 95% CI, 1.11–4.87) and versus non-exposed workers at another plant (risk ratio, 6.69; 95% CI, 1.71–26.2) were elevated. Relative to non-exposed workers (3 deaths), liver cancer mortality increased by estimated cumulative serum levels of PFOA by tertile, with risk ratios of 3.07 (95% CI, 0.31–30.0; 1 death), 8.39 (95% CI, 1.40–50.3; 2 deaths), and 9.28 (95% CI, 2.07–41.5; 4 deaths), by tertile of increasing serum level. Death from liver cirrhosis was also markedly elevated, based on 6 deaths (SMR, 1.71; 95% CI, 0.77–3.81; and risk ratio, 3.87; 95% CI, 1.18–12.7), compared with the unexposed workers cohort. [The Working Group noted that the authors suggested that the excess of cirrhosis could be due to high exposure to PFOA, as PFOA is a liver toxin. The Working Group noted the excess of cirrhosis could be a sign of confounding by alcohol, which is also associated with liver cancer.]

[The Working Group also noted that the number of liver cancer deaths in the occupational cohort mortality study by Consonni et al. (2013) was too small to be informative. The study by Girardi and Merler (2019) also had only a small number of cases, making it less informative, but was notable for the strong exposure–response relation, with very high PFOA exposure, and good exposure estimation.]

Li et al. (2022a) studied liver cancer incidence in Ronneby, Sweden, in 60 507 residents. One third of households were exposed to relatively high levels of both PFOS and, to a lesser extent, PFOA from drinking-water contaminated by nearby military firefighting operations (based on a subset with serum levels, PFOS being the most elevated). The authors were unable to separate exposures to different PFAS, particularly PFOS and PFHxS (Section 2.1.13). In a sample of 3084 Ronneby residents and 226 non-Ronneby residents, the geometric means for the high-exposure group in Ronneby (*n* = 2052) were 199, 176, and 11 ng/mL for PFOS, PFHxS, and PFOA, respectively. For men who never resided in a high-exposure area, the SIR for liver cancer (area surrounding Ronneby used as a reference) was 1.12 (95% CI, 0.72–1.66; 24 cases), and for women

it was 0.98 (95% CI, 0.45–1.86; 9 cases). For men ever living in a high-exposure area, the SIR was 1.52 (95% CI, 0.70–2.89; 9 cases) and for women the corresponding estimate was 1.52 (95% CI, 0.41–3.88; 4 cases).

[The cohort study in Ronneby, Sweden, by Li et al. (2022a) had a larger number of cases (*n* = 25) compared with other studies reporting on liver cancer after PFOA or PFOS exposure, but a limitation was the ecological assignment of exposure on the basis of residence, and some uncertainty regarding the role of PFOS versus that of PFHxS, another PFAS that was present at high levels in the drinking-water.]

Goodrich et al. (2022) conducted a nested case–control study of PFOA and PFOS (baseline measurements), and HCC not of viral origin, in a large multiethnic cohort, with 50 cases and 50 controls (Section 2.1.16). Geometric mean concentrations of plasma PFOA and PFOS did not differ between cases and controls, and the use of continuous measures of PFOA and PFOS did not show any statistically significant positive associations with liver cancer. When restricting the definition of exposure to above the 85th percentile for PFOS (54.9 ng/mL, which corresponded to the 90th percentile in NHANES) and PFOA (8.6 ng/mL), exposure was associated markedly with liver cancer for PFOS (OR for PFOS, 4.50; 95% CI, 1.20–16.00), but not for PFOA (OR for PFOA, 1.20; 95% CI, 0.52–2.80), after adjusting for the matching variables of age, sex, race or ethnicity, and study site. However further adjustment for BMI lowered the OR for high PFOS (> 54.9 ng/mL) relative to low PFOS to 2.90 (95% CI, 0.78–10.00).

(b) Case–control studies

Vieira et al. (2013) conducted two case–control studies of incident liver cancer among residents of 13 counties in Ohio and West Virginia, USA, which included both contaminated and non-contaminated water districts near the same Parkersburg polymer-production plant in West Virginia that was the source of contamination in the population studied by Barry et al. (2013) (see Section 2.1.22). In the first case–control study, cases and controls (all other cancer cases excluding kidney, pancreatic, testicular, and liver cancers) obtained from both Ohio and West Virginia cancer registries were compared with regard to residence in a contaminated or non-contaminated water district. The liver cancer OR for exposed water district residents was 1.1 (95% CI, 0.7–1.6; 23 exposed cases) versus residents in non-contaminated water districts. These authors also conducted a separate case–control study among Ohio residents; cases were participants with liver cancer and controls were participants with other cancers in the Ohio counties, again excluding kidney, pancreatic, testicular, and liver cancers. Exposure in the second study was based on estimated individual serum levels of PFOA at specific addresses at specific points in time. The methods for estimating individual serum PFOA levels from linked environmental, exposure, and toxicokinetics models are described in detail elsewhere (Shin et al., 2011a). The environmental models integrated facility emissions data; fate, and transport characteristics of PFOA; addresses of cases and controls; and hydrogeological properties of the study area to estimate PFOA air and water concentrations from 1951 through 2008. Exposure was the estimated individual serum level 10 years before the diagnosis dates for cases and controls. Relative to non-contaminated water districts (50 cases), the ORs for those with low, medium, and high exposure 10 years before diagnosis were 1.1 (95% CI, 0.4–3.1; 4 cases), 0.9 (95% CI, 0.3–2.5; 4 cases), and 1.0 (95% CI, 0.3–3.1; 3 cases), respectively (there were no cases among those with very high exposure).

Cao et al. (2022) studied 203 cases of incident liver cancer compared with 203 hospital-based controls in a hospital in China during 2019–2021. [The Working Group noted that the study did not mention how controls were matched.] Serum PFOA and PFOS were measured in study

participants. [The Working Group noted that the timing of collection with respect to cancer diagnosis was not reported.] In cases and controls combined, the mean serum PFOS and PFOA concentrations were 9.8 ng/mL and 8.3 ng/mL, respectively. Log-transformed PFOS (a continuous variable) was associated with liver cancer (OR, 2.609; 95% CI, 1.179–4.029), after adjustment for covariates, but log-transformed PFOA was not (OR, 1.036; 95% CI, 1.002–1.070). [The Working Group considered that the base of log transformation of PFOS was 10; however, it was not specified in the manuscript.]

[The Working Group noted that the three case–control studies had large numbers of cases but suffered from other limitations. The study by Vieira et al. (2013), which included two different case–control studies, had a fairly large number of cases in the first study (179 cases, 23 exposed) but was limited by an ecological assignment of exposure. The second case–control study in Vieira et al. (2013) had fewer cases (61 cases, 11 exposed), with better assessment of estimated individual exposure levels, but was again limited by small numbers of exposed cases. The two other case–control studies by Goodrich et al. (2022) and Cao et al. (2022) showed some positive associations but had their own limitations. In the nested case–control study by Goodrich et al. (2022), positive findings for PFOS were observed only when baseline plasma concentrations were dichotomized between the top 15% and the bottom 85% and were diminished after control for BMI. In the other case–control study by Cao et al. (2022), serum levels were available only at time of diagnosis, making etiological inference difficult.]

(c) Meta-analysis

Seyyedsalehi and Boffetta (2023) published a meta-analysis of liver cancer in relation to PFAS that included all the studies cited in the present monograph as well as two ecological studies judged to be not informative, and one other that was a predecessor of the study by Steenland and Woskie (2012) cited here [for those reasons, the Working Group did not considered this meta-analysis to be informative].

2.5.2 Pancreatic cancer

See Table S2.5 (Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636).

A total of nine studies investigating the association between PFAS (mainly PFOA) and cancer of the pancreas are presented below according to three different types of population: three studies among workers in chemical plants producing or using PFOA (Steenland and Woskie, 2012; Consonni et al., 2013; Raleigh et al., 2014), three from communities surrounding a plant from which there had been environmental release of PFOA and contamination of public and private water supplies (Barry et al., 2013; Vieira et al., 2013; Li et al., 2022a), and three from studies in the general population with background exposures (Eriksen et al., 2009; Winquist et al., 2023; Zhang et al., 2023).

Raleigh et al. (2014) investigated mortality and cancer incidence among APFO-production workers (n = 4668) compared with tape and abrasives production workers (n = 4359) in two manufacturing facilities owned by the same company in Minnesota, USA, between 1947 and 2002 (see Section 2.1.1). Hazard ratios for mortality using the unexposed workers as the referent were calculated for quartile-based categories of PFOA exposure created using a task-based JEM; the hazard ratios were 0.32 (95% CI, 0.08–1.35), 0.89 (95% CI, 0.34–2.31), 0.82 (95% CI, 0.32–2.12), and 1.23 (95% CI, 0.50–3.00) on the basis of 18 deaths from pancreatic cancer observed in all the exposed categories. Hazard ratios for incidence in exposed workers compared with unexposed workers were 0.13 (95% CI, 0.02–1.03; 1 case) for quartiles 1 and 2 combined and 1.36 (95% CI,

0.59–3.11; 9 cases) for the upper two quartiles combined (Raleigh et al., 2014).

Steenland and Woskie (2012) studied mortality among 5791 workers employed for ≥ 1 day at a polymer-production plant in Parkersburg, West Virginia, USA, between 1948 and 2002 (see Section 2.1.3). For exposed workers compared with other non-exposed workers at other plants within the same company and region, SMRs calculated for pancreatic cancer by quartile of cumulative serum PFOA level (estimated using a JEM) were 1.18 (95% CI, 0.32–3.03; 4 cases), 1.02 (95% CI, 0.28–2.61; 4 cases), 1.09 (95% CI, 0.35–2.54; 5 cases), and 0.92 (95% CI, 0.30–2.16; 5 cases) from the lowest to the highest quartile categories, respectively.

[Both of these occupational cohort studies (Steenland and Woskie, 2012; Raleigh et al., 2014) had the advantage of the ability to evaluate associations with PFOA in a population exposed to levels much higher than those in the general population; however, a major limitation for both was the small number of observed cases.]

Eriksen et al. (2009) conducted a case–cohort study within a prospective cohort of men and women from the general population in Denmark. Eligible participants were aged 50–65 years at enrolment. The investigators measured PFOA and PFOS concentrations in plasma samples collected before cancer diagnosis (Section 2.1.4). IRRs were calculated on the basis of 128 cases of pancreatic cancer and 772 subcohort participants and were adjusted for age, sex, smoking status, smoking intensity, smoking duration, dietary fat intake, and fruit and vegetable intake. IRRs for pancreatic cancer were 1.55 (95% CI, 0.85–2.80) and 0.91 (95% CI, 0.51–1.65) in the highest quartiles of plasma PFOA and PFOS concentration, respectively, compared with the lowest quartile. The IRR per increase in PFOA of 1 ng/mL was 1.03 (95% CI, 0.98–1.10) and that per increase in PFOS of 10 ng/mL was 0.99 (95% CI, 0.86–1.14). [The strengths of this study included a relatively large number of cases and adjustment for potential confounders such as smoking. Because the PFOA and PFOS measurements were from samples collected before diagnosis, concentrations were less likely to be influenced by the presence of cancer. However, a single measurement at enrolment may not reflect exposure at crucial windows in cancer development. Since the study was carried out among the general population with background exposure levels, exposure contrasts might be too small to detect an association.]

Within communities surrounding a plant from which there had been environmental release of PFOA and contamination of public and private water supplies, the C8 Science Panel (Section 2.1.5) conducted a cohort study of a total of 32 254 community residents and workers exposed to PFOA from a fluoropolymer-production plant in the Mid-Ohio Valley on the border of West Virginia and Ohio, USA (Barry et al., 2013). For community participants, annual estimates of cumulative serum PFOA concentrations were estimated from 1952 to 2011 using a model by Shin et al. (2011a, b). For the workers, estimates of occupational PFOA exposure were calculated as described in Woskie et al. (2012) and were combined with estimates of environmental exposure. Self-reported cancer according to the surveys administered in 2008–2011 was verified through medical records and cancer registry review. The hazard ratio per unit of natural log-transformed estimated cumulative PFOA serum concentration was 1.00 (95% CI, 0.78–1.29; 24 cases) after adjustment for time-varying smoking, time-varying alcohol consumption, sex, education, birth year (5-year calendar intervals), and age. [The strengths of this study included its use of individual-level exposure modelling using lifetime residential history, the validation of the exposure modelling, the wide range of PFOA exposure levels, and control for potential confounders such as smoking. The main limitation was the small number of cases of incident pancreatic cancer.

In addition, community members and workers who died before enrolment would not have been included, owing to the design of the study as a survivor cohort. This might lead to the potential underascertainment of cancers with a high fatality rate in this population. However, given that PFOA exposure was considered unlikely to be related to survival time, the impact of this aspect of the study design on the resulting risk estimates was likely to be minimal (Barry et al., 2015).]

Consonni et al. (2013) conducted a mortality study in the pooled international TFE cohort that included 5879 male workers who were ever employed or employed for a minimum of 6 or 12 months at one or more of six TFE-production sites in North America and Europe between 1950 and 2002 (see Section 2.1.6). Causes of death were ascertained from 1950 through 2008. Semiquantitative levels of work-related exposure to TFE and APFO were assessed by a plant- and job-specific exposure matrix with yearly estimates (in arbitrary units) of exposure from the start of TFE production until the end of 2002. Among the subset of workers who had ever been exposed to APFO (n = 4205), the SMR using national rates as a referent was 1.05 (95% CI, 0.51–1.94; 10 deaths). In addition, the SMRs for groups with low, medium, and high cumulative APFO exposure were 0 (0 deaths), 1.30 (95% CI, 0.35–3.33, 4 deaths), and 1.84 (95% CI, 0.67–4.00, 6 deaths), respectively (P for trend, 0.34). [Consonni et al. (2013) studied work-related exposure to TFE and/or APFO, and high correlations were observed between exposure to TFE monomer (IARC Group 2A; IARC, 2016) and PFOA, which precludes evaluation of effects of the individual compounds. This study observed fewer than 20 cases, and the small number was a major limitation.]

The Ronneby Register cohort (see Section 2.1.13 for details) comprised 60 507 individuals who had ever lived in the Ronneby municipality during the period when drinking-water was contaminated with a mixture of PFAS, mainly PFOS, PFHxS, and PFOA (1985–2013) (Li et al., 2022a). Cancer incidence data were obtained through linkage to the Swedish Cancer Register (1985–2016). SIRs for incident pancreatic cancer among residents who had ever lived in a highly exposed area were 0.46 (95% CI, 0.17–1.01; 6 cases) for men and 0.81 (95% CI, 0.39–1.50; 10 cases) for women, using the regional external population as the referent. Groups of residents who had ever lived in the contaminated area were subdivided by the number of years living at an ever-high area, and calendar year-, age-, and sex-adjusted hazard ratios compared the ever-high group to the never-high group comprising residents who had never lived in the contaminated area. Hazard ratios for this internal comparison were below unity. [The strengths of this study included the large general population sample with complete ascertainment and follow-up, owing to high-quality Swedish population registers with complete population coverage, and a strong documented exposure contrast. The limitations of this study were the mixed exposure profile without the possibility to single out effects caused by specific compounds, the small number of cases, and the lack of information on important confounders such as smoking. Additionally, SIRs from the external comparisons might be viewed as ecological comparisons.]

Zhang et al. (2023) conducted two independent nested case–control studies within the ATBC Cancer Prevention Study and the PLCO Cancer Screening Trial (Sections 2.1.11 and 2.1.19). Prediagnostic serum samples were measured for relative levels of PFOA and PFOS among 251 matched pairs from ATBC comprising male smokers aged 50–69 years at baseline (1985–1988) in Finland who were followed until December 2011, and 360 matched pairs from PLCO comprising men and women, mostly non-smokers, aged 55–74 years at baseline (1993–2001) in the USA who were followed until 15 May 2010. ORs for pancreatic ductal

adenocarcinoma were adjusted for age and date at blood draw, smoking, diabetes, and BMI. ORs were 2.37 (95% CI, 1.24–4.51) and 1.82 (95% CI, 0.82–4.03) in the highest quintiles of serum PFOA and PFOS concentrations, respectively, compared with the lowest quintile in ATBC. The ORs per SD increase were 1.27 (95% CI, 1.04–1.56) and 1.13 (95% CI, 0.88–1.45) for PFOA and PFOS, respectively. For PLCO, the ORs per SD increase were below unity for both PFOA and PFOS. ORs for only men who had ever smoked or were still in the habit of smoking were lower than those for all participants. [The strengths of this study included prediagnostic serum samples, the relatively large number of cases, and adjustment for potential confounders such as smoking. The limitations of this study included low-level exposure with a small exposure contrast. The Working Group noted that there was unexplained inconsistency between the results for the ATBC and the PLCO for male smokers only.]

Winquist et al. (2023) conducted a case–cohort study within the ACS CPS-II LifeLink Cohort (Section 2.1.21). Prediagnostic serum samples were collected during 1998–2001, and participants (median age, 70 years) were followed for cancer incidence until June 2015. Serum concentrations of PFAS were measured for 172 cases of pancreatic cancer and 999 subcohort participants, and hazard ratios were calculated with adjustment for age and year at blood draw, education, race or ethnicity, smoking, and alcohol use. Hazard ratios (95% CI) for pancreatic cancer per concentration doubling were 0.94 (95% CI, 0.74–1.21) and 0.87 (95% CI, 0.70–1.10) for PFOA and PFOS, respectively. In sex-specific analyses, hazard ratios per PFOA doubling were 0.71 (95% CI, 0.52–0.96) and 1.14 (95% CI, 0.78–1.67) for men and women, respectively, although similar hazard ratios for both sexes were observed for PFOS. [The strengths of this study included prediagnostic serum samples, the relatively large number of cases, and adjustment for potential confounders such as smoking. The study limitations included low-level exposure with a small exposure contrast. In addition, because of its design as a survivor cohort, this study would not have included some people who may have had PFOA- or PFOS-related cancer, especially those who developed cancers earlier in life in a susceptible exposed population. This survivor bias would have biased the results downwards (i.e. towards the null or even towards inverse associations).]

Vieira et al. (2013) conducted two case–control studies of 18 different incident cancers during the years 1996–2005 among residents of 13 counties in Ohio and West Virginia, USA, including both contaminated and non-contaminated water districts near the same polymer-production plant in Parkersburg, West Virginia, USA, that was the source of contamination in the population studied by Barry et al. (2013) (see Section 2.1.22). In the first case–control study, cases and controls (all other cancer cases excluding cancers of the kidney, liver, pancreas, and testis) were compared with regard to residence in a contaminated or non-contaminated water district. The OR for pancreatic cancer was 1.0 (95% CI, 0.8–1.3; 58 exposed cases) after adjustment for age, sex, diagnosis year, insurance provider, and smoking status. In the second case–control study, restricted to the Ohio data because of availability of geocoded street addresses, serum PFOA concentrations were estimated by environmental, exposure, and toxicokinetics models designed by Shin et al. (2011a, b). The ORs for pancreatic cancer in the low, medium, high, and very high exposure categories compared with the unexposed, calculated after adjustment for age, race, sex, diagnosis year, insurance provider, and smoking status, were 1.3 (95% CI, 0.7–2.3; 12 exposed cases), 0.9 (95% CI, 0.5–1.7; 10 exposed cases), 1.1 (95% CI, 0.6–2.3; 9 exposed cases), and 0.6 (95% CI, 0.1–2.5; 2 exposed cases), respectively. [The Working Group noted that the studies by Barry et al. (2013) and Vieira et al. (2013) were overlapping rather than independent studies in

that the same geographical areas and some of the same cases were included in both analyses, although the extent of overlap was unknown. The strengths of this study were the large number of incident cancers from cancer registries and the reasonably large number of exposed cases in the contaminated water districts. The second case–control study based in Ohio benefited from being able to estimate serum levels for individuals on the basis of a validated model. The limitations were the assignment of an ecological exposure (by water district) in the first case–control study and the somewhat arbitrary assumption in the second case–control study that the estimated serum levels 10 years before case diagnosis were the most relevant, as well as the assumption that cases and controls had remained in the same residence for 10 years. Additionally, the control group included cases of other cancers (bladder, brain, female breast, cervix, leukaemia, lung, melanoma, multiple myeloma, NHL, ovary, prostate, thyroid, and uterus), which may not be representative of the source population because of differences in lifestyle and socioeconomic status among cancer cases. In particular, it might bias estimates towards the null, if any of the included cancers were in fact associated with PFOA. Otherwise, the Working Group considered these potential differences in confounders to be unlikely to have substantive effects in this population with a very high exposure.]

2.5.3 Colorectal cancer and other cancers of the digestive tract (other than liver and pancreas)

See Table S2.5 (Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636).

Five occupational cohort studies (Alexander et al., 2003; Leonard et al., 2008; Lundin et al., 2009; Consonni et al., 2013; Steenland et al., 2015; Girardi and Merler, 2019) and three studies from communities surrounding a plant from which there had been environmental release of PFOA and contamination of public and private water supplies (Barry et al., 2013; Vieira et al., 2013; Li et al., 2022a) investigated the association between PFOA or PFOS (or both) and cancers of the colorectum and other digestive organs (oesophagus and stomach).

Olsen et al. (2004) studied workers at two manufacturing plants between 1993 and 1998 in Decatur, Alabama, USA. "Episode of care" was identified using health claim data between 1993 and 1998 and was compared between 652 workers at a fluorochemical-production plant (exposed group) and 659 workers at a film plant (non-exposed group). [Episode of care is not a definitive measure of risk because it could include cases of incident cancer, prevalent cancer, and tentatively diagnosed cancer. Mortality in the same company was reported in a study by Alexander et al. (2003) included in the present monograph. Therefore, the study by Olsen et al. (2004) was judged to be uninformative.]

Innes et al. (2014) conducted a cross-sectional study to examine the association between serum concentrations of PFOA and PFOS and self-reported colorectal cancer diagnosis, verified by chart review for 47 359 participants in a comprehensive health survey between 2005 and 2006 by the C8 Health Study Project. [Since the participants in this study overlapped with those in a cohort study by Barry et al. (2013), and since prevalent cases were used as the case group and the serum concentrations of these participants were influenced by the presence and/or treatment of cancer, the study was judged to be uninformative.]

Among the occupational cohort studies was a study by Lundin et al. (2009) of mortality among of 3993 workers at an APFO-production plant in Cottage Grove, Minnesota, USA, between 1947 and 1997, with follow-up until 2002 (see Section 2.1.1). Using rates for the state of Minnesota as a referent, SMRs were calculated

according to classification of jobs by exposure to APFO. For colon cancer, SMRs for "never", "ever probable/never definite", and "ever definite" exposure groups were 1.30 (95% CI, 0.75–2.12; 16 deaths), 0.88 (95% CI, 0.42–1.62; 10 deaths), and 1.07 (95% CI, 0.13–3.86; 2 deaths), respectively. For rectal cancer, SMRs for "never" and "ever probable/never definite" exposure groups were 0.40 (95% CI, 0.01–2.22; 1 death) and 1.28 (95% CI, 0.26–3.76; 3 deaths), respectively (0 deaths in the "ever definite" category). For oesophageal cancer, SMRs for "never", "ever probable/never definite", and "ever definite" exposure groups were 0.59 (95% CI, 0.07–2.13; 2 deaths), 0.31 (95% CI, 0.01–1.70; 1 death), and 1.54 (95% CI, 0.04–8.57; 1 death), respectively. For stomach cancer, SMRs for "never" and "ever probable/never definite" exposure groups were 0.74 (95% CI, 0.15–2.15; 3 deaths) and 1.06 (95% CI, 0.29–2.71; 4 deaths), respectively (0 deaths in the "ever definite" category).

Alexander et al. (2003) studied the mortality of a cohort of 2083 production workers who were exposed to PFOS at a plant in Decatur, Alabama, USA, that produced speciality films and fluorochemicals, and who had worked for ≥ 1 year at the plant between 1961 and 1997 (Section 2.1.2). Using rates for the state of Alabama as referent, SMRs for all cohort members were 0.30 (95% CI, 0.01–1.66; 1 death) for colon cancer and 1.76 (95% CI, 0.21–6.35; 2 deaths) for oesophageal cancer. In addition, the SMR for cohort members ever employed in a low-exposure job, but never a high-exposure job, was 1.43 (95% CI, 0.04–7.94; 1 death) for colon cancer.

Leonard et al. (2008) investigated mortality among 6027 workers exposed to PFOA at a polymer-production plant in Parkersburg, West Virginia, USA (see Section 2.1.3). Eligible workers were employed at the plant for ≥ 1 day between 1948 and 2002 and were followed for mortality from 1948 to 2002. SMRs were computed in comparison to the US population, the West Virginia state population, and an eight-state regional employee population from the same company on the basis of 17 deaths from colon cancer, 5 from rectal cancer, 4 from oesophageal cancer, and 3 from stomach cancer. SMRs estimated using three different reference populations were less than unity except for that for rectal cancer using the reference population of workers from the other regional facilities within the same company (SMR, [1.321]; 95% CI, [0.429–3.082]). Steenland and Woskie (2012) reported an extension of this study by an additional 6 years of follow-up and comprehensive quantitative exposure assessment. Steenland et al. (2015) conducted an incidence study of a subset of the PFOA-exposed workers (n = 3713) in Steenland and Woskie (2012). Rate ratios for quartiles of cumulative serum PFOA level estimated by JEM were calculated by adjusting for age, year of birth, sex, race, education, BMI, and time-varying smoking and alcohol consumption. Compared with those in the lowest quartile, the rate ratios in the second, third, and highest quartiles were 0.58 (95% CI, 0.18–1.87), 1.43 (95% CI, 0.49–4.19), and 1.20 (95% CI, 0.39–3.62), respectively, on the basis of 41 cases of incident colorectal cancer (P for trend, 0.68).

The C8 Health Study (Section 2.1.5) included a total of 32 254 community residents and workers exposed to PFOA from a polymer-production plant in the Mid-Ohio Valley, USA (Barry et al., 2013). Cumulative serum PFOA concentrations were estimated for community residents and workers, taking into account community and occupational exposure, and cancer diagnosis was assessed through self-reported questionnaire and validation through medical-record review and cancer registry data (Barry et al., 2013). Hazard ratios per unit natural log-transformed estimated cumulative PFOA serum concentration were 0.99 (95% CI, 0.92–1.07; 264 cases) for incident colorectal cancer, 0.96 (95% CI, 0.70–1.32; 15 cases) for incident oesophageal cancer, and 0.72 (95% CI, 0.45–1.14; 12 cases) for incident stomach cancer, after adjustment

for time-varying smoking, time-varying alcohol consumption, sex, education, birth year (5-year calendar intervals), and age.

In the pooled international TFE cohort study, follow-up was conducted for mortality (1950–2008) of 5879 male workers who were ever employed or employed for a minimum of 6 or 12 months at one of six TFE-production sites in North America and Europe between 1950 and 2002 (Section 2.1.6). Among the subset of workers who had ever been exposed to APFO (n = 4205), the SMR using national rates as the referent was 1.44 (95% CI, 0.72–2.57) for cancer of the oesophagus, whereas SMRs for cancers of the stomach, colon, and rectum were below or around unity (Consonni et al., 2013). In addition, SMRs for oesophageal cancer for groups of low, medium, and high cumulative APFO exposure were 1.62 (95% CI, 0.44–4.14; 4 deaths), 1.54 (95% CI, 0.42–3.93; 4 deaths), and 1.16 (95% CI, 0.24–3.39; 3 deaths) (P for trend, 0.60).

Girardi and Merler (2019) reported on mortality among 462 male employees who had worked for ≥ 6 months before 2009 at a factory manufacturing PFOA, PFOS, and other chemicals in Trissino, Veneto, Italy (Section 2.1.9). They were followed for mortality from 1970 to 2018. SMRs were calculated in comparison with the regional mortality rates, and mortality risk ratios were estimated by a Poisson regression model using rates from non-exposed workers in other plants. For colon cancer, the SMR was 1.72 (95% CI, 0.72–4.14) and the mortality risk ratio was 2.84 (95% CI, 0.74–10.9), based on 5 deaths; for oesophageal cancer, the SMR was 2.31 (95% CI, 0.68–6.50) and the mortality risk ratio was 3.62 (95% CI, 0.59–22.3), based on 3 deaths; and for stomach cancer, the SMR was 1.30 (95% CI, 0.42–4.02) and the mortality risk ratio was 2.43 (95% CI, 0.54–10.9), based on 3 cases.

[The strengths of the incidence study by Steenland et al. (2015) included use of estimated average annual serum PFOA concentrations and adjustment for several potential confounders such as smoking, alcohol drinking, and BMI. In contrast, the five occupational cohort studies of cancer mortality (Alexander et al., 2003; Leonard et al., 2008; Lundin et al., 2009; Consonni et al., 2013; Girardi and Merler, 2019) included small numbers of deaths (fewer than 17) and lack of adjustment for important confounders such as smoking, alcohol drinking, and BMI. The strengths of the study by Barry et al. (2013) included a relatively large number of cases of colorectal cancer and control for potential confounders such as smoking, but the small number of cases of oesophageal and stomach cancer was a limitation of this study. In addition, Consonni et al. (2013) studied work-related exposure to TFE and APFO and noted high correlations between exposure to TFE monomer (IARC Group 2A, IARC, 2016) and PFOA, which precluded evaluation of the effects of the individual compounds.]

The Ronneby Register cohort (Section 2.1.13) comprised 60 507 individuals who had ever lived in the Ronneby municipality during a period when drinking-water was contaminated with a mixture of PFAS, mainly PFOS, PFHxS, and PFOA (1985–2013), and incidence data were linked to Swedish Cancer Register (1985–2016) (Li et al., 2022a). Using the regional external population as the referent, SIRs for rectal cancer among residents who had ever lived in the contaminated area were 1.25 (95% CI, 0.89–1.69; 41 cases) for men and 1.33 (95% CI, 0.91–1.88; 32 cases) for women. SIRs for stomach cancer were 1.10 (95% CI, 0.70–1.64; 24 cases) for men and 1.03 (95% CI, 0.55–1.76; 13 cases) for women. For colon and oesophageal cancer, SIRs were below or around unity for both men and women. The group of residents who had ever lived in the contaminated area was subdivided by the number of years living at an ever-high area, and calendar year-, age-, and sex-adjusted hazard ratios were calculated, comparing the ever-high group with the never-high group of residents who had never lived in the contaminated area. Hazard ratios for

the ever-high group were 1.25 (95% CI, 0.95–1.64) for rectal cancer and 1.14 (95% CI, 0.79–1.66) for stomach cancer. In addition, hazard ratios for short-high (1–10 years) and long-high (≥ 11 years) exposure were 1.16 (95% CI, 0.80–1.69) and 1.34 (95% CI, 0.94–1.90) for rectal cancer, respectively, and 0.86 (95% CI, 0.51–1.46) and 1.56 (95% CI, 0.95–2.55) for stomach cancer, respectively. [The strengths of this study included the large general population sample with complete ascertainment and follow-up, owing to the high-quality Swedish population registers with complete population coverage, and the strong documented exposure contrast. The study limitations were the mixed exposure profile without the possibility to single out effects caused by specific compounds, the small numbers of cases, and the lack of information on important confounders such as smoking, alcohol drinking, and BMI. Additionally, SIRs from the external comparisons might be viewed as ecological comparisons.]

Vieira et al. (2013) conducted two case–control studies among residents of 13 counties in Ohio and West Virginia, USA (Section 2.1.22). In the first case–control study, after adjustment for age, sex, diagnosis year, smoking status, and insurance provider, the odds ratio for colorectal cancer was 0.9 (95% CI, 0.8–1.0; 383 exposed cases). In the second case–control study, restricted to the Ohio data, and after adjustment for age, race, sex, diagnosis year, smoking status, and insurance provider, ORs for colorectal cancer for the categories with low, medium, high, and very high exposure compared with the unexposed were 1.0 (95% CI, 0.8–1.3; 72 exposed cases), 0.9 (95% CI, 0.7–1.2; 64 exposed cases), 1.3 (95% CI, 1.0–1.7; 63 exposed cases), and 0.6 (95% CI, 0.3–1.0; 13 exposed cases), respectively. [The strengths of the study by Vieira et al. (2013) also included the large number of incident colon cancers from cancer registries and the reasonably large number of exposed cases in the contaminated water districts.]

2.6 Cancers of the brain and lymphatic and haematopoietic tissue

2.6.1 Cancers of the eye and brain, and other cancers of the nervous system

See Table S2.6 (Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636).

The Working Group identified four cohort studies and two case–control studies investigating the risk of brain cancer associated with PFOA or PFOS exposure. Two of the cohort studies included occupational cohorts (Lundin et al., 2009; Consonni et al., 2013), one of the cohort studies included the C8 Health Project cohort (Barry et al., 2013), and one of the cohort studies included the Ronneby Register cohort (Li et al., 2022a). The case–control studies were population-based (Vieira et al., 2013). In addition, the Working Group reviewed one case–control study on retinoblastoma (Chen et al., 2024).

(a) Cohort studies

A cohort study was conducted on mortality among 3993 employees of an APFO-manufacturing facility located in Cottage Grove, Minnesota, USA (Lundin et al., 2009) (see Section 2.1.1). During the follow-up until 2002, 807 decedents were identified. Using the rates for the state of Minnesota as the referent, SMRs were calculated for different jobs classified by exposure to APFO (the ammonium salt of PFOA). Only 7 deaths were observed for cancer of the central nervous system, with 5 deaths assigned to the "ever probable/never definite" exposure group and 2 deaths assigned to the "never" exposure group. The SMRs for the "ever probable/never definite" exposure group and the "never" exposure group were 1.16 (95% CI, 0.37–2.70) and 0.44 (95% CI, 0.05–1.59), respectively. [The Working Group noted that the significant limitations of

the study included the small occupational cohort with a limited number of deaths and the crude exposure assessment by job classification, which made this study uninformative for cancers of the central nervous system.]

Barry et al. (2013) focused on PFOA exposure and incident cancers among community residents and workers exposed to PFOA from a chemical plant, using the C8 Health Project cohort in combination with the cohort of workers from the polymer-production plant in Parkersburg, West Virginia, USA (see Section 2.1.5). The study population comprised 28 541 community members and 3713 workers, with 32 254 participants in the entire cohort. Cancer cases were captured by self-report by the participant and confirmed by medical chart review or state cancer registry matching in Ohio and West Virginia. The number of reported cases of primary brain cancer was 33. The analysis included 17 cases of validated primary brain cancer for whom there was complete covariate information. The authors calculated cumulative PFOA serum concentration estimates for each community participant on the basis of regional historical data. For participants who had ever worked in the polymer-production plant in Parkersburg, a JEM was applied to estimate occupational exposure levels and combined with estimated serum levels from residential exposure to contaminated drinking-water. A proportional hazards regression model was applied in a stratified analysis adjusting for age, time-varying smoking, time-varying alcohol consumption, sex, education, and birth year. Risk estimates based upon models in which exposure was unlagged or lagged 10 years were similar. The hazard ratios for a 1-unit increase in natural log-transformed cumulative exposure in relation to brain cancer were 1.13 (95% CI, 0.84–1.51) for unlagged exposure and 1.06 (95% CI, 0.79–1.41) for exposure lagged by 10 years in the whole cohort. For community residents, increased exposure to PFOA was associated with a slightly increased risk of brain cancer (HR, 1.14; 95% CI, 0.78–1.65; 13 cases), whereas for the workers, there was no clear evidence of a trend in risk of brain cancer (HR, 0.82; 95% CI, 0.26–2.59; 4 cases). [The Working Group noted as strengths the large cohort, strong exposure contrast, assessment of individual cumulative PFOA exposure, and lagged analyses. Limitations included the self-reported cancer cases, no evaluation of co-exposure to other PFAS in residents, and wide confidence intervals in the estimate for occupational workers.]

The association between occupational exposure to PFOA and mortality from brain cancer was investigated in the pooled international TFE worker cohort, in which data were pooled from workers from one or more of six TFE-production sites in North America and Europe (Consonni et al., 2013) (see Section 2.1.6). The epidemiology departments or the local health unit performed ascertainment of vital status and cause of death through record linkage or individual follow-up procedures. Exposure assessment was performed by a personal semiquantitative estimate using a JEM. There were 4 cases of brain cancer among 4205 men who had ever been exposed to PFOA, and the SMR for brain cancer associated with exposure to PFOA was 0.64 (95% CI, 0.17–1.63), using national rates as the referent. [The Working Group noted as strengths the inclusion of all TFE-production sites worldwide, and the complete enrolment and follow-up data. Limitations included the high correlations between TFE and PFOA exposure and the small number of cases of brain cancer observed, which limited the informativeness of this study.]

Li et al. (2022a) investigated cancer incidence in the Ronneby Register cohort, a community of residents with high-level environmental exposure to a mixture of PFAS, in Sweden. By the end of the follow-up (31 December 2016), the study had identified 150 cases of incident brain cancer (80 men and 70 women) (see Section 2.1.13). All information on brain cancer diagnosis was

obtained from the nationwide Swedish Cancer Register. To facilitate comparison, Ronneby residents were assigned to mutually exclusive groups, "never-high" and "ever-high", based on whether they were exposed to PFAS-contaminated water at their residence. When comparing the study population to the general population of Blekinge County excluding Ronneby, the incidence of brain cancer was increased in the "ever-high" group among men (SIR, 1.29; 95% CI, 0.83–1.93) but decreased in the "never-high" group among women (SIR, 0.73; 95% CI, 0.55–0.96). In internal comparisons, the "ever-high" group was further subdivided by the time period of high exposure ("early-high" in 2004 or earlier, "late-high" in 2005 or later) and duration of time in a high-exposure area ("short-high" for ≤ 10 years, and "long-high" for ≥ 11 years). Hazard ratios for early-high and late-high were 1.20 (95% CI, 0.78–1.84) and 1.31 (95% CI, 0.76–2.26), respectively, and those for short-high and long-high were 1.06 (95% CI, 0.66–1.69) and 1.50 (95% CI, 0.92–2.44), respectively. [The Working Group noted as strengths the large study population, strong exposure contrast, and unbiased inclusion. The main limitations included the small number of cases, the crude exposure assessment (not including individual water intake or other sources of exposure than drinking-water), the mixed exposure profile, and the limited information on potential confounders such as smoking habits, BMI, and occupational exposure.]

(b) Case–control study

A case–control study was conducted using cancer registry data for residents of counties in Ohio and West Virginia surrounding the polymer-production plant in Parkersburg, West Virginia, USA, from which PFOA had been emitted into drinking-water sources (Vieira et al., 2013) (see Section 2.1.22). The study included incident cancer cases drawn from registry data from 1996 through 2005. Controls comprised all other cancers in the study data set, except cancers of the kidney, pancreas, testis, and liver. There were 506 cases of brain cancer in the final data set, of which 150 came from Ohio. All people with a cancer diagnosis were classified as exposed (living within a contaminated water district) or unexposed (not living in contaminated water districts) using geocoding. The AORs varied among the water districts exposed to contaminated water, with the AOR for the overall exposure risk being 1.0 (95% CI, 0.8–1.3). In a second case–control study, the authors restricted the analysis to Ohio participants for whom annual PFOA serum concentrations could be estimated on the basis of an existing PFOA exposure prediction model. Individual-level annual exposure was categorized as very high, high, medium, low, and unexposed. Using the unexposed group as the reference category, the AORs for high, medium, and low individual-level exposures were 0.6 (95% CI, 0.2–1.6), 1.8 (95% CI, 1.1–3.2), and 1.5 (95% CI, 0.8–2.7), respectively. No cases of brain cancer occurred in the group with very high exposure. Findings were similar in various sensitivity analyses (e.g. using cumulative PFOA serum exposure instead of annual exposure; using exposure level for exposure estimates that did not account for latency; including cases of kidney, liver, pancreatic, and testicular cancer in the control group). [The Working Group noted that the strengths of the study included its focus on a population with high PFOA exposure, the strong contrast in exposure levels, and the estimation of individual-level exposure for a subset of the population. Limitations included the use of other cancers as the reference group and the potential for exposure misclassification (reliance on the address at the time of diagnosis rather than a complete residential history in analyses among Ohio participants). However, both these limitations would be expected to have resulted in a bias towards the null.]

A population-based case–control study was conducted that included 501 children aged < 5 years with a diagnosis of retinoblastoma

between 1983 and 2013 identified and randomly selected from the California Cancer Registry (Chen et al., 2024). Controls (n = 899) were selected from California birth rolls and frequency-matched to cases on year of birth. For cases and controls, neonatal dry blood samples were available, collected from the newborn heel-stick test, which is done 12–48 hours after birth for neonatal genetic screening. This sampling is a routine procedure, for > 99% of all neonates in California, and samples are stored by the California Newborn Screening Program for genetic disease. The blood spot was used for quantification of PFOA, PFOS, and PFNA. Outliers for PFAS measurement identified through a principal component analysis were excluded (n = 10), leaving a total of 497 cases and 893 controls for the analysis. Children with a PFOA concentration above the mean, compared with those with a concentration below the mean, had a higher risk of retinoblastoma (OR, 1.16; 95% CI, 0.90–1.50), particularly so for those born from US-born mothers (OR, 1.41; 95% CI, 1.00–2.02). Children with a neonatal heel-stick PFOS concentration of above the mean, compared with those with a concentration of below the mean, had a 29% higher risk of retinoblastoma (OR, 1.29; 95% CI, 1.00–1.67), with risk being elevated in both US-born and Mexico-born mothers. When restricting to unilateral retinoblastoma cases, the OR for a PFOS concentration of above the mean versus below the mean was 1.42 (95% CI, 1.03–1.97), whereas for bilateral retinoblastoma cases the OR was 1.14 (95% CI, 0.82–1.62). [The Working Group noted the limited sample size for the stratified analysis by mother's birthplace. The population-based design and the use of prediagnostic samples collected for medical reasons unrelated to the case status was a strength of this study, since it minimized selection bias and provided a measurement of PFOA and PFOS exposure unrelated to diagnosis or treatment. Such measurements are probably representative of fetal exposure; however, uncertainty remained concerning the capture of the relevant window of exposure for cancer development. In addition, PFAS were measured by a semiquantitative non-targeted method, which limited comparability across studies.]

2.6.2 Cancers of lymphatic and haematopoietic tissue and other cancers

See Table S2.6 (Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636).

Five occupational cohort studies have investigated mortality for cancers of lymphatic and haematopoietic tissue, melanoma, lung, or mesothelioma (Alexander et al., 2003; Leonard et al., 2008; Lundin et al., 2009; with later follow-up of mortality for selected cancers by Steenland and Woskie, 2012; Consonni et al., 2013; Steenland et al., 2015, Girardi and Merler, 2019). Two cohort studies (Barry et al., 2013; Li et al., 2022a) and one case–control study (Vieira et al., 2013, partly overlapping with Barry et al., 2013) addressing highly exposed community residents have investigated the incidence of cancers of lymphatic and haematopoietic tissues and melanoma according to PFOA and/or PFOS exposure. A large US case–cohort study of the general population with low background exposure examined a range of cancers of lymphatic and haematopoietic tissue (Winquist et al., 2023). A small case–control study with cross-sectional sampling of exposure data by Lin et al. (2020) examined associations between germ cell tumours in preschool children and maternal serum concentrations of PFOA and PFOS. [The Working Group noted the unclear methods used for control selection and some very high PFOA measurements that were not discussed. This study was considered uninformative for the evaluation of human cancer hazard and was not considered further.]

Lundin et al. (2009) conducted a mortality study among 3993 workers at an APFO-production plant in Cottage Grove, Minnesota, USA, between 1947 and 2002 (see Section 2.1.1). Using the rates for the state of Minnesota as the referent, SMRs were calculated according to classification of jobs by exposure to APFO (the ammonium salt of PFOA) in three categories: never, ever probable/never definite, and ever definite. Of 29 deaths from cancers of lymphatic and haematopoietic tissue, only one was among those definitely exposed to APFO (SMR, 0.37; 95% CI, 0.01–2.08) and 14 were probably, but never definitely exposed (SMR, 0.96; 95% CI, 0.53–1.61). None of the 3 deaths from lymphosarcoma-reticulosarcoma or 13 deaths from other lymphatic and haematopoietic cancers were among the definitely exposed, but 2 deaths (SMR, 1.80; 95% CI, 0.22–6.51) and 5 deaths (SMR, 0.71; 95% CI, 0.23–1.66), respectively, were among the probably, but never definitely, exposed for these cancer types. The single death from Hodgkin lymphoma was observed in the group of workers who had never been exposed. For leukaemia, 7 of 12 deaths were among the probably but never definitely exposed (SMR, 1.27; 95% CI, 0.51–2.61) with only 1 death among the definitely exposed (SMR, 0.96; 95% CI, 0.02–5.34). [The Working Group noted that this study was focusing on mortality, and its informativeness with respect to specific, relatively rare, cancers was limited by the small numbers and the crude exposure assessment that did not allow for analysis of cumulative exposure or lagged analyses.]

Alexander et al. (2003) studied mortality from cancers of lymphatic and haematopoietic tissues combined and melanoma between 1961 and 1998 among 2083 workers enrolled from 1961 through 1997 at the PFOS facility in Decatur, Alabama, USA (see Section 2.1.2 for a full description). The median duration of follow-up was 25.9 years, and a total of 4 deaths from lymphatic and haematopoietic cancer, 3 deaths from melanoma, and 15 deaths from respiratory system cancers were identified. Workers were classified as highly exposed to PFOS (and PFOA) according to a company-specific JEM based upon a survey of PFOS serum measurements and included a subset of workers in the chemical division, whereas workers in the film-producing division were unexposed at work. The geometric mean serum PFOS concentration for chemical division employees was 0.9 ppm [900 ng/mL] and for film division employees it was 0.1 ppm [100 ng/mL]. Using the Alabama state population as the referent, SMRs for lymphatic and haematopoietic cancers were not increased in the entire cohort including both chemical and film divisions, or in ever potentially highly exposed employees (Table S2.6, Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636). The SMR for melanoma was increased in both the entire cohort and exposed employees, but estimates were based on few (≤ 3) exposed cases. Mortality was not elevated from respiratory system cancers (all trachea, bronchus, and lung) overall or in any exposure category.

[The Working Group noted that this study had complete data for a highly exposed occupational cohort with long follow-up, but numbers of less-frequent cancers, such as cancers of lymphatic and haematopoietic tissue and melanoma, were low and did not allow estimation of an association with PFOS with reasonable precision. The exposure assessment was rather crude, without assessment of cumulative exposure, and co-exposures to potential carcinogens and other fluorochemicals were likely. Therefore, the Working Group considered that this study provided limited information for the evaluation of cancers of lymphatic and haematopoietic tissue, melanoma, or respiratory system cancers.]

Leonard et al. (2008) studied mortality from several specific cancers of lymphatic and haematopoietic tissue and from melanoma among 6027 workers (men, 81%) who had ever worked at the polymer-production facility in

Parkersburg, West Virginia, USA, between 1948 and 2002. With follow-up until 2002, mortality was not elevated for melanoma (3 deaths) for workers versus any of the three reference groups considered. A later follow-up until 2008 was published by Steenland and Woskie (2012) (see Section 2.1.3 for a full description). Only the latest follow-up data for NHL, leukaemia, lung cancer, and mesothelioma are reported here (Steenland and Woskie, 2012). The latest follow-up for melanoma is also reported in Table S2.6, as in Steenland et al. (2015). The mean follow-up was 30 years, and 14, 14, 84, and 6 deaths from NHL, leukaemia, lung cancer, and mesothelioma, respectively, were observed. SMRs were computed using the US population and an eight-state regional employee population from the same company (other workers in the same company and region) as referents.

Compared with other workers in the same company and region, increases were not observed for NHL (SMR, 1.05; 95% CI, 0.57–1.76), leukaemia (SMR, 1.05; 95% CI, 0.57–1.76), or lung cancer (SMR, 0.78; 95% CI, 0.62–1.64) in the PFOA-exposed cohort. SMRs according to individual cumulative PFOA exposure estimates without a lag did not indicate dose–response associations for these causes of death. Mortality from mesothelioma was elevated in the cohort (SMR, 2.85; 95% CI, 1.05–6.20), especially in the fourth quartile of cumulative PFOA exposure (5 deaths).

[The Working Group noted that this was the largest of the three US occupational PFAS cohorts (partly because there was no restriction with respect to duration of employment) and was characterized by a high degree of completeness of case ascertainment and cohort follow-up. A major strength of the updated follow-up was estimation of individual cumulative serum PFOA levels. The magnitude of occupational exposure to suspected or known human carcinogens such as asbestos was not quantified, but some co-exposure could not be ruled out.]

Barry et al. (2013) evaluated the risk of cancers of lymphatic and haematopoietic tissue and of melanoma in 28 541 community residents in the Mid-Ohio Valley, USA, who were exposed to PFOA in drinking-water as a result of emissions from the polymer-production plant in Parkersburg, West Virginia, and in 3713 employees working at this plant (a total of 32 254 individuals; men, 46%) (see Section 2.1.5). The average duration of follow-up after age 20 years was 33 years and during this period 66, 136, 241, and 108 cases of incident leukaemia, lymphoma, melanoma, and lung cancer, respectively, were identified. Adjusted hazard ratios for selected cancers of lymphatic and haematopoietic tissue and melanoma were computed by proportional hazard regression by estimated cumulative PFOA exposure (continuous variable). The risk of leukaemia, lymphoma (type not specified), melanoma, and lung cancer did not increase with increasing estimated cumulative exposure (Table S2.6, Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636). Risk estimates based upon models where exposure was unlagged, lagged 10 years, or lagged 20 years were similar (data for a 20-year lag were not reported in the manuscript). Results based on all self-reported cancer cases were similar to those for based on validated cases only (the results of the analysis using validated cases only were not reported in the manuscript). [The Working Group noted that these findings in this large community cohort with individual assessment of cumulative exposure did not consistently indicate that PFOA is associated with increased risk of these cancers at exposure levels encountered in a community with mainly high environmental exposure.]

Consonni et al. (2013) investigated cause-specific mortality rates in an international occupational cohort of 5879 male TFE workers of whom 4205 were also exposed to APFO (see Section 2.1.6). An individual semiquantitative

estimate of cumulative TWA airborne exposure was assigned from a study-specific JEM. In total, 49 deaths from lung cancer and 19 deaths from lymphatic and haematopoietic cancer were identified during follow-up from 1950 to 2008 in workers who had ever been exposed to APFO.

Using national rates as the referent, SMRs for lymphatic and haematopoietic tissue cancers combined, for NHL, and for multiple myeloma were not elevated in male workers who had ever been exposed to APFO. Mortality from leukaemia was increased (SMR, 1.61; 95% CI, 0.88–2.88), but without indications of increasing risk with increasing cumulative exposure. Lung cancer mortality was lower in workers than in the reference population (SMR, 0.73; 95% CI, 0.54–0.97).

[The Working Group noted that this cohort included all TFE-production sites worldwide during the entire period of production and benefited from almost complete enrolment and follow-up data. The informativeness of this study was limited. Internal analyses were not performed. Analyses stratified by level of cumulative exposure only included few cases (e.g. 3 or 4 cases of leukaemia in tertiles of cumulative exposure).]

Girardi and Merler (2019) reported mortality from cancers of lymphatic and haematopoietic tissue and lung in 1970–2018 among 462 male employees enrolled from 1960 through 2008 at a factory manufacturing PFOA, PFOS, and other chemicals in Trissino, Veneto, Italy (see Section 2.1.9 for details). A cohort of railroad workers from the geographical region constituted the reference group. For the factory-worker and railroad-worker cohorts, the mean duration of employment was 12.5 and 9.7 years, respectively, and the mean length of follow-up was 31.7 and 34.3 years, respectively. Loss to follow-up was < 3%. The geometric mean for PFOA was 4048 ng/mL, highest among PFOA operators (geometric mean, 8826 ng/mL; range, 335–86 300 ng/mL).

Using both regional and reference factory data as referents, mortality from cancers of lymphatic and haematopoietic tissue (7 deaths) was increased in the entire factory-worker cohort and increased with increasing estimated cumulative level of PFOA exposure, as indicated by tertile analysis (Table S2.6, Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636). Mortality from lung cancer (6 deaths) was not elevated in the factory-worker cohort compared with either reference group.

[The Working Group noted that this study had the advantage of complete data for an occupational cohort with high-level exposure, long follow-up, biological monitoring data, and estimates of cumulative exposure to PFOA. Subsets of employees seemed to have the highest recorded levels of PFOA among the available PFAS cohorts so far. Major limitations were that few samples were available to model some job categories; the small size of the factory-worker cohort with only 7 deaths from cancers of lymphatic and haematopoietic tissue among 462 employees followed for about 32 years, resulting in imprecise confidence intervals; inability to distinguish the effects of different PFAS compounds and other potential carcinogenic co-exposures; and limited confounding control. Factory workers were exposed to several chemicals in addition to PFOA and PFOS.]

Li et al. (2022a) reported sex-stratified risk estimates for the most common specific cancers of lymphatic and haematopoietic tissue, melanoma, and lung cancer among 60 507 residents (15 811 highly exposed; men, 52%) of Ronneby municipality, Sweden, reporting on follow-up from 1985. PFOA constituted only a minor proportion of the PFAS (mainly PFOS and PFHxS) that contaminated the drinking-water (for details, see Section 2.1.13).

The SIR, adjusted for sex, age and calendar year, for NHL was not increased in male or female

Ronneby residents who had ever been exposed to highly contaminated drinking-water, but the internal analysis within the Ronneby municipality revealed an elevated hazard ratio for residents with high-level exposure for > 10 years and for residents with high-level exposure during the latest period, where contamination levels were assumed to be higher, relative to residents who had never been exposed to highly contaminated water. The latter risk estimates were uncertain, with broad confidence intervals including unity.

The SIRs for multiple myeloma and chronic lymphocytic leukaemia were not increased in residents who had ever been exposed (men or women), and no consistent increase in risk was seen according to time or duration of exposure in residents ever living in a highly contaminated district. The SIR for chronic myeloid leukaemia was increased in Ronneby residents with low-level (only men) or high-level exposure, but numbers were low and precluded more detailed analysis.

The SIR for melanoma was increased in Ronneby residents with low-level (only men) or high-level exposure, and internal analysis indicated higher risk among residents with high-level exposure for > 10 years and especially for residents with high-level exposure during the latest period (HR, 1.54; 95% CI, 1.09–2.19).

[The Working Group noted that major strengths included complete registration of the cohort, no loss to follow-up, and a long follow-up period. Major limitations were the crude ecological exposure assessment, without individual estimates related to PFOS exposure.]

Winquist et al. (2023) conducted a case–cohort study within the ACS prospective CPS-II LifeLink Cohort, with measurement of PFOA, PFOS, and several other PFAS in prediagnostic serum samples collected during 1998–2001. Overall, there was no increase in the incidence of lymphatic and haematopoietic cancers associated with serum PFOA or PFOS serum concentrations. [The Working Group noted several strengths, including the case–cohort design, large sample size, good cancer ascertainment via registries and examination by histological subtype, and prediagnostic serum samples. Limitations were mainly the low exposure levels, narrow exposure contrast, and probable attenuation of risk estimates because of delayed blood sampling relative to time of enrolment.]

Vieira et al. (2013) conducted a case–control study to investigate the risk of 18 cancers in a community sample with relatively high exposure to PFOA because of contamination of drinking-water by the polymer-production plant in Parkersburg, West Virginia, USA. Using all other cancers except kidney, testicular, liver, and pancreatic as controls, odds ratios were estimated for exposed versus unexposed and for subsets of exposed across districts (see Section 2.1.18).

The odds of NHL were elevated among exposed residents in contaminated water districts (152 cases) relative to the unexposed, but the excess was limited to the very-high and medium exposure categories. Leukaemia (72 exposed cases), multiple myeloma (36 exposed cases), and melanoma (168 exposed cases) were not associated with exposure in contaminated water districts, and the odds of these cancers did not increase with increasing exposure category.

Lung cancer (632 exposed cases) was associated with exposure to contaminated water (OR, 1.2; 95% CI, 1.1–1.3), but the elevation was observed only in the high-exposure category and not in the very-high exposure category.

[The Working Group noted that this was a relatively large study population with a strong exposure contrast and with estimates of individual-level exposure for a subset of the population. Limitations included the use of other cancers as controls, which may cause bias towards the null if PFAS exposure is a risk factor for the cancers in the control group, or the opposite if PFAS exposure is associated with risk factors – for instance, smoking and alcohol consumption – that are probably more prevalent in the cancer controls than in the background population.

Individual-level exposure misclassification was most likely to be independent of the cancer outcome, with probable bias towards the null as a result.]

2.7 Cancer of all sites combined

See Table S2.7 (Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636).

There were seven cohorts that contributed evidence on PFOA and/or PFOS exposure and the risk of cancer overall. Five of these were occupational cohorts that used JEMs to estimate exposure and were focused primarily on men and on cancer mortality. In contrast, Li et al. (2022a) examined overall cancer incidence in residents with high environmental exposure, and Wen et al. (2022) evaluated serum PFOA and PFOS levels in relation to mortality using NHANES, which was more representative of exposure levels in the general US population. All these studies used data linkages to ascertain cancer outcomes, most commonly using information from death certificates. [The Working Group noted that although this approach had the benefit of often providing very complete outcome data, the focus on cancer mortality did not provide much information on the relation between PFAS exposure and specific cancers that have a longer survival time after diagnosis. Additionally, the studies in this section considered all cancer diagnoses as a single outcome, a heterogeneous category that may mask important associations with individual cancer outcomes.]

Raleigh et al. (2014) evaluated overall cancer mortality in an occupational cohort that included 4668 workers exposed to PFOA at an APFO factory in Cottage Grove, Minnesota, USA, between 1947 and 2002 and a comparison group of 4359 employees who were unexposed workers at a tape and abrasive production facility in Saint Paul, Minnesota (see Section 2.1.1, PFOA-production workers). Individual inhalation exposure was estimated using a JEM created from expert evaluation and industrial hygiene data. Mortality information was obtained from the NDI. There were 332 cancer deaths identified among the exposed workers. Overall cancer mortality for individuals working at the exposed plant (Cottage Grove, SMR, 0.87; 95% CI, 0.78–0.97) was lower than that for workers at the unexposed location (Saint Paul, SMR, 1.04; 95% CI, 0.95–1.13). Higher APFO exposure was not associated with a higher SMR for overall cancer mortality (quartile 4, SMR, 0.92; 95% CI, 0.71–1.16). [The Working Group noted that although this study had individual cumulative air exposure assessment with some evidence of this exposure metric being correlated with serum level, unlikely co-exposure to TFE, and a relatively higher number of overall cancer mortality cases compared with the individual cancer types, the heterogeneous nature of the outcome limited the inference from these findings. The study also lacked data on workers who left Minnesota or Wisconsin or on potential confounding factors such as smoking, which, if associated with occupational exposure, may have led to residual confounding.]

Alexander et al. (2003) studied a population of 3512 PFOS-exposed production workers at a plant in Alabama, USA (see Section 2.1.2, PFOS-production workers). 2083 participants were identified who had worked for ≥ 1 year between 1961 and 1997. Mortality follow-up was conducted using linkage to the NDI. The individual's PFOS exposure was estimated on the basis of job history and information from a subset (n = 232) for whom blood samples had been collected in 1998 and PFOS levels measured. Based on this subset, all workers were categorized according to their possible exposure (no workplace exposure, low potential exposure, or high potential exposure). Of the 2083 workers who met the criterion of working ≥ 1 year at the plant, 39 cancer deaths (total deaths, 145) were observed. The SMR for all

cancer deaths was 0.72 (95% CI, 0.51–0.98). It was similar when limiting to employees who were ever employed in a high-exposure job (SMR, 0.84; 95% CI, 0.50–1.32; 18 deaths), as well as for those who were ever employed in a low-exposure job but never in a high-exposure job (SMR, 0.52; 95% CI, 0.19–1.14), or those who worked in a high-exposure job for ≥ 1 year (SMR, 0.84; 95% CI, 0.46–1.41). [The Working Group noted that this study used a JEM informed by a subset of workers with blood measurements and had a strong exposure contrast but few deaths and no cancer incidence data. This study evaluated mortality using an NDI linkage, which would have underestimated associations with specific cancer types that have more favourable survival after diagnosis. The heterogeneous nature of the outcome limited the inference from these findings. Furthermore, the study was conducted predominantly in men and had limited control for confounding by factors such as smoking, which, if associated with occupational exposure, may have led to residual confounding.]

Steenland and Woskie (2012) conducted a mortality study among a cohort of PFOA-exposed workers at the polymer-production plant in Parkersburg, West Virginia, USA (see Section 2.1.3). There were 5791 workers who were employed for ≥ 1 day between 1948 and 2002 and who had sufficient work histories to allow for estimation of PFOA exposure. PFOA exposure was estimated using information on work history and from a subset with serum PFOA measurements. This cohort was highly exposed, with estimated serum PFOA concentrations that were two orders of magnitude higher than those in the general population. SMRs were calculated comparing workers in the cohort to workers at other factories in the same company in a similar geographical region and to the general US population. A total of 1084 deaths were observed during follow-up from 1952 to 2008; of these, 304 were determined to be cancer-related, ascertained via linkage to the NDI or from death certificate data. Relative to workers at other factories within the same region and company, mortality for all cancer types in participants in the Parkersburg polymer-production plant cohort was not elevated overall or when considering quartiles of estimated exposure to PFOA (e.g. quartile 4, SMR, 0.94; 95% CI, 0.76–1.16). The consideration of either a 10-year lag (e.g. quartile 4, SMR, 0.92; 95% CI, 0.73–1.15) or 20-year lag (data not reported) did not alter conclusions. [The Working Group noted that this study included a highly exposed cohort with long follow-up period and used a comparison group that included other workers, which may have attenuated any healthy-worker effect. Another strength of the study was the detailed exposure assessment using an enhanced JEM with serum exposure levels based on measurements from workers. However, the study did not evaluate the incidence of all cancers combined. Using cancer mortality data from linkages may underestimate associations with specific types of incident cancer, particularly those with more favourable survival after diagnosis. The heterogeneous nature of the outcome was a main limitation. There was limited control for confounding, which could have led to residual confounding if lifestyle factors such as smoking were related to occupational status in the cohort.]

Consonni et al. (2013) evaluated cancer mortality in a cohort of workers who were employees at six TFE-production sites in North America and Europe and were exposed to APFO (the ammonium salt of PFOA) as part of the manufacturing process between 1950 and 2002 (see Section 2.1.6). Job-specific exposure matrices based on the potential for exposure were used to estimate semiquantitative exposure to both TFE and PFOA. Vital status was obtained until 2008 using a variety of methods and linkages across the various geographical locations where the factories were located. Among 4205 workers who had ever been exposed to APFO, there were a total of 534 deaths, including 159 deaths from cancer. Overall, there was no association

between all-cancer mortality and cumulative estimated APFO exposure, when comparing with a national referent (e.g. highest cumulative exposure, SMR, 0.78; 95% CI, 0.59–1.02). Co-exposure to high levels of TFE and high levels of APFO was also not associated with an elevated SMR (0.81; 95% CI, 0.60–1.06). [The Working Group noted that although this study was a comprehensive population of international TFE workers at the time it was conducted, it used a semiquantitative exposure assessment with no validation of estimated exposures. The study was limited to men and did not include information on potential confounding factors such as smoking status, which, if associated with occupational exposure, could have led to residual confounding. It was also difficult to discern whether any observed effects in this study would be caused by TFE (IARC Group 2A, IARC, 2016), if present, or by PFOA, given the high correlation between the exposures. Using cancer mortality data from linkages may underestimate associations with specific types of incident cancer, particularly those with more favourable survival after diagnosis. The heterogeneous nature of the outcome limited the inference from these findings.]

Girardi and Merler (2019) investigated mortality in a cohort of 462 PFAS-exposed workers at a factory in Trissino, Veneto, Italy, and compared mortality rates with those for regional general populations and 1383 railroad workers who were not exposed to PFAS compounds (see Section 2.1.9). PFAS exposure was estimated using a JEM, which was informed in part by serum PFOA concentrations. Exposure was categorized into tertiles of estimated PFOA and was also evaluated on the basis of categories of exposure (ever at PFAS department, never at PFAS department, and in offices). Vital status was obtained from death certificates, for deaths between 1970 and 2018. This was a highly exposed occupational cohort (n = 120 with measured PFOA; geometric mean, 4048 ng/mL), with 107 deaths observed, 42 of which were from cancer. There was no excess mortality observed when compared with regional rates for comparison overall (all cancers, SMR, 1.00; 95% CI, 0.74–1.36) although the overall cancer mortality risk was elevated when compared to that for the railworkers (risk ratio, 1.32; 95% CI, 0.91–1.91). There was little evidence of association with categorical estimates of PFAS exposure, with imprecise increases in the SMR for the highest estimated tertile of PFOA (SMR, 1.22; 95% CI, 0.79–1.87) and among those ever working in a PFAS department (SMR, 1.46; 95% CI, 0.85–2.51), when using regional rates as the referent. However, the estimate for the highest tertile of PFOA was more pronounced when the railroad workers were used as the referent (risk ratio, 1.65; 95% CI, 1.02–2.65), and an increase was also evident for those ever working in a PFAS department (risk ratio, 1.97; 95% CI, 1.10–3.54). [The Working Group noted that this was a highly exposed cohort, for which serum levels were used in conjunction with a JEM to inform the exposure classification. The population was limited to men and although the study did not include any information on confounders such as smoking, it used both national rates and an unexposed worker population to reduce the impact of the healthy-worker effect, which may also limit residual confounding. However, despite a long follow-up period, there were few cancer-related deaths, and the use of death certificates to ascertain cancer mortality data may underestimate possible associations with specific types of incident cancer, particularly those with more favourable survival after diagnosis. The heterogeneous nature of the outcome limited the inference from these findings.]

Li et al. (2022a) examined overall cancer incidence in more than 60 000 individuals who lived in Ronneby municipality in Sweden in 1985–2013 (see Section 2.1.13). This study population included approximately one third who were exposed to water contaminated with a mixture of PFAS compounds, mainly PFOS, PFHxS, and, to a lesser extent, PFOA. The exposure assessment

was based on annual residential addresses and information on drinking-water supply, and cases were identified using cancer registry linkage until 2016. There were 5702 cases of cancer identified. There was no evidence that exposure to highly contaminated PFAS drinking-water was associated with excess incidence, as SIRs were around or below the null. SIRs were also similar for both the "never-high" and "ever-high" exposure groups, defined on the basis of living at an address supplied with PFAS-contaminated water, compared with an external reference group. In the internal cohort comparison analysis, there was little difference in the hazard ratios for overall cancer incidence according to estimated exposure duration or timing of exposure, although there may have been a slight increase for high exposures between 2005 and 2013 (late period) (HR, 1.09; 95% CI, 0.99–1.20) but not for high exposures between 1985 and 2004 (early period). Li et al. (2022a) also conducted sensitivity analyses further adjusting for highest education attained. Potential confounding by smoking was partly accounted for, since duration of education and smoking are highly correlated (Eek et al., 2010). [The Working Group noted that this study had a large general population sample with a high environmental level of PFAS exposure and near-complete registry-based case identification. Other strengths included the use of both an external reference group and internal comparisons. However, the exposure assessment was limited by not having any individual-level measurements of exposure and by including areas that were contaminated by multiple PFAS, which limited inferences regarding associations with individual compounds. The minimal information on individual-level confounders, except for education (which was included in sensitivity analyses), may not be as important in this context, given that exposure was determined on the basis of the water distribution system.]

Wen et al. (2022) evaluated the association between mortality and serum measurements of PFOA and PFOS using data from NHANES, which is a continuously conducted and nationally representative cross-sectional survey designed to represent the non-institutionalized US population (see Section 2.1.15). Blood samples were collected in 1999–2014, and participants were followed up for mortality using linkage to the NDI until the end of 2015. Of the 1251 deaths that occurred during the study follow-up period, 248 were from cancer. Increasing serum PFOA levels were not related to higher incidence of cancer-related mortality (PFOA tertile 3 versus tertile 1, HR, 1.06; 95% CI, 0.68–1.71). In contrast, increasing PFOS level was related to higher adjusted hazard ratio for overall cancer-related mortality in a dose–dependent manner (PFOS tertile 2 versus tertile 1, HR, 1.26; 95% CI, 0.75–2.06; PFOS tertile 3 versus tertile 1, HR, 1.75; 95% CI, 1.10–2.83), adjusting for the other measured PFAS in addition to sex, age, race or ethnicity, education, smoking status, physical activity, hypertension, healthy eating index, creatinine clearance rate, serum total cholesterol, and serum cotinine. [The Working Group noted that the strengths of this investigation were the use of a nationally representative population with serum measurements of PFOS and PFOA, with probable complete ascertainment of cancer mortality. Despite relatively good control for potential confounders, including other PFAS, the Working Group noted that the analysis did not adjust for calendar time, which is important given temporal trends in PFAS concentrations. Other limitations included the short follow-up time for some of the participants, which may not reflect the relevant etiological window, and, for some individuals, the blood sample may have been collected after cancer diagnosis and treatment, since participants with cancer at baseline were not excluded. There was also a relatively small number of cancer-related deaths, and the focus on overall cancer mortality, a heterogeneous outcome, may mask associations for individual cancer types.]

2.8 Evidence synthesis for cancer in humans

This section provides a synthesis of the epidemiological evidence on cancer in humans exposed to PFOA or PFOS. The synthesis is based upon a total of 36 epidemiological studies available to the Working Group.

The first epidemiological study addressing risk of cancer associated with exposure to PFAS was an occupational mortality study in a cohort of workers manufacturing APFO (the ammonium salt of PFOA), in the Cottage Grove plant in Minneapolis, Minnesota, USA (Gilliland and Mandel, 1993). This study was published more than five decades after large-scale manufacture of PFOA was initiated.

2.8.1 Studies evaluated

The epidemiological evidence on the carcinogenicity of PFOA and PFOS in humans is available from studies with three different exposure settings. First, occupational exposure of workers in chemical plants manufacturing or using PFOA or PFOS; second, high environmental exposure in communities contaminated by emissions from chemical plants or other specific sources, such as the use of aqueous firefighting foam; and last, background exposure of the general population. Studies within these three settings typically have different epidemiological designs with different strengths and limitations; thus, comparing findings for a particular cancer site across the various exposure settings may assist causal inference. The studies that the Working Group considered the most informative and hence to which the most weight was given when balancing the evidence for carcinogenicity in humans were large cohort and nested case–control studies from all three major exposure settings.

(a) Occupational cohort studies

Chemical plants manufacturing or using PFOA or PFOS were established at three major sites in USA and some facilities in Europe from the late 1940s onwards, and follow-up studies of worker cohorts from these sites contributed substantially to the evidence on human carcinogenicity of PFOA and, to a lesser degree, of PFOS. The three US cohorts were the PFOA-manufacturing facility in Cottage Grove, Minnesota (Gilliland and Mandel, 1993; Lundin et al., 2009; Raleigh et al., 2014), the polymer-production plant in Parkersburg, West Virginia (Leonard et al., 2008; Steenland and Woskie, 2012; Steenland et al., 2015), and the fluorochemical-production facility in Decatur, Alabama (Alexander et al., 2003; Alexander and Olsen, 2007). A number of plants in Europe and the Parkersburg polymer-production facility were included in the pooled international TFE cohort (Consonni et al., 2013), and a small PFAS-manufacturing plant in the Veneto region, Italy, contributed data on workers with extremely high PFOA serum concentrations (Girardi and Merler, 2019).

The occupational cohort studies were distinguished by PFOA or PFOS serum concentrations that were up to two orders of magnitude higher in workers than in the background population, with exposure contrasts facilitating the evaluation of exposure–response relations. The occupational cohorts generally had several decades of follow-up since first exposure, and exposure profiles were dominated by only a few PFAS compounds, depending on manufacturing processes. Thus, occupational exposures to either PFOA and PFOS were usually confined largely to one or the other, without being mixed. However, co-exposure to other PFAS and TFE was possible in some studies. Some occupational cohorts had information on blood concentrations of PFOA and/or PFOS, which were used to develop company-specific JEMs estimating serum levels

across time and jobs. This provided reliable information, given that blood levels represent an internal dose resulting from all exposure routes and may be superior to exposure metrics based on air concentrations that account for exposure only by the inhalation route.

A major limitation of occupational cohort studies is their relatively small sample sizes for specific cancers, inability to address cancers of the breast and female genital tract (since most chemical-plant workers are men), and the use of mortality rather than incidence by most of the studies. Mortality studies provide weaker data on etiology and generally smaller sample sizes than do incidence studies, especially for cancers with a low fatality rate. Another concern with occupational studies is that some are based on cross-sectional samples excluding workers at risk before cohort enrolment, although this will affect cancers with a low fatality rate to a lesser extent and may create only a rather weak downward bias for cancers with a high rate of fatality. Moreover, mortality studies often lack histological data, which might lead to attenuation of risk estimates for all cases combined if the risk is associated only with certain histological subtypes. Despite these caveats, the Working Group considered three occupational studies to be particularly informative, i.e. those by Alexander and Olsen (2007), Raleigh et al. (2014), and Steenland et al. (2015). All three used incidence data and provided risk estimates for a range of cancers according to cumulative quantitative exposure metrics, although the study by Alexander and Olsen (2007) still suffered from small numbers.

(b) Studies of high environmental exposure

These studies can be particularly informative because, like occupational studies, they may enable detection of health effects that may be less marked in general population studies with low exposures. Only three high-level environmental exposure studies were available: Barry et al. (2013), Vieira et al. (2013), and Li et al. (2022a). Exposure levels and contrasts in these settings were between the low background levels of the general population and the very high occupational levels. They benefited from large study populations, resulting in more precise risk estimates and high comparability of exposed and unexposed people recruited from the same geographical regions.

The C8 Science Panel Project included a group of workers (11.5% of the cohort) with occupational exposure to primarily PFOA at a polymer-production plant in Parkersburg, West Virginia, USA, and residents in the Mid-Ohio Valley USA, which was contaminated by emissions from this facility from the late 1940s until about 2005 (Barry et al., 2013). This Mid-Ohio Valley cohort had an important strength in its modelling of individual cumulative serum PFOA concentrations from birth onwards based on a large number of parameters, including plant emission data, measured drinking-water levels, residential histories, individual consumption of tap water, and toxicokinetic data for PFOA in humans. The estimated PFOA serum concentrations correlated well with a large number of PFOA measurements made in 2005 and 2006. The serum concentrations of PFOA, PFHxS, and PFNA were elevated by about 500%, 75% and 40%, respectively, compared with US background levels, whereas the PFOS serum concentration was not increased (Frisbee et al., 2009).

The case–control study of West Virginia and Ohio residents (Vieira et al., 2013) somewhat overlapped the C8 Science Panel study. Since this non-nested case–control study was based upon cancer registry records from a longer period and from larger geographical areas, the total number of cancer cases was higher than those of the analyses by Barry et al. (2013). This provided more accurate risk estimates, which is particularly important when considering rare cancer types. However, a limitation was the reference group that comprised people with other cancers

(except pancreatic, kidney, testicular, and liver cancer), which may attenuate risk estimates if these other cancers are also associated with PFAS. Also, exposure misclassification was of some concern, because the residential address used to assign exposure based on the same model used in Barry et al. (2013) was known only at the time of diagnosis (for details, see Section 2.1.22). However, since the error could mostly be of Berkson type, exposure misclassification might not cause substantial attenuation of risk estimates (Armstrong, 1998).

The third study, in the Ronneby Register cohort, included more than 60 000 community residents living in an area where parts of the population received drinking-water contaminated with PFAS from a nearby airfield (Li et al., 2022a). In contrast to the Mid-Ohio Valley cohort, the Ronneby Register cohort used a crude assignment of exposure based on earlier and current residential addresses, but it was supported by large exposure contrasts in PFAS concentrations in drinking-water across the various water supplies of the municipality. Another limitation of the Ronneby Register cohort, which is in general an issue for most non-occupational PFAS studies, was overlapping exposures to various PFAS. Whereas Ronneby had very high levels of PFOS and PFHxS, levels of PFOA greatly overlapped those of the unexposed Swedish population in the region.

(c) *Studies in the general population with background exposure*

Studies of subsets of the general population were often case–control studies nested within large cohorts or trials created for other purposes. With this design it is possible to cost-effectively sample large series of cases of a specific cancer; to take advantage of individual data on social, lifestyle, and health issues of particular relevance for a specific cancer; to use frozen blood samples to obtain prediagnostic measurements of contaminants; and to limit potential bias and confounding by matching on relevant characteristics. The main limitation pertaining to population-based studies is low exposure levels, low exposure contrasts, and background exposure to numerous other PFAS. Several chlorinated persistent organic pollutants are also widespread and have even longer biological half-lives than do PFAS, but the two classes of chemicals do not share physicochemical characteristics and in general serum concentrations are not correlated. Positive findings that are not corroborated in studies of high-exposure contrast (e.g. occupational or high environmental exposures) may seem contradictory, although for many carcinogens it has been shown that risk increases greatly with increasing levels at low exposure and then tails off or reaches a plateau at higher exposures (Stayner et al., 2003; Lanphear, 2017; Steenland et al., 2022). Suggested biological explanations include saturation of metabolic pathways, enhanced detoxification, and greater DNA repair efficiency at higher exposure levels (Stayner et al., 2003). Increasing exposure measurement error with increasing level of exposure can also result in the exposure–response relation reaching a plateau (Stayner et al., 2003). Healthy-worker survivor bias may also be a factor reducing the apparent risk in occupational cohort studies.

Despite limitations, several case–control studies nested within large cohorts were considered informative for this evaluation. They included studies based upon the Danish Diet, Cancer, and Health Cohort, addressing associations of PFOA and PFOS with cancers of the urinary bladder, prostate, liver, and pancreas in men (Eriksen et al., 2009); four studies based on the intervention arms in the PLCO Trial, addressing cancers of the kidney (Shearer et al., 2021), breast (Chang et al., 2023), prostate (Rhee et al., 2023a), and pancreas (Zhang et al., 2023); the US Air Force servicemen cohort, addressing testicular cancer (Purdue et al., 2023); two studies based on the US MEC, addressing HCC (Goodrich et al., 2022) and kidney cancer (Rhee

et al., 2023b); a study based in the ATBC Study in Finland, on pancreatic cancer (Zhang et al., 2023); a study of women in the FMC, addressing thyroid cancer (Madrigal et al., 2024); a case–cohort study on the association between PFAS and cancers of the kidney, pancreas, breast, prostate, and lymphatic and haematopoietic tissue among participants in the ACS CPS-II LifeLink Cohort (Winquist et al., 2023); and a small nested case–control study evaluating thyroid cancer in New York, USA (van Gerwen et al., 2023). Finally, four nested case–control studies with prediagnostic PFAS measurements, which addressed risk of breast cancer in population samples with a low level of exposure, i.e. a study of women in the French education system (E3N; Mancini et al., 2020a); the Danish National Birth Cohort (Ghisari et al., 2017); the US Child Health and Development Cohort (Cohn et al., 2020); and the Dongfeng-Tongji cohort of female retirees from a large motor company in China (Feng et al., 2022).

A number of hospital-based and non-nested case–control studies were considered less informative, because the control groups did not clearly represent the same population from which the cases were chosen, resulting in potential unpredictable bias. Moreover, the exposure assessment in these studies was based on postdiagnostic measurements of PFAS in blood samples, which are expected to provide less-reliable information on exposure during the relevant time windows than do prediagnostic baseline samples. Risk estimates may be biased if prodromal disease states, the fully developed disease, or the treatment affect serum concentrations of PFAS (this is labelled reverse causation), but little is known on this issue and the direction of bias, if any, is unpredictable. For these reasons, such case–control studies and one nested case–control study (Hurley et al., 2018) were given less weight when balancing the epidemiological evidence for causal associations for cancers of the breast (Wielsøe et al., 2017; Tsai et al., 2020; Itoh et al., 2021; Li et al., 2022b; Velarde et al., 2022), thyroid (Liu et al., 2022; Li et al., 2023), prostate (Hardell et al., 2014) and liver (Cao et al., 2022).

2.8.2 Exposure assessment quality considerations

Information on individual cumulative exposure to PFOA and/or PFOS that enabled analyses of exposure–response relations including lagged analyses was considered of critical importance for the evaluation of epidemiological studies on the carcinogenicity of PFOA and PFOS. A systematic description and appraisal of exposure assessment in all available epidemiological studies is provided in Section 1.6.1.

Among nine occupational cohort studies in which exposure assessment primarily relied on job history, three studies focusing on PFOA and one study on PFOS used quantitative estimates of cumulative exposure (Alexander and Olsen, 2007; Steenland and Woskie, 2012; Raleigh et al., 2014; Steenland et al., 2015). These estimates were derived from company-specific JEMs informed by industrial hygiene and/or biological measurements and accounted for temporal shifts in exposure levels. In particular, the approach used in the study by Steenland and Woskie (2012) (and Steenland et al., 2015), which incorporated industrial hygiene and biological measurements into modelled serum concentrations, was considered superior to the others.

The inevitable misclassification of exposure related to group-based exposure assignment in these studies may not necessarily cause attenuation of risk estimates towards the null. Depending on the degree of Berkson-type measurement error, it may primarily result in unbiased but less precise risk estimates (Armstrong, 1998). However, errors involved in group mean exposure measurement used in JEMs also could cause bias towards or away from the null. Exposure assessment in the small cohort of PFAS-manufacturing workers in Italy was also modelled via cumulative PFOA serum

concentrations based upon a JEM informed by measurements, but the effects of considerable co-exposure to other PFAS compounds were not accounted for by the analyses (Girardi and Merler, 2019). Other occupational cohort studies applying crude or semiquantitative assignments of exposure levels and without quantitative estimates of individual cumulative exposure were considered at higher risk of exposure misclassification for lifetime exposure and therefore provided less-reliable risk estimates (Alexander et al., 2003; Leonard et al., 2008; Lundin et al., 2009; Consonni et al., 2013).

Among the three studies addressing risk of cancer in residents living in areas contaminated by local PFOS and/or PFOA emissions, one study of the population in the Mid-Ohio Valley, West Virginia, USA, was considered particularly informative because of the modelled annual serum PFOA concentrations from birth onwards (Barry et al., 2013), and another partly overlapping study assessed exposure 10 years before diagnosis, making the exposure assessment slightly less informative (Vieira et al., 2013). The Ronneby Register cohort study in Sweden had well-documented, strong contrasts among residents with respect to PFOS and PFHxS serum concentrations, but the exposure assessment was entirely based upon timing and duration of residence at contaminated and uncontaminated addresses and did not allow estimation of the effects of the individual compounds (Li et al., 2022a).

All studies addressing risk of cancer in general population samples used the concentration of PFAS in at least one blood sample as a proxy for cumulative exposure. This approach is supported by the long biological half-lives of PFOA and PFOS in humans (see Section 4.1 for details) and some indications of high stability of blood concentrations across several years within individuals who provided repeated samples (Blake et al., 2018; Purdue et al., 2023; Rhee et al., 2023a). Furthermore, simulation studies based on available data with repeated measurements up to 8 years apart indicated that bias towards the null because of non-differential misclassification would be modest (Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636).

2.8.3 Co-exposures to other agents of relevance to cancer hazard identification

Mutually independent information on the carcinogenicity of PFOA and PFOS in humans in the available epidemiological studies was obtained by two main approaches.

First, some occupational and environmental settings were associated with exposure to specific PFAS compounds at levels many times as high as background levels, whereby co-exposure to other PFAS compounds above background levels was unlikely, given the characteristics of the production processes and sources of exposure. This applied to the occupational cohorts of workers at the APFO-producing plant in Cottage Grove, Minnesota, USA (Raleigh et al., 2014); the studies of fluoropolymer-production workers in Parkersburg, West Virginia, USA (Steenland and Woskie, 2012; Steenland et al., 2015); and the C8 Science Panel cohort of workers and residents of contaminated areas of the Mid-Ohio Valley, USA (Barry et al., 2013; Vieira et al., 2013). In all these studies, PFOA serum concentrations were substantially elevated above background levels, whereas PFOS serum concentrations were not. Serum concentrations of PFHxS and PFNA were also somewhat above background levels in Mid-Ohio Valley residents, but the correlation with PFOA was modest, indicating that exposure via a source other than the plant in Parkersburg, West Virginia, was likely (Frisbee et al., 2009). Moreover, co-exposure to TFE (classified in IARC Group 2A; IARC, 2016) may have occurred at some European workplaces (Consonni et al., 2013) but was considered unlikely at the plant in

Parkersburg because use was strictly controlled under normal operations (Steenland and Woskie, 2012). For PFOS, there were no occupational or environmental settings without some co-exposure to other PFAS compounds or carcinogens. The occupational cohort of fluorochemical-production workers in Decatur, Alabama was characterized by high exposure to PFOS, but exposure to several other fluorochemicals including PFOA was possible or likely (Alexander et al., 2003; for details, see Table S1.22, Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636). Likewise, the Ronneby Register cohort was characterized by PFOS exposures an order of magnitude above background levels, but levels of PFHxS were also substantially higher than background levels whereas PFOA levels were not (Li et al., 2022a).

Second, in all studies of general population samples, PFAS exposure was mixed. Most studies estimated exposure by measurement of PFAS compounds in one or more blood samples, and estimates were typically provided for both PFOA and PFOS. In some studies, mutual adjustment was performed for the effects of other PFAS compounds (Cohn et al., 2020; Shearer et al., 2021; Wen et al., 2022; Chang et al., 2023; Purdue et al., 2023; Rhee et al., 2023a, b; Madrigal et al., 2024), which helped to identify individual effects. The correlation coefficients of PFOA and PFOS in the above studies ranged from 0.50 (Wen et al., 2022) to 0.70 (Rhee et al., 2023a), and therefore the possibility of unstable statistical models or overadjustment was unlikely. The same concern applied to correlations between PFOA, PFOS, and other common legacy PFAS (Shearer et al., 2021; Rhee et al., 2023b).

2.8.4 Bias and confounding

Exposure- and outcome-dependent selection into studies was not considered to be an important source of bias in the most informative studies available for this evaluation. Most occupational studies were based upon rosters kept by major companies (Alexander and Olsen, 2007; Raleigh et al., 2014; Steenland et al., 2015), and the large, nested case–control studies mostly used existing independent databases or public registries to define the study populations (see Table 2.1). Selection bias in terms of a healthy-worker effect (sometimes viewed as a confounder) and healthy-worker survivor bias is of concern when considering occupational studies. Both would be expected to lead to downward bias. The former bias can be mitigated by using internal rather than external comparisons, and the latter is of less concern if there is little evidence that high exposure is associated with leaving employment in a highly exposed job or altogether. Furthermore, compared with studies on other chronic diseases, studies on cancer may be less susceptible to both healthy-worker effects and healthy-worker survivor effects.

The C8 Science Panel cohort of workers and residents in the PFOA-contaminated Mid-Ohio Valley area in Ohio and West Virginia was considered particularly informative for this evaluation because of its size, validated estimates of cumulative internal exposure, large exposure contrast, and extensive covariate information. It was mainly based upon a cross-sectional population sample of residents alive at the time of interview and with most at-risk years occurring before baseline interviews. Selection bias was unlikely because the participation rate was about 80%, and the data did not indicate preferential participation of residents from contaminated areas who had a history of cancer (Barry et al., 2013). Moreover, simulation analyses demonstrated that lacking information about fatalities occurring in the population before enrolment would not affect risk estimates (Barry et al., 2015), unless survival after diagnosis was associated with exposure level, judged a priori to be unlikely.

In most studies, case identification and ascertainment were based upon population-wide cancer registries, death certificates, or death registries (or a combination of these), and in one study the additional data from personal recall of cancer were verified by medical records (Barry et al., 2013). The approaches for case identification in general were not expected to introduce major outcome misclassification.

Only a few informative studies distinguished subtypes of specific cancers, mainly for breast cancer (Hurley et al., 2018; Mancini et al., 2020a; Chang et al., 2023). Examples of environmental exposures causing risk of some but not of other specific cancer subtypes are few (e.g. wood dust causes adenocarcinoma but rarely causes squamous cell carcinoma of the sinonasal cavity; IARC, 2012). Considering that PFAS may modulate endocrine regulation and signalling (for details, see Section 4.2.8), there is a rationale for examination of receptor-defined subtypes of, particularly, breast cancer. However, since the effects of PFAS may depend on endogenous hormone levels and may be inhibitory in some situations but stimulatory in others, it is difficult to put forward a priori hypotheses, which complicates the interpretation of epidemiological findings. There is no mechanistic evidence indicating that the main subtypes of testicular cancer (seminoma, and non-seminoma) have different etiologies in young men in whom these tumours develop from carcinoma in situ in cells of developmental origin (Rajpert-De Meyts, 2006). It is disputed whether subtypes of testicular cancer for which incidence peaks later in adulthood have different etiologies (Coupland et al., 1999; Stang, 2009). At present, studies addressing specific cancer subtypes are mainly explorative and foremost of importance as starting points for forthcoming studies.

Demographic characteristics such as race, ethnicity, sex, age, residence area, socioeconomic status and calendar period are strong determinants of cancer and are also associated with PFAS exposure in the general population (Steenland et al., 2009; Eriksen et al., 2011; Buekers et al., 2018; Momenimovahed and Salehiniya, 2019; Rhee et al., 2023b). With few exceptions, these factors were controlled by design and/or analysis in the highly informative studies. Some studies, mainly nested case–control studies (for details, see Table 2.1), also accounted for the effects of smoking, alcoholic beverage consumption, and BMI, based on data collected by personal interview (Barry et al., 2013); however, such information was not available in the occupational cohort studies, the case–control studies by Vieira et al. (2013), and the Ronneby Register cohort study (Li et al., 2022a). In occupational studies, internal analyses and comparisons of exposed with unexposed workers in the same types of jobs from nearby plants during the same calendar period mitigated confounding due to differences in social and lifestyle factors, whereas confounding in studies of heterogeneous populations not accounting for these factors may result in bias in an unpredictable direction. Nested case–cohort and case–control studies designed to address one or more specific cancers often included information on a range of determinants of these specific cancers, such as hepatitis for primary liver cancer (Goodrich et al., 2022); hypertension and possible reduced glomerular filtration for kidney cancer (Shearer et al., 2021); reproductive factors for breast cancer (e.g. Ghisari et al., 2017; Cohn et al., 2020; Mancini et al., 2020a; Chang et al., 2023); or specific occupational exposures for bladder cancer (Eriksen et al., 2009).

As the main potentially confounding factors, sex, age, time, geography, socioeconomic status, and possibly race or ethnicity were measured and analysed with high accuracy, residual confounding by these factors was considered unlikely. Cooking practices such as frying and consumption of a number of food items (such as eggs, potatoes, red meat, snacks, and vegetables) have been associated with serum concentrations of PFOA and PFOS in a number of

studies. In a general population sample, these factors explained 14% and 24% of the variation in concentrations of PFOA and PFOS, respectively (Eriksen et al., 2011). Various foodstuffs have also been associated with some cancers, and therefore confounding by diet (with unpredictable magnitude and direction) cannot be ruled out in the general population studies, whereas confounding by diet was very unlikely in the occupational studies and the studies of communities with high-level exposure, because the dietary intake of PFAS was marginal compared with the main source of exposure.

2.8.5 Specific cancer sites and exposure to PFOA

(a) Kidney cancer

Three partly overlapping studies of workers and residents in West Virginia and Ohio, USA, have consistently shown increased risk of kidney cancer in relation to occupational and/or high-level environmental exposure (Steenland and Woskie, 2012; Barry et al., 2013; Vieira et al., 2013). The occupational mortality study reported an SMR for fluoropolymer workers in the highest exposed quartile of estimated cumulative PFOA serum concentrations compared with unexposed workers of 2.66 (95% CI, 1.15–5.24; 8 deaths) with indications of an exposure–response relation (Steenland and Woskie, 2012). The highly informative cohort study of workers and residents found an increasing risk of incident kidney cancer with increasing cumulative PFOA serum levels, albeit with borderline statistical significance (Barry et al., 2013). The adjusted hazard ratio for the fourth quartile of cumulative PFOA serum concentrations versus the first was 1.58 (95% CI, 0.88–2.84; 105 cases; linear trend test, $P = 0.18$; using the log continuous PFOA serum concentration, $P = 0.10$). Findings were consistent with results of the third partly overlapping study from this geographical area, a register-based case–control study in Ohio that reported an adjusted odds ratio for incident kidney cancer in exposed people in the highest PFOA serum concentration quartile (110–655 µg/L [ng/mL]), versus the unexposed, of 2.0 (95% CI, 1.0–3.9; total, 246 cases) (Vieira et al., 2013). In this study, there was some concern about the appropriateness of the control group, which comprised people with all other cancers excluding those of the testis, liver, and pancreas.

The results of two (less informative) occupational cohort studies did not corroborate or refute the above findings. The cohort study of APFO workers at the Cottage Grove facility in Minneapolis, Minnesota, USA did not find indications of an increased incidence of kidney cancer in exposed workers. The hazard ratio for the fourth quartile versus the unexposed workers (Saint Paul plant) was 0.73 (95% CI, 0.21–2.48; 16 exposed cases) and there was no indication of increasing risk across increasing quartiles of exposure (see Table 2.2). However, the wide confidence intervals were not incompatible with the effects observed in the earlier studies (Raleigh et al., 2014). The exposure metric was based upon air measurements of PFOA, which may be less reliable than biological measurements if exposure occurs through pathways other than inhalation or if there is large variation in pulmonary absorption of PFOA due to, for instance, differential use of respiratory protection equipment or high pulmonary ventilation in some physically demanding jobs. The international study of mortality in TFE-production workers (Consonni et al., 2013) was not informative because of the semiquantitative exposure assessment and the small number of cases ($n = 10$).

Unlike the above five studies of highly exposed populations, two nested case–control studies and a case–cohort study using a single prediagnostic PFOA serum concentration addressed risk associated with the much lower background exposure of the general US population (Shearer et al., 2021; Rhee et al., 2023b; Winquist et al., 2023). The study based upon the

PLCO Trial cohort reported an adjusted odds ratio for RCC (constituting about 80–90% of all kidney cancers) in the highest exposure quartile (> 7.3–27.2 µg/L [ng/mL]) versus the lowest (< 4.0 µg/L [ng/mL]) of 2.63 (95% CI, 1.33–5.20) (Shearer et al., 2021). Adjusted for other PFAS compounds, the OR was 2.19 (95% CI, 0.86–5.61). This relative risk for RCC observed in the general population was similar to that for kidney cancer observed among people with an exposure more than one order magnitude higher. If these associations are causal, this indicates a non-linear exposure–response relation with a steep increase in risk at very low exposure levels, which tails off or even reaches a plateau with higher exposure (Steenland et al., 2022). The other nested case–control study of an ethnically diverse US population with background exposure levels did not find an association between prediagnostic PFOA serum concentrations and risk of incident RCC overall (OR for a 1-unit increase in PFOA serum concentration on the $\log_2$ scale, 0.89; 95% CI, 0.67–1.18), but – consistent with the earlier findings – the risk was elevated in White participants (23% of the study population), albeit with wide confidence intervals (adjusted OR for a 1-unit increment in PFOA serum concentration on the $\log_2$ scale, 2.12; 95% CI, 0.87–5.18; Rhee et al., 2023b). Finally, the case–cohort study conducted within the CPS-II LifeLink Cohort (in which 98% of participants were White) found no increased risk for all kidney cancers (HR for continuous $\log_2$-plasma PFOA concentrations was 1.08; 95% CI, 0.88–1.33, 156 cases; Winquist et al., 2023). For RCC, the hazard ratio was 1.06 (95% CI, 0.83–1.35). Among women (38% of the case–cohort group), for all kidney cancers there was an increased hazard ratio of 1.33 (95% CI, 0.97–1.83; 65 cases) and for RCC it was 1.54 (95% CI, 1.05–2.26; 42 cases). Of note was that this was a “survivor cohort”, in which the median age when follow-up started was 70 years, about 8 years after enrolment began in the CPS-II. At age 40–60 years, the rate of RCC is twice as high in men as in women, which could have contributed to a differential survivor effect by sex (Mancini et al., 2020b; NCI, 2023).

The Working Group concluded that increased risks of kidney cancer overall or RCC in relation to PFOA exposure were reported by two independent and highly informative studies (Barry et al., 2013; Shearer et al., 2021). These studies included large study populations and long follow-up, across which there were large exposure contrasts spanning background, high environmental, and extremely high occupational exposure. There was comprehensive individual-level assessment of cumulative exposure in one of these studies (Barry et al., 2013). Exposure–response relations were observed overall in these two independent populations. The findings were not corroborated overall by those of two other less-informative occupational studies (Raleigh et al., 2014; Consonni et al., 2013), and only among subgroups in two other general population studies (Rhee et al., 2023b; Winquist et al., 2023). In the random-effects meta-analysis conducted by the Working Group, which was based on six studies (three of which were from the Mid-Ohio Valley, as well as Shearer et al., 2021, Rhee et al., 2023b, and Winquist et al., 2023) a meta-rate ratio per 10 ng/mL of 1.16 (95% CI, 0.98–1.38; $I^2 = 0.91$) was estimated for PFOA. The limitations of the meta-analysis were estimation of the linear exposure–response relation from two categorical data points, assumptions about the duration of exposure in three studies, assumptions about the midpoint in the high-exposure category in three studies, and lack of independence of three of the studies.

Taken together, the body of epidemiological evidence indicated that a causal association between PFOA and RCC is credible, but the evidence was not considered sufficiently consistent to rule out chance and bias with confidence. The studies did not allow for an evaluation of kidney cancers of non-RCC histology subtype.

(b) Testicular cancer

The cohort study of the highly exposed Mid-Ohio Valley population with PFOA exposure substantially above background levels (Barry et al., 2013) and the nested case–control study of US Air Force servicemen with exposure levels in the range of the general US population (Purdue et al., 2023) were considered the most informative for the evaluation of testicular cancer. Barry et al. (2013) reported an adjusted hazard ratio for incident testicular cancer of 1.34 (95% CI, 1.00–1.79) for a 1-unit increase in natural log-transformed serum concentrations in unlagged analyses. This observation was not corroborated by Purdue et al. (2023), who reported an OR for testicular germ cell tumour (TGCT; the most common type of testicular cancer) of 0.8 (95% CI, 0.5–1.4), comparing the highest exposed quartile with the lowest, based on 530 cases and matched controls. There was no indication of an exposure–response relation (P for trend, 0.86), and similar results were observed in an analysis of the second sample collected in a subset of the population. The range of measured serum PFOA levels in 2005–2006 was 0.25–4752 ng/mL (median, 24.2 ng/mL) for residents in the study by Barry et al. (2013) compared with a geometric mean of 5.8 ng/mL for controls in the study by Purdue et al. (2023). Therefore, the higher exposure contrast in the former study may explain the discrepant findings. Moreover, the study of Air Force servicemen did not control for residential area, which may cause bias in an unpredictable direction. Findings in the study by Vieira et al. (2013) were compatible with an increased risk of testicular cancer, but the cancer cases included somewhat overlapped the cases in the study by Barry et al. (2013), and the study offered no improvements in design or analysis.

One additional population was exposed to high levels of PFOA (and to a much lesser extent other PFAS) resulting from industrial contamination in the Veneto region of Italy, and serum concentration data (more than 18 000 measurements in 2016 among those aged 14–39 years) were reported by municipality (n = 21) by Pitter et al. (2020). Orchiectomies by the same groupings of municipality in the Veneto region between 1997 and 2014 were reported separately (Sistema Epidemiologico Regionale, 2016). Orchiectomy was found to have high sensitivity and positive predictive value for testicular cancer in this region (Sistema Epidemiologico Regionale, 2016). The Working Group combined the serum and orchiectomy rate data and observed a strong positive correlation (Spearman correlation, 0.57; P = 0.006; 21 cases) between serum PFOA concentrations and rates of orchiectomy (standardized on age by 5-year age groups from ages 15 to 54 years to the overall regional rate) by municipality.

Few other studies addressed the of the association between PFOA exposure and testicular cancer. Results of two occupational mortality studies were also compatible with an increased risk but were based on very few cases (< 3), not permitting detailed analysis (Steenland and Woskie, 2012; Consonni et al., 2013). Moreover, these studies of mortality were considered less informative, owing to the high survival rate for testicular cancer, since mortality-based risk estimates reflect a mix of etiological and prognostic factors.

The Working Group concluded that there were indications in two independent populations for an increased risk of testicular cancer associated with PFOA serum concentrations in residents with a high level of exposure. In the third informative study, a null association was seen, but exposure levels were at background in this population. Overall, the Working Group concluded that a positive association between PFOA and testicular cancer is credible. However, chance and/or bias could not be ruled out as explanations for these findings, given the small number of cases in the few available studies and that one of the positive studies was of ecological design.

(c) Bladder cancer

Two occupational cohort studies (Raleigh et al., 2014; Steenland et al., 2015), the cohort study of the Mid-Ohio Valley population with high exposure (Barry et al., 2013), and the partly overlapping registry-based case–control study (Vieira et al., 2013) provided data on the incidence of bladder cancer in relation to individual-level estimates of cumulative PFOA exposure. None of these studies that included large study populations with a strong exposure contrast indicated an increased risk of bladder cancer in relation to PFOA exposure, and the results were consistent with those of the Danish Diet Cancer and Health Cohort study (Eriksen et al., 2009) and a large US case–cohort study (Winquist et al., 2023). The former study only addressed low-level background exposure and did not adjust for co-exposure to PFOS, but PFOS was not associated with increased risk. Finally, the international occupational mortality study did not observe an increased risk of fatal bladder cancer (Consonni et al., 2013), and some indication of increased risk of fatal bladder cancer in an occupational cohort study (Steenland and Woskie, 2012) was not corroborated by the subsequent incidence study that had an improved exposure assessment (Steenland et al., 2015).

The Working Group concluded that the epidemiological evidence in aggregate did not indicate a positive association between PFOA at environmental or occupational exposure levels and urinary bladder cancer, but noted that the occupational cohort studies in particular include few exposed cases, limiting informativeness, and that exposure misclassification may have biased associations towards the null.

(d) Prostate cancer

Altogether, six studies on the risk of prostate cancer and PFOA exposure were fairly consistent in reporting null or inverse associations regardless of study design, type of population (background exposure, high environmental or occupational exposure), method of exposure assessment (estimates of external exposure using various approaches or measurements of serum concentration) or outcome (incident cases or mortality) (see Section 2.3.2 for details). This collection of studies included highly informative studies with a large exposure contrast and lifelong estimates of cumulative PFOA serum concentrations (Barry et al., 2013); high comparability of exposed and non-exposed (Rhee et al., 2023a); extensive control for potential confounding, also including education, BMI and diet (Eriksen et al., 2009); a large study size (Winquist et al., 2023); and examination of more aggressive (i.e. fatal) prostate cancer (Steenland and Woskie, 2012; Raleigh et al., 2014), although cases were few in the latter. One study of incident prostate cancer in an occupational cohort found a higher risk in the second to fourth quartiles based on estimated cumulative PFOA exposure, compared with the lowest quartile, but without a consistent exposure–response trend (Steenland et al., 2015).

An inherent issue in all studies of incident prostate cancer was detection bias in populations undergoing different levels of medical surveillance, because of the common occurrence of latent disease that may be detected by blood assays, and which may cause bias in an unpredictable direction.

The Working Group concluded that the results of several studies addressing diverse populations in different countries and with different designs fairly consistently did not indicate an association between exposure to PFOA and prostate cancer, but considering exposure misclassification that most probably caused bias towards the null, issues related to outcome ascertainment, and latency periods of < 30 years in several studies, the epidemiological evidence did not preclude that such associations may exist.

(e) Breast cancer

The available evidence included two occupational cohort studies with high-level exposure to PFOA but with few cases of incident (Raleigh et al., 2014) or fatal (Steenland and Woskie, 2012) breast cancer, one large cohort study addressing high-level environmental exposure of community residents on the basis of modelled estimates of individual cumulative lifelong serum PFOA concentrations (Barry et al., 2013), and a case–control study of Mid-Ohio Valley residents with a high level of exposure (Vieira et al., 2013, partly overlapping Barry et al., 2013). These studies found no associations between PFOA exposure and breast cancer overall but did not separately evaluate pre- and postmenopausal cancer or subtypes of breast cancer. Moreover, three large cohort or nested case–control studies addressing background exposure of the general population did not report increased risk of incident breast cancer with increasing prediagnostic PFOA serum concentrations, either overall or for pre- or postmenopausal breast cancer, when these were analysed separately (Ghisari et al., 2017; Cohn et al., 2020; Chang et al., 2023). These studies extensively controlled for confounders; however, most could not address risk for specific subtypes. A large case–cohort study of female retirees from a motor vehicle company in China (Dongfeng-Tongji cohort; see Section 2.1.14 for details) reported higher risk of incident breast cancer with higher levels of prediagnostic PFOA plasma concentrations (Feng et al., 2022). The hazard ratio for the highest quartile of PFOA serum concentration versus the lowest was 1.69 (95% CI, 1.05–2.70), with a positive trend. It was not clear how this selected sample of retirees compared with the general population of Chinese women and how selection might influence risk estimates. Moreover, a large cohort study in France of primarily teachers (E3N cohort; see Section 2.1.10 for details) found an increased risk with wide confidence intervals for postmenopausal cancer in the second quartile of prediagnostic PFOA serum concentrations (OR, 1.69; 95% CI, 0.89–3.21) but not in higher quartiles (Mancini et al., 2020a). There was no indication of an exposure–response relation, and risk estimates were not adjusted for PFOS, which also was related to risk of breast cancer in this study.

The association between PFOA and breast cancer was also studied in a large, nested case–control study of US teachers (CTS; see Section 2.1.8 for details, Hurley et al., 2018) and five non-nested case–control studies that all evaluated associations with background exposure of the general population (Wielsøe et al., 2017; Tsai et al., 2020; Itoh et al., 2021; Velarde et al., 2022; Li et al., 2022b). In spite of methodological strengths, such as extensive control for potential confounding, including a range of known determinants related to reproduction, lifestyle, and other environmental contaminants in some of the studies, all were based on measurement of PFOA in postdiagnostic blood samples, and only three studies specified whether efforts were made to collect samples before treatment (Tsai et al., 2020; Velarde et al., 2022; Li et al., 2022b). Moreover, several studies were limited by small sample sizes and control groups with questionable representativeness of the population from which cases were recruited. Findings with respect to breast cancer overall in relation to PFOA exposure were diverse in these six studies – some studies provided indications of increased risk (Wielsøe et al., 2017; Li et al., 2022b), others did not (Hurley et al., 2018; Tsai et al., 2020; Itoh et al., 2021; Velarde et al., 2022).

Breast cancer is a heterogeneous tumour type, and subtypes defined by different ER or PR characteristics may have different etiologies (Yager and Davidson, 2006). Therefore, it might assist causal inference to distinguish risk according to tumour subtype – not least because there is evidence that PFAS compounds may interfere with receptor-mediated hormonal signalling (see Section 4.2.8 for details). Five studies of incident

breast cancer in general population samples provided risk estimates according to breast cancer receptor characteristics, but with inconsistent results. The large, nested case–control studies of the E3N cohort (Mancini et al., 2020a) and the PLCO cohort (Chang et al., 2023) both used prediagnostic serum samples and found some indications of increased risk related to all receptor subtypes (Mancini et al., 2020a; ER+, ER–, PR+, PR–) or to some but not others (Chang et al., 2023; ER–, PR–, ER–/PR–) but without an exposure–response relation, with limited statistical power and without adjustment for the effects of other PFAS (except Chang et al., 2023). Among three case–control studies that used cross-sectional sampling of blood specimens, Li et al. (2022b) reported increased risk with an exposure–response pattern for ER+ and PR+, but not for ER– and PR–. Itoh et al. (2021) reported null or reduced risk in all examined receptor type combinations (ER+/PR+; ER+/PR–; and ER–/PR–), and Tsai et al. (2020) found (with one exception) null or reduced risk in ER+ and ER– subtypes. Although some of these studies were distinguished by extensive adjustment for potential confounders, including both known determinants for breast cancer and, in some cases, other persistent organic compounds (Itoh et al., 2021), they had other methodological drawbacks (for details, see Section 2.4 and Table 2.4). A general issue pertaining to many studies examining receptor subtypes was low statistical power, which complicates causal inference. Similarly, the only study with analyses stratified by polymorphisms in selected xenobiotic and metabolizing genes had limited informativeness because of insufficient statistical power (Ghisari et al., 2017). Finally, a systematic review and meta-analysis included 18 papers of which 11 were eligible for meta-analysis (Chang et al., 2024). The summary rate ratio per 1-unit increase in natural log-transformed serum or plasma PFOA concentration was 0.95 (95% CI, 0.77–1.18) in analyses including all risk estimates. Excluding studies that assessed exposure after diagnosis of breast cancer revealed a summary rate ratio of 1.16 (95% CI, 0.96–1.40). There was considerable heterogeneity across studies.

The Working Group concluded that the most informative epidemiological studies showed a slightly elevated but uncertain association with PFOA. Overall, the two most informative studies (Mancini et al., 2020a; Chang et al., 2023) were null overall but were the only prospective studies that examined postmenopausal breast cancer cases by ER/PR receptor status. Both found non-linear positive associations with ER– and PR– postmenopausal breast cancer. The statistical power was low in studies examining associations with specific tumour subtypes or stratified by levels of endogenous hormone levels (pre- or postmenopausal cancer), limiting the ability to identify causal associations. Moreover, there were few data on risk at exposure levels above background. Overall, despite some evidence of associations for certain subgroups, the available epidemiological evidence was not considered sufficiently consistent to permit a conclusion to be made about the presence of a causal association between exposure to PFOA and breast cancer.

(f) Thyroid cancer

The study of residents with high environmental exposure in the Mid-Ohio Valley, USA, included 86 cases of validated incident cancer and was considered the most informative of a total of seven studies providing data on risk of incident cancer of the thyroid gland (Barry et al., 2013). This study found indications of increased risk of incident thyroid cancer overall but no exposure–response relation, attenuated 10-year lagged risk estimates, and wide confidence intervals (HR per unit cumulative serum PFOA concentration, natural log scale, no lag, 1.10; 95% CI, 0.95–1.26). The corresponding hazard ratio in the subset of workers with substantially higher exposure at the polymer-production plant in Parkersburg,

West Virginia, USA, was 1.93 (95% CI, 1.00–3.71) but with strong attenuation in 10-year lagged analyses based on 8 cases (Barry et al., 2013). The partly overlapping case–control studies in West Virginia and Ohio based on 343 cases did not observe an increased risk of incident thyroid cancer in relation to PFOA exposure (Vieira et al., 2013). A case–control study nested within a cohort of nulliparous pregnant women in Finland found no associations overall but weak associations in women aged > 40 years at diagnosis (OR, 1.20; 95% CI, 0.71–2.01) (Madrigal et al., 2024), and a small nested case–control study likewise reported null results (van Gerwen et al., 2023). Two occupational mortality studies of predominantly male workers were considered uninformative because of low numbers and because mortality is a less appropriate outcome measure because of the high survival rate for thyroid cancer (Leonard et al., 2008; Lundin et al., 2009). Finally, two case–control studies in China addressing background exposure levels reported strong inverse associations between postdiagnostic PFOA serum concentrations and risk of thyroid cancer (Liu et al., 2022; Li et al., 2023).

The Working Group concluded that there was no consistent epidemiological evidence for increased risk of thyroid cancer in relation to occupational or environmental exposure to PFOA across the available studies, which generally had small numbers of cases.

(g) Liver cancer

Of the nine studies addressing the association between PFOA and liver cancer, the cohort study of residents with high environmental exposure in Mid-Ohio Valley, USA, (Barry et al., 2013) and the Diet, Cancer and Health Cohort (Eriksen et al., 2009) were considered particularly informative. These cohorts provided incidence data, had large and well-characterized study populations with high completeness of verified cases, validated lifelong estimates of cumulative internal exposure or prediagnostic serum sampling, and meticulous control for confounding, including adjustment for tobacco smoking, alcoholic beverage consumption, and – in one study – occupation as a waiter or cook, which have been associated with risk of liver cancer. These studies found no associations between levels of PFOA exposure and risk of liver cancer. This finding was also consistent with results of the Ohio and West Virginia cancer registry-based case–control studies (Vieira et al., 2013; for details, see Section 2.1.22), which was also in a highly exposed population (overlapping with Barry et al., 2013). Six other studies of liver cancer reported similar essentially null results but were considered less informative. Four of these were occupational cohort studies with very high exposure contrast but with too few cases for causal inference (Steenland and Woskie 2012; Consonni et al., 2013; Raleigh et al., 2014; Girardi and Merler, 2019). A nested case–control study of the MEC was distinguished by its study of non-viral HCC and found no associations with prediagnostic PFOA plasma concentrations (Goodrich et al., 2022). Finally, a hospital-based case–control study in China was less informative because of various methodological limitations (Cao et al., 2022; for details, see Section 2.5.1). The Working Group concluded that most findings for liver cancer were null, and that most studies, including the one positive high-exposure study (Girardi and Merler, 2019), had few cases.

(h) Pancreatic cancer

Two occupational cohort studies of mortality (Steenland and Woskie, 2012; Raleigh et al., 2014) and incident cancer (Raleigh et al., 2014), a cohort study of a community with high environmental exposure (Barry et al., 2013), and the large nested case–cohort or case–control studies addressing background exposures in the general population in Denmark (Eriksen et al., 2009), in the US PLCO cohort (Zhang et al., 2023), and the US CPS-II LifeLink Cohort

(Winquist et al., 2023) found no indications of increased risk of pancreatic cancer in relation to PFOA exposure. In contrast, a case–control study of male smokers nested within a cancer prevention study in Finland reported an overall increased risk of pancreatic cancer (OR per SD increase in PFOA on the $\log_{10}$ scale, 1.27; 95% CI, 1.04–1.56) (Zhang et al., 2023). The reasons for these discrepant findings compared with earlier studies were unknown. Men within the PLCO cohort who had ever smoked did not have an increased risk of pancreatic cancer (Zhang et al., 2023; see Table S2.5; Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636). Finally, in the international occupational mortality study of TFE synthesis and polymerization workers, the SMR for workers with the highest cumulative exposure estimate versus the national reference rate was 1.84 (95% CI, 0.67–4.00; 10 exposed cases) (Consonni et al., 2013).

The Working Group concluded that the epidemiological evidence on risk of pancreatic cancer at high levels of occupational exposure to PFOA concerned very few exposed cases and that findings in studies addressing high environmental and background levels were generally null.

(i) Colorectal cancer and cancers of the digestive tract other than liver and pancreas

The most informative occupational cohort study that investigated risk of colorectal cancer found increased incidence in the third and fourth quartiles versus the first quartile of estimated cumulative PFOA serum concentrations but with wide confidence limits and without an exposure–response relation (Steenland et al., 2015). The results of four occupational mortality studies were conflicting, but all reported on very few exposed cases (Leonard et al., 2008; Lundin et al., 2009; Consonni et al., 2013; Girardi and Merler, 2019). The case–control study in West Virginia and Ohio, USA, reported an increased incidence of colorectal cancer in participants with high, but not very high, estimated PFOA serum levels (OR, 1.3; 95% CI, 1.0–1.7; Vieira et al., 2013), but these findings were not corroborated by those of the partly overlapping study of Mid-Ohio Valley residents (Barry et al., 2013). Risk of oesophagus and stomach cancer was addressed by the Mid-Ohio Valley study (Barry et al., 2013), with essentially null findings, and by four occupational mortality studies (Leonard et al., 2008; Lundin et al., 2009; Consonni et al., 2013; Girardi and Merler, 2019). The findings of these studies were conflicting but all – except Consonni et al. (2013) – included fewer than 5 exposed cases (see Section 2.5.3 and Table S2.5 for details; Annex 4, Supplementary material for Section 2, Cancer in Humans, online only, available from: https://publications.iarc.who.int/636).

The Working Group concluded that there was no clear or consistent epidemiological evidence for an increased risk of cancer of the colorectum, oesophagus, or stomach in relation to PFOA exposure. There were no studies in low-exposure general populations. In occupational studies or studies on high environmental exposure, there were very few exposed cases, resulting in highly uncertain risk estimates.

(j) Cancers of lymphatic and haematopoietic tissue

The study of residents with high environmental exposure in the Mid-Ohio Valley, USA, found no indications of increased risk of incident leukaemia or non-specified lymphoma (Barry et al., 2013). These findings were fairly consistent with those from a US case–cohort study (Winquist et al., 2023) and with the mortality study of the fluoropolymer worker cohort in the Parkersburg polymer-production plant, which did not find indications of increased risk of fatal NHL or leukaemia in exposed workers (Steenland and Woskie, 2012). In contrast, the case–control studies in West Virginia and Ohio, USA, reported

an increased incidence of NHL among exposed groups and provided some indications of an exposure–response relation. The OR for residents with the highest estimated PFOA serum concentration, assuming 10-year residency and latency, versus unexposed residents was 1.8 (95% CI, 1.0–3.4; Vieira et al., 2013). The reason for this discrepancy was unknown, but the cohort study with modelled and validated lifelong cumulative exposure assessment was considered to be the most informative. Other occupational mortality studies added little to the evidence because of few exposed cases, resulting in very imprecise risk estimates, reporting risk for other subgroups of cancers of lymphatic and haematopoietic tissue, crude exposure assessment, and other methodological issues (Gilliland and Mandel, 1993; Lundin et al., 2009; Consonni et al., 2013; Girardi and Merler, 2019; for details, see Section 2.6.3).

The Working Group concluded that the studies addressing effects at high occupational exposure levels included very few exposed cases of cancers of lymphatic and haematopoietic tissue and that the strongest evidence on risk related to high environmental levels did not indicate an increased risk. Overall, the epidemiological evidence was insufficient to permit causal inference and to exclude the possibility that causal associations between PFOA exposure and cancer of lymphatic and haematopoietic tissue may exist.

(k) Other cancer types

One study of community residents with high exposure found weak indications of increased risk of brain cancer (Vieira et al., 2013), but findings were not corroborated by those of the most informative study of highly exposed Mid-Ohio Valley residents (Barry et al., 2013). Two occupational mortality studies did not find an increased risk, but there were 5 or fewer deaths in exposed people (Lundin et al., 2009; Consonni et al., 2013). No increased risk for melanoma associated with PFOA exposure was reported in the study of residents with high environmental exposure in the Mid-Ohio Valley, USA (Barry et al., 2013; Vieira et al., 2013). Two occupational cohort mortality studies included very few exposed cases of melanoma, resulting in very imprecise risk estimates (Leonard et al., 2008; Steenland et al., 2015). A positive association between PFOA exposure and mesothelioma was reported in one study of a polymer-production plant in Parkersburg, West Virginia, USA (Steenland and Woskie, 2012), but this finding was not replicated in other studies and was likely to be caused by exposure to asbestos at the plant. Some indication of an increased risk of lung cancer in relation to exposure to PFOA was reported in one study (Vieira et al., 2013), but five other studies, including some that were highly informative, did not find an increased risk (Alexander et al., 2003; Steenland and Woskie, 2012; Barry et al., 2013; Consonni et al., 2013; Girardi and Merler, 2019). The available epidemiological evidence base for evaluation of cancer at these organ sites was sparse and generally null. Finally, a case–control study found indications of an increased risk of retinoblastoma (adjusted OR per IQR increase in blood PFOA, 1.03; 95% CI, 0.97–1.09) in a population with background exposure levels (Chen et al., 2024).

(l) All sites combined

Three occupational cohort studies did not find indications of increased mortality from all types of cancers combined in analyses based upon internal comparisons or comparisons with non-exposed workers, which reduce the likelihood that risk estimates were attenuated because of primary healthy-worker selection or healthy-worker survivor bias (Steenland and Woskie, 2012; Consonni et al., 2013; Raleigh et al., 2014). These results are in line with those of the NHANES 1999–2014 cohort addressing general population background exposure, in which cancer mortality was not associated with PFOA serum levels (Wen et al., 2022). In contrast

to these five studies that all had high statistical power, one small study in Italy of perfluorocarbon-production workers who had the highest serum levels of PFOA ever published provided some, but inconsistent, indications of increased mortality from all cancers combined (Girardi and Merler, 2019).

The Working Group noted that null associations observed in studies of overall risk of cancer are of minimal value when it comes to causal inference of cancer etiology, because specific compounds such as PFOA cannot be expected to contribute to the occurrence of all cancer types, and therefore associations with any specific cancer type may be masked by null associations with other cancer types.

2.8.6 Specific cancer sites and exposure to PFOS

(a) Kidney cancer

The Swedish Ronneby Register cohort study of residents with high environmental exposure found some evidence for an increased risk of incident kidney cancer; when comparing with unexposed residents, the hazard ratio for residents with longer exposure was 1.47 (95% CI, 0.87–2.49) and for residents with more recent exposure was 1.85 (95% CI, 1.00–3.40) (Li et al., 2022a). However, the study did not distinguish the effects of PFOS (the predominant PFAS in drinking-water in this population) from those of other PFAS compounds that were also present at exposure levels above background (Li et al., 2022a). Of the two large nested case–control studies of general populations with substantial lower background exposure and with prediagnostic blood samples, one study observed an association in analyses not adjusted for other PFAS; however, neither study observed associations after adjustment for other PFAS (Shearer et al., 2021; Rhee et al., 2023b). A third large nested population-based case–control study also found no association overall, in men or women (Winquist et al., 2023). The only occupational cohort study that primarily addressed PFOS exposure (Alexander and Olsen, 2007) did not report kidney cancer data.

The Working Group considered that the epidemiological evidence on the association between PFOS and kidney cancer was too sparse to permit evaluation.

(b) Testicular cancer

The study of US Air Force servicemen exposed to levels comparable to those in the general population was the only available study with a sufficient number of cases that addressed the risk of TGCT (the most common type of testicular cancer) related to PFOS exposure and adjusted for other PFAS (Purdue et al., 2023). In an analysis that included the entire study population (using first or only samples), the adjusted OR for the highest versus the lowest exposed quartile was 1.8 (95% CI, 0.9–3.6; *P* for trend, 0.15), whereas the OR for the subset of the population with repeated blood samples (second blood sample only; about one third of the participants) was 4.6 (95% CI, 1.4–15.1; *P* for trend, 0.009). These estimates were adjusted for other PFAS. The reasons for these discrepant results – if not due to chance – were unknown. The men with repeated samples had accumulated more exposed years, but PFOS measurement levels were similar between the two samples, and both were similar to background levels (e.g. as measured in NHANES). Both measurements were obtained by analysis of samples collected on average about 5 years before diagnosis. The Ronneby Register cohort study reported a hazard ratio of 1.51 (95% CI, 0.56–4.03; 45 incident cases) among residents with the longest exposure compared with residents in the same municipality who were not exposed to contaminated drinking-water (Li et al., 2022a). Although PFOS was the main contaminant of drinking-water among Ronneby residents, it was not possible to distinguish the effects of PFOS

from those of other PFAS compounds that were also present at levels above background.

The Working Group concluded that in the two available studies, an imprecise or inconsistent positive association was observed between PFOS exposure and cancer of the testis. Overall, the evidence did not permit the evaluation of a causal association between PFOS and testicular cancer because there were too few informative studies, unexplained inconsistencies between findings, or potential confounding by other PFAS compounds (i.e. PFHxS).

(c) Bladder cancer

An occupational cohort study of PFOS-exposed workers at a chemical plant in Alabama, USA, found indications of an increased incidence of bladder cancer in workers with the highest cumulative PFOS exposure in internal comparisons (Alexander and Olsen, 2007), but these included only a small number of cases, and co-exposure to other PFAS was likely. Incomplete registration and ascertainment of diagnoses may have caused non-differential misclassification of the outcome and bias towards the null. The Ronneby Register cohort study reported moderately elevated risks of bladder cancer among residents with later and longer exposure to PFOS-contaminated drinking-water compared with unexposed residents (HR, 1.50; 95% CI, 0.98–2.28; and HR, 1.39; 95% CI, 0.95–2.02; respectively) (Li et al., 2022a). The group-based exposure assessment not accounting for individual variation limited the options to explore risk at the full range of exposure. The Danish Diet, Cancer, and Health Cohort study (Eriksen et al., 2009) and a US case–cohort study (Winquist et al., 2023) did not find increased risk of bladder cancer. Eriksen et al. (2009) applied a more stringent design including detailed adjustment for smoking and several occupations that have been associated with bladder cancer. Although the average exposure was lower than in Ronneby, exposure levels were overlapping. The Working Group concluded that there were findings indicating an increased risk of bladder cancer in two studies of workers and residents with high environmental exposure, but not in two studies of populations with lower (background) levels of PFOS exposure. The Working Group concluded that there were too few studies available to permit a conclusion to be drawn about the association between PFOS and bladder cancer.

(d) Prostate cancer

Two highly informative cohort studies of background exposure levels in the general Danish and US population investigated the association between PFOS and risk of incident prostate cancer (Eriksen et al., 2009; Rhee et al., 2023a). Eriksen et al. reported moderately increased risks of prostate cancer in the three upper quartiles compared with the lowest quartile, but exposure–response analyses did not provide solid evidence for a linear trend (IRR per 10 ng/mL increase in PFOS concentration, 1.05; 95% CI, 0.97–1.14). The US population-based study reported an OR per unit increase on $\log_2$ scale of 0.99 (95% CI, 0.79–1.23) (Rhee et al., 2023a). Other environmentally exposed population studies did not provide substantial additional information (Hardell et al., 2014; Li et al., 2022a; Winquist et al., 2023). In particular, there were no data available from occupational cohorts with much higher exposure levels.

The Working Group concluded that two large cohort studies of the general population found no consistent evidence for increased risk of prostate cancer in relation to PFOS exposure, but there were no available data at higher exposure levels in an occupational setting.

(e) Breast cancer

Almost all the available studies on associations between PFOS and breast cancer were based upon general population samples, which limited causal inference because of the narrow ranges of exposure. Findings in the most informative

nested case–control and case–cohort studies based upon large samples, incident data, prediagnostic serum PFOS measurements, and good confounder control were partly conflicting. The study conducted in the French E3N Cohort (Mancini et al., 2020a) provided some indications of an increased risk of overall breast cancer at higher PFOS exposure levels. This association appeared to be stronger and linear when restricted to hormone receptor-positive breast cancers. Similarly, in the US PLCO cohort (Chang et al., 2023), PFOS appeared to be positively associated only with hormone receptor-positive breast cancers. These results were not confirmed in the case–cohort study conducted in the Dongfeng-Tongji cohort in China (Feng et al., 2022), or in the CPS-II LifeLink Cohort (Winquist et al., 2023); however, these two studies did not explore the association by hormone-receptor tumour subtype. The only informative available study of populations with higher environmental exposure, the Swedish Ronneby Register cohort study, did not find associations between exposure to PFAS (including PFOS) and overall breast cancer risk, but did not investigate associations with specific tumour subtypes (Li et al., 2022a). Findings in several case–control studies were less informative because of methodological issues relating to postdiagnostic or post-treatment PFOS measurements, and potential confounding (for details, see Section 2.4 and Table 2.4).

In summary, there was little evidence of an association between PFOS exposure and breast cancer overall. However, the two most informative studies (Mancini et al., 2020a; Chang et al., 2023), which were the only prospective studies to examine the association by hormone-receptor tumour subtype, found an imprecise but increased risk of hormone receptor-positive breast cancers associated with higher levels of PFOS. This finding was somewhat contradicted by the null findings among postmenopausal women in the Dongfeng-Tongji cohort (Feng et al., 2022) and the CPS-II cohort (Winquist et al., 2023), which did not stratify by receptor status (most postmenopausal breast cancers are hormone receptor-positive). Given the inconsistencies across studies, the Working Group considered that the available evidence on risk of breast cancer associated with PFOS exposure was inconclusive.

(f) Thyroid cancer

The only studies available to the Working Group were the Swedish Ronneby Register cohort study of residents with high environmental exposure (Li et al., 2022a); a case–control study nested within the FMC including nulliparous women from the general population (Madrigal et al., 2024); a case–control nested within the Bio*Me* cohort (van Gerwen et al., 2023); and two case–control studies on risk related to background exposure of the general population (Liu et al., 2022; Li et al., 2023). The Ronneby study reported an increased risk of thyroid cancer (type unspecified) in exposed women (SIR based on regional reference rates, 2.08; 95% CI, 1.19–3.38; 16 exposed cases) but not in men (3 exposed cases). The FMC study did find indications of an increased risk of papillary thyroid cancer among women diagnosed at age < 40 years; however, when adjusted for exposure to other PFAS, the association was greatly attenuated – the OR for serum PFOS increment by $\log_2$ in women aged < 40 years at diagnosis was 1.14 (95% CI, 0.68–1.93; 185 cases). Although PFOS was present at by far the highest concentrations in contaminated drinking-water in the Ronneby municipality, the effects of other PFAS could not be accounted for in this study (Li et al., 2022a), the study by van Gerwen et al. (2023) had very small numbers in the longitudinal subsample, and the two case–control studies were less informative because of postdiagnostic measurements of exposure, potential for bias related to selection of reference groups, or few cases (Liu et al., 2022; Li et al., 2023).

The Working Group noted that there were inconsistent indications of positive associations between PFOS exposure and thyroid cancer in women, but that, overall, studies were too few to permit an evaluation of causal associations.

(g) Liver cancer

Of the five studies addressing the association between PFOS and liver cancer, the nested case–cohort study of the Danish Diet, Cancer, and Health Cohort (Eriksen et al., 2009) was considered particularly informative because of the large well-characterized study population, high completeness of verified cases, prediagnostic serum sampling, and meticulous control for confounding. No association between levels of PFOS exposure and risk of liver cancer was identified. This study was limited by low exposure levels and rather narrow exposure contrast, but its findings were consistent with those of the Ronneby Register cohort study that addressed much higher environmental exposure (Li et al., 2022a). Also, the Ronneby study did not find an increased risk of liver cancer but included < 10 exposed cases. An occupational mortality study was not informative because it included even fewer cases (Alexander et al., 2003). In contrast, the nested case–control study in the MEC, which was distinguished by its study of non-viral HCC, found some indications of an increased, BMI-adjusted, risk for higher plasma PFOS concentrations (> 54.9 ng/mL, corresponding to the NHANES 90th percentile) compared with lower concentrations (OR, 2.90; 95% CI, 0.78–10.00), but without a clear exposure–response relation (Goodrich et al., 2022) and a risk estimate based upon a post-hoc grouping of exposure. Finally, a Chinese hospital-based case–control study was less informative because of various methodological limitations (Cao et al., 2022; for details see Section 2.5.1).

The Working Group concluded that the most informative studies found no associations between PFOS exposure and risk of liver cancer and, overall, the available epidemiological studies were too few to permit an evaluation of causal associations.

(h) Other cancer types

One cohort study of residents with high environmental exposure (Li et al., 2022a) and two large studies of background exposure (Eriksen et al., 2009; Zhang et al., 2023) found no indications of increased risk of pancreatic cancer. An occupational mortality study of PFOS-production workers in Decatur, Alabama, USA, found no increased risk of fatal cancer of the digestive organs and peritoneum combined, but estimates were based on only 5 deaths (Alexander et al., 2003). The Ronneby Register cohort found no significant increase in the incidence of cancer of the colon, rectum, oesophagus, or stomach in residents with high environmental exposure (Li et al., 2022a). The only available study on associations between PFOS and brain cancer was the Ronneby Register cohort study, which found weak indications of an increased risk of incident brain cancer among highly exposed residents (Li et al., 2022a), but risk estimates were imprecise and the effects of PFOS – which was present at highest concentrations in contaminated drinking-water – and other PFAS present at lower concentrations could not be distinguished. Two occupational cohort studies of fatal cancer of lymphatic and haematopoietic tissue in workers with exposure to PFOS were not informative because of the crude exposure assessments and very few cases (Alexander et al., 2003; Girardi and Merler, 2019). The Ronneby Register cohort study had greater statistical power, but lower exposure levels did not reveal any increased risk of several specific types of cancer of lymphatic and haematopoietic tissue, including NHL, multiple myeloma, and chronic lymphocytic leukaemia, whereas residents who had ever been exposed had a higher risk of chronic myeloid leukaemia but with too few cases to allow more detailed analysis (Li et al., 2022a). One occupational

cohort study addressed risk of fatal melanoma, but the few cases resulted in very imprecise estimates (Alexander et al., 2003). The Ronneby Register cohort study had higher statistical power and found some evidence for an increased risk of melanoma in the subset of residents with the latest ever-high exposure to PFAS-contaminated drinking-water (HR, 1.54; 95% CI, 1.09–2.19) but not in those with the longest ever-high exposure (HR, 1.14; 95% CI, 0.80–1.64), compared with those with no exposure. The findings were not corroborated or refuted by other studies. Finally, a case–control study did not find an increased risk of retinoblastoma (adjusted OR per IQR increase in blood PFOS, 1.02; 95% CI, 0.95–1.09) in a population with background exposure levels (Chen et al., 2024).

The Working Group noted that there were too few studies available for the evaluation of associations between PFOS exposure and melanoma, retinoblastoma, and cancers of the pancreas, colon, rectum, oesophagus, stomach, brain, or lymphatic and haematopoietic tissue.

(i) All sites combined

Overall cancer mortality was not increased among PFOS-exposed workers at the PFOS-production facility in Decatur, Alabama, USA (Alexander et al., 2003), and this was consistent with results from the Ronneby Register cohort study in which combined cancer incidence was not elevated in a population of residents with above-background exposure to PFOS in particular, but also PFHxS and PFOA to a lesser degree (Li et al., 2022a). On the other hand, overall cancer mortality was increased among members of the NHANES 1999–2014 cohort who had the highest serum PFOS values compared with the lowest tertile (Wen et al., 2022), and a small study in Italy with low statistical power reported some inconsistent indications of increased all-cancer mortality in workers with very high exposure to PFOS as well as other PFAS (Girardi and Merler, 2019).

The Working Group noted that the results of the few available studies were conflicting and that the number of informative epidemiological studies was too few to permit an evaluation of the evidence on PFOS and PFOA exposure and risk of all cancers combined.

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3. CANCER IN EXPERIMENTAL ANIMALS

3.1 Perfluorooctanoic acid (PFOA)

Perfluorooctanoic acid (PFOA) was previously evaluated by the *IARC Monographs* programme in 2014 and the evaluation was published in Volume 110 (IARC, 2016). In its evaluation at that time, the Working Group concluded that there was *limited evidence* in experimental animals for the carcinogenicity of PFOA. Since the previous evaluation of PFOA by the *IARC Monographs* Programme, there have been new studies investigating the occurrence of cancer in experimental animals in relation to exposure to PFOA.

3.1.1 Mouse

See Table 3.1.

(a) Oral administration (drinking-water)

A cancer promotion study using the KC mouse model was conducted by Kamendulis et al. (2022). A mouse model was developed by selectively introducing a *Kras^{G12D}* mutation in pancreatic ductal cells using a Cre-lox technology, i.e. *PDX-1-Cre;LSL-Kras^{G12D}* transgenic mouse model (KC model) (Hingorani et al., 2003). This KC mouse model spontaneously develops pancreatic intraepithelial neoplasia (PanIN), mimicking human lesions as progression through four stages. At 9 months, 80% of mutant mice have PanIN lesions (considered to be a cancer precursor lesion), and eventually develop invasive and metastatic adenocarcinoma (Hingorani et al., 2003).

Groups of male and female *LSL-Kras^{G12D}; Pdx-1 Cre* (KC) transgenic mice (age, 8 weeks) were treated with PFOA (specifically, the ammonium salt; purity, 96%) at a concentration of 5 ppm in drinking-water for 4 or 7 months. The numbers of mice [number of each sex not reported] were 10 and 11 at 6 months, and 9 and 9 at 9 months, for the control and PFOA groups, respectively. [The Working Group noted that the administered dose of PFOA in milligrams per kilogram body weight (bw) was not reported.] Controls received tap water. PFOA exposure did not significantly alter body weight at either time point in treated mice compared with controls. No information on survival or food consumption was reported. At the end of the feeding period, the mice were killed (at age 6 or 9 months). PanIN grade, inflammation score and stroma evaluation were performed by pathologists blinded to the experimental groups on haematoxylin-and-eosin-stained slides using light microscopy to evaluate each section.

Administration of PFOA in drinking-water did not cause a significant increase in the incidence of any type of neoplasm in either males or females.

In the same study, there was a significant increase in both the PanIN lesion area (58%) and the number of PanIN lesions per mm^2 of pancreas

Table 3.1 Studies of carcinogenicity in mice exposed to PFOA

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Initiation–promotion (tested as promoter) Mouse (transgenic), *LSL-Kras^{G12D};Pdx-1 Cre* (KC) (M, F) (combined) 2 mo 6 mo Kamendulis et al. (2022)	Oral administration (drinking-water) PFOA (ammonium salt), 96% Tap water 0, 5 ppm, for 4 mo 10, 11 NR, NR	No significant increase in tumour incidence in treated animals		*Principal strengths*: end-points studied at two time points (age 6 and 9 mo, see below); PFOA measured in serum and pancreatic tissue at both time points; PanIN grade, inflammation score and stroma evaluation performed by pathologists blinded to the treatment. *Principal limitations*: intake of drinking-water was not measured, thus the PFOA dose was not known; data were combined for males and females; only one dose used; no survival data; short duration of exposure; limited number of animals per group. *Other comments*: the mean PanIN grade did not significantly differ between control and PFOA-treated mice at 6 mo; the composite histopathology severity score derived by incorporating PanIN lesion stage, inflammation and stromal density, was significantly increased at 6 mo; the lesion number per area was significantly increased.
Initiation–promotion (tested as promoter) Mouse (transgenic), *LSL-Kras^{G12D};Pdx-1 Cre* (KC) (M, F) (combined) 2 mo 9 mo Kamendulis et al. (2022)	Oral administration (drinking-water) PFOA (ammonium salt), 96% Tap water 0, 5 ppm for 7 mo 9, 9 NR, NR	No significant increase in tumour incidence in treated mice		*Principal strengths*: end-points studied at two time points (age 6 and 9 mo); PFOA measured in serum and pancreatic tissue at both time points; PanIN grade, inflammation score, and stroma evaluation performed by pathologists blinded to the treatment. *Principal limitations*: intake of drinking-water was not measured, thus the PFOA dose was not known; data were combined for males and females; only one dose used; no survival data; short duration of exposure; small number of animals per group. *Other comments*: the mean PanIN grade did not significantly differ between control and PFOA-treated mice at 9 mo; the composite histopathology severity score, derived by incorporating PanIN lesion stage, inflammation, and stromal density, was not significantly increased at 9 mo; the lesion number per area was not significantly increased.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J-*Apc*$^{Min/+}$ (M) Day 1 of gestation 11 wk Ngo et al. (2014)	Gavage PFOA (ammonium salt), ≥ 98% Water 0, 0.01, 0.1, 3.0 mg/kg bw per day 15, 3, 19, 0 15, 3, 19, 0	*Small intestine (duodenum, jejunum or ileum)* Tumour incidence: 15/15, 3/3, 19/19, 0/0 Tumour multiplicity: 146.7 ± 72.4, 128.0 ± 127.1, 82.2 ± 38.3, NR *Colon* Tumour incidence: 12/15 (80%), 3/3 (100%), 17/19 (89%), 0/0 Tumour multiplicity: 2.5 ± 2.2, 4.0 ± 3.5, 2.4 ± 2.2, NR	 NS NS NS NS	*Principal strengths*: males and females studied; multiple doses used; analysed background levels of PFOA in feed and drinking-water; analysed internal doses of PFOA; tested stability of PFOA; blocks of PFOA administration were compared statistically. *Principal limitations*: no histopathological examination of the small intestinal tumours was performed; small number of mice per group. *Other comments*: study of transplacental exposure; increase in the incidence and multiplicity of spontaneous tumours was studied in this mouse model; small intestinal tumours were observed in all *Min*/+ mice in all experimental groups, including the vehicle group, demonstrating 100% incidence in this end-point, as is usual in this mouse model.
Full carcinogenicity Mouse, C57BL/6J-*Apc*$^{Min/+}$ (F) Day 1 of gestation 11 wk Ngo et al. (2014)	Gavage PFOA (ammonium salt), ≥ 98% Water 0, 0.01, 0.1, 3.0 mg/kg bw per day 23, 15, 26, 2 23, 15, 26, 2	*Small intestine (duodenum, jejunum or ileum)* Tumour incidence: 23/23, 15/15, 26/26, 2/2 Tumour multiplicity: 151.0 ± 102.3, 102.9 ± 40.7, 119.5 ± 73.0, 84.0 ± 36.8 *Colon* Tumour incidence: 9/23 (39%), 8/15 (53%), 17/27 (63%), 1/2 (50%) Tumour multiplicity: 0.6 ± 1.0, 0.8 ± 1.1, 1.1 ± 1.1, 1.0 ± 1.4	 NS NS NS NS	*Principal strengths*: males and females studied; multiple doses used; analysed background levels of PFOA in feed and drinking-water, analysed internal doses of PFOA, tested stability of PFOA, blocks of PFOA administration were compared statistically. *Principal limitations*: no histopathological examination of the small intestinal tumours was performed; small number of mice per group; and short duration. *Other comments*: study of transplacental exposure; increase in incidence and multiplicity of spontaneous tumours was studied in this mouse model; small intestinal tumours were observed in all *Min*/+ mice in all experimental groups including the vehicle group, demonstrating 100% incidence in this end-point, as is usual in this mouse model.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Mouse, CD-1 (F) Exposure on days 1–17 of gestation Killed at 18 mo Filgo et al. (2015)	Gavage PFOA (ammonium salt), > 98% (pure, linear product) Deionized water Control, 0.01, 0.1, 0.3, 1, 5 mg/kg bw, once per day 29, 29, 37, 26, 31, 21 29, 29, 37, 26, 31, 21	*Liver*		*Principal strengths*: three mouse strains used; multiple doses used. *Principal limitations*: only females were studied; number of tumours per animal was not reported; no statistical comparison between blocks of mice was reported; there was no statement that the dams were randomized to the treatment groups. *Other comments*: for mice killed before 18 mo when tumours were counted, only the percentage of mice born, which is unknown, was stated; thus, the numbers of mice reported at the start and surviving are both the numbers surviving at 18 mo and included in the study.
		Hepatocellular adenoma		
		0/29, 0/29, 1/37 (2.7%), 4/26* (15.38), 0/31, 1/21 (4.8%)	**P* < 0.05, Fisher exact test	
		Hepatocellular adenoma, multiple		
		0/29, 1/29 (3.4%), 0/37, 0/26, 0/31, 0/21	NS	
		Hepatocellular carcinoma		
		0/29, 0/29, 0/37, 1/26 (3.8%), 0/31, 1/21 (4.8%)	NS	
		Haemangiosarcoma		
		0/29, 0/29, 0/37, 1/26 (3.8%), 0/31, 2/21 (9.5%)	*P* < 0.01, Cochran–Armitage trend test	
		Histiocytic sarcoma		
		0/29, 0/29, 1/37 (2.7%), 0/26, 1/31 (3.2%), 1/21 (4.8%)	NS	
		Lymphoma		
		1/29 (3.4%), 0/29, 0/37, 1/26 (3.8%), 1/31 (3.2%), 1/21 (4.8%)	NS	
Full carcinogenicity Mouse, 129/Sv wildtype (F) Exposure on days 1–17 of gestation Killed at age 18 mo Filgo et al. (2015)	Gavage PFOA (ammonium salt), > 98% (pure, linear product) Deionized water Control, 0.1, 0.3, 0.6, 1 mg/kg bw, once per day 10, 10, 8, 6, 8 10, 10, 8, 6, 8	*Liver*		*Principal strengths*: three mouse strains used; multiple doses used. *Principal limitations*: only females were studied; number of tumours per mouse was not reported; no statistical comparison between blocks of mice was reported; there was no statement that the dams were randomized to the treatment groups. *Other comments*: for mice killed before 18 mo when tumours were counted, only the percentage of mice born, which is unknown, was reported. Thus, the numbers of mice given at start and surviving are both the numbers surviving at 18 mo and included in the study.
		Adenoma		
		0/10, 0/10, 0/8, 0/6, 0/8	NS	
		Haemangiosarcoma		
		0/10, 0/10, 0/8, 0/6, 0/8	NS	
		Histiocytic sarcoma		
		0/10, 1/10 (10%), 0/8, 0/6, 0/8	NS	
		Ito cell tumour		
		0/10, 0/10, 0/8, 0/6, 0/8	NS	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Mouse, 129/Sv PPARα knockout (F) Exposure on days 1–17 of gestation Killed at age 18 mo Filgo et al. (2015)	Gavage PFOA (ammonium salt), > 98% (pure, linear product) Deionized water Control, 0.1, 0.3, 1, 3 mg/kg bw, once per day 6, 10, 10, 9, 9 6, 10, 10, 9, 9	*Liver*		*Principal strengths*: three mouse strains used; multiple doses used. *Principal limitations*: only females were studied; number of tumours per mouse was not reported; no statistical comparison between blocks of mice was reported; there was no statement that the dams were randomized to the treatment groups. *Other comments*: for mice killed before 18 mo when tumours were counted, only the percentage of mice born, which is unknown, was reported. Thus, the numbers of mice given at start and surviving are both the numbers surviving at 18 mo and included in the study.
		Hepatocellular adenoma		
		0/6, 1/10 (10%), 1/10 (10%), 1/9 (11%), 2/9 (22%)	NS	
		Haemangiosarcoma		
		1/6 (16.7%), 0/10, 0/10, 0/9, 0/9	NS	
		Histiocytic sarcoma		
		0/6, 0/10, 0/10, 0/9, 0/9	NS	
		Ito cell tumour		
		0/6, 0/10, 1/10 (10%), 0/9, 0/9	NS	

bw, body weight; F, female; M, male; mo, month(s); NR, not reported; NS, not significant; PanIN, pancreatic intraepithelial neoplasia; PFOA, perfluorooctanoic acid; PPARα, peroxisome proliferator-activated receptor alpha; ppm, parts per million; vs, versus; wk, week(s).

(twofold) in the group receiving PFOA versus the controls at 6 months, but not at 9 months. [The Working Group noted that PanIN progresses to pancreatic ductal adenocarcinoma through stages characterized by morphological changes and nuclear atypia (see Hezel et al., 2006).] [The Working Group noted that this model effectively examines the shortening of latency by the treatment. The Working Group also noted that this study included two time points and measured PFOA concentrations in the serum and tissue at both time points; however, there was a lack of information on the number of animals per sex (males and females were combined); a limited number of animals (for most end-points, the number of mice per group was 9–11); a lack of information on survival and on randomization to treatment groups; one dose only was used; and there was a lack of information on the exact dose of PFOA ingested, since the intake of drinking-water was not measured.]

(b) Transplacental exposure

Ngo et al. (2014) examined the tumorigenic effects of gestational exposure to PFOA (specifically, the ammonium salt) in C57BL/6J-*Apc*$^{Min/+}$ mice, a mouse model that develops spontaneous intestinal tumours because of a heterozygous *Min* mutation in the tumour suppressor gene adenomatous polyposis coli (*Apc*). These mice are sensitive to chemicals that mutate or delete (parts of) the remaining wildtype *Apc* allele, and it is a model both for the inherited disorder, familial adenomatous polyposis, and for sporadic colorectal cancer. The wildtype C57BL/6J-*Apc*$^{+/+}$ dams were mated to heterozygous C57BL/6J-*Apc*$^{Min/+}$ males. Pregnant wildtype females were treated with PFOA (purity, ≥ 98%) by gavage at a dose of 0, 0.01, 0.1, or 3.0 mg/kg bw per day on days 1–17 of gestation. Insufficient rates of pregnancy and littering and low F_1 survival were observed in the first experimental block – block 1, 0 (vehicle, distilled water), 0.1, and 3.0 mg/kg per day; 104 exposed dams (age, 7–8 weeks) – thus, a second block was added for which the PFOA exposure was lower – block 2, 0, 0.01, and 0.1 mg/kg per day; 100 exposed dams (age, 9–10 weeks). The PFOA solutions were made separately for the two experimental blocks, and the gavage volumes for all doses were below 1 mL/100 g bw. The PFOA solutions were tested by chemical analysis and found to be stable during the experiment. Furthermore, the tap water (used as drinking-water for the mice) and both the breeding and maintenance diets, as well as the distilled water (used as the vehicle for PFOA), were analysed and showed very low background PFOA levels (picograms per litre and picograms per gram in water and feed, respectively). Internal exposure was quantified (1 or 2 mice per time point) in the dams on day 18 of gestation, postnatal day 23 (block 1) or postnatal days 26–28 (block 2), and F_1 pups on postnatal days 25–27 (depending on the block). The limit of quantification (LOQ) for PFOA was 0.05 ng/mL serum. Although minimal data were generated, they confirmed that the internal exposure within dams and pups increased with dose and decreased with time post-dosing (day 18 of gestation versus postnatal day 23 in dams). Serum concentrations of PFOA were significantly increased in mice exposed to PFOA, with mice in the control groups for both ages exhibiting an average PFOA concentration of 0.003 µg/mL, whereas serum concentrations in PFOA-treated KC mice aged 6 or 9 months were 41.96 ± 16.45 and 26.35 ± 17.53 µg/mL, respectively. PFOA concentrations in pancreatic tissue were also elevated (in the range of nanograms per milligram protein) in mice treated with PFOA. For *Min*/+ F_1 male offspring, the numbers of mice in each dose group were 15, 3, 19, and 0, in the groups exposed to PFOA at a dose of 0 (vehicle, water), 0.01, 0.1, or 3.0 mg/kg bw, respectively. The numbers of *Min*/+ F_1 female offspring obtained in each dose group (both blocks together) were 23, 15, 26, and 2 in the groups exposed in utero to PFOA at dose of 0 (vehicle, water), 0.01, 0.1, or 3.0 mg/kg bw, respectively. For the dams weighed

on days 1–18 of gestation, there were no differences in body weight as area under the curve (AUC, arbitrary units) between the experimental groups, in either experimental block 1 or 2, and there was no difference in body weight between the two experimental blocks. For the pups aged 3–18 days, including both *Min*/+ and wildtype (+/+) mice of both sexes, there were some significant differences in body weight between the treatment groups but in varying directions; thus, there were no consistent differences in pup body weight AUC for the pups between experimental blocks 1 and 2. Considering the individual pups in both experimental blocks, PFOA at doses of 3.0 and 0.1 mg/kg bw per day decreased the pup body weight compared with that of pups treated with the vehicle only (water). The offspring were weaned when aged 21 days and housed as a litter per cage, males and females separately. They were genotyped for the heterozygous *Min*/+ mutation using DNA collected from ear punches. The $Apc^{+/+}$ mice were not expected to develop intestinal tumours at age 11 weeks and were used for studies on non-cancer end-points. All C57BL/6J-$Apc^{Min/+}$ offspring mice were killed at age 11 weeks, before the onset of serious anaemia caused by their spontaneous tumours (based on experience with this model), and were used to study the effects of PFOA on intestinal tumorigenesis. The number, diameter, and localization of tumours in the small intestine and colon were measured by transillumination in an inverse light microscope. The reviewer scored lesions at 20× magnification and was blinded to mouse treatment. The diameter of the tumours was scored using an eyepiece graticule. Statistical analysis of incidence was performed on both an individual and litter basis; furthermore, the two experimental blocks were combined in the analysis if no statistical differences in incidence were found between them.

Neoplastic lesions (tumours of the small intestine) were observed in all *Min*/+ mice in all experimental groups including the vehicle group, demonstrating 100% incidence of this end-point, as is usual in this mouse model. PFOA, at any dose, did not cause a significant increase in the number of small intestine tumours, compared with the vehicle (control). In male *Min*/+ mice only, treated with PFOA at 0.01 mg/kg bw, the small intestine tumours were larger in size than those in mice that were treated with the vehicle. Most of the small intestine tumours were localized in the distal two thirds, i.e. in the middle and distal parts, of the small intestine, irrespective of treatment or sex, as seen in previous experiments with *Min*/+ mice (see Andreassen et al., 2002). [The Working Group noted that there was no clear linear dose–response relation in the number and size of small intestine tumours and that these results were found both when the data were analysed with individual mice or with the litter as the statistical unit.]

The incidence of colon tumours at the individual level showed no significant differences between experimental blocks 1 and 2. The only significant difference between the treatment groups was that the group treated with PFOA at 0.1 mg/kg bw had a higher incidence of colon tumours than did the group treated with the vehicle, for males and females together, in experimental block 1 ($P = 0.039$, Fisher exact test, two-tailed probability). However, when this result was tested with the litter as the statistical unit, it did not reach statistical significance. There were no statistically significant differences in the number or diameter of colon tumours between experimental blocks 1 and 2 on the individual level, and therefore the data from both experimental blocks were evaluated together. There were no significant differences in the number of tumours of the colon in mice from any of the groups treated with PFOA compared with that in mice in the vehicle group. The experimental design, such as duration of the study with termination at 11 weeks, was based on previous experience with this model. [The Working Group noted that, usually, when *Min*/+ mice are killed

at age 11 weeks, most intestinal tumours identified are adenomas (see Moser et al., 1990).] [The Working Group noted that this study used both sexes, multiple doses, tested PFOA stability, and analysed the internal dose and the background levels of PFOA in feed and drinking-water. However, no histological examination of tumours of the small intestine or colon was performed.]

Filgo et al. (2015) studied liver toxicity in CD-1 and 129/Sv strains of mice treated with PFOA (specifically, the ammonium salt; purity, > 98%) administered orally (by gavage) after gestational exposure. Both wildtype and peroxisome proliferator-activated receptor alpha (PPARα)-knockout transgenic 129/Sv mice were used. Two blocks of time-pregnant CD-1 mice (12, 12, 14, 13, 12, and 6 dams) were treated with distilled water (vehicle control), or PFOA at a dose of 0.01, 0.1, 0.3, 1, or 5 mg/kg bw, respectively, resulting in a final number of 29, 29 37, 26, 31, and 21 female offspring per group, respectively, surviving to age 18 months. Some mice died before 18 months (28%, 17%, 16%, 28%, 24%, and 22% of the numbers at the beginning of the experiment from the control group and groups treated with PFOA at 0.01, 0.1, 0.3, 1, and 5 mg/kg bw, respectively), because of sudden unknown causes (found on check; 28% of early deaths) or severe dermatitis (common to CD-1 mice; 32%) and other health problems (40%) that required pre-emptive euthanasia. [The Working Group noted that the percentages of early death in this study were reported to be in line with survival rates reported in several other studies of control CD-1 mice aged 18 months, with an average death rate before 18 months of 21.7% (see Giknis and Clifford, 2010).] For the 129/Sv mice, three blocks of animals were used, each separated by 2–3 weeks. 129/Sv wildtype mice were dosed with vehicle, or PFOA at 0.1, 0.3, 0.6, or 1 mg/kg bw, resulting in a final number of 10, 10, 8, 6, and 8 female offspring surviving to age 18 months (to be consistent with the CD-1 mice) and included in the necropsy (from 7, 7, 5, 3, and 5 pregnant dams, respectively). PPARα-knockout mice were dosed with vehicle, or PFOA at 0.1, 0.3, 1, or 3 mg/kg bw, resulting in a final number of 6, 10, 10, 9, and 9 offspring (from 5, 9, 8, 7, and 9 pregnant dams), respectively. Different dose ranges were used for the three strains because of differences in strain sensitivities to PFOA. The highest dose used per strain was selected to minimize developmental toxicities and litter loss (see Abbott et al., 2007). The lower doses were selected such that resulting adolescent mice would have PFOA blood serum concentrations comparable to those reported for highly exposed humans (see Macon et al., 2011). PFOA was administered to all mice by oral gavage on days 1–17 of gestation. To determine the dose amounts, the dams were weighed daily before dosing. At birth, the pups were individually weighed and sexed. Pups within a treatment group were pooled and randomly redistributed among the dams of their respective treatment groups, and litters were equalized to 10 male and female pups. [The Working Group noted that litter effects could not be evaluated because of the cross-fostering that only occurred in the CD-1 mice.] Among the CD-1 mice, small litters (fewer than 4 pups) were excluded from the study. Pups were weaned at age 21 days, and only female offspring were retained in this study and housed 3–5 mice per cage. At 18 months, all mice underwent full necropsy, and livers were collected from all surviving mice in the exposure groups. [The Working Group noted that the mice that died for various reasons before 18 months were not included in this study because of inconsistencies in age and quality of tissues that could be retrieved.] Liver sections underwent a pathology peer review by a team of board-certified veterinary pathologists (pathology working group) to determine the incidence of neoplastic and non-neoplastic lesions, and "INHAND" (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) liver nomenclature was used when evaluating liver lesions (Thoolen et al., 2010). [The

Working Group noted that this study was not designed as a liver carcinogenesis study but was the consequence of finding liver tumours when investigating unscheduled deaths of PPARα-knockout mice in which no tumours were expected to be found. In addition, mice that died before termination of the study were not examined and, therefore, potential tumours in the liver were not included that could have affected the statistical analysis.]

Neoplastic lesions were present in female CD-1 mice treated with PFOA. The incidence of hepatocellular adenoma – 0/29, 0/29, 1/37 (2.7%), 4/26 (15.4%), 0/31, and 1/21 (4.8%) for the groups at 0 (control), 0.01, 0.1, 0.3, 1, and 5 mg/kg bw, respectively – was significantly increased ($P < 0.05$, Fisher exact test) at 0.3 mg/kg bw, and exceeded the upper bound of the range for historical controls – 3/897 (0.3%); range, 0–2% – reported by Giknis and Clifford (2010). There was one mouse with hepatocellular adenoma (multiple) in the group at the lowest PFOA dose. [The Working Group noted that there was a significant increase in the incidence of adenoma at the intermediate dose (0.3 mg/kg bw) compared with controls and that the upper bound of the range for historical controls was exceeded, but that there was no significant trend in the incidence of adenoma. Therefore, the Working Group was uncertain about the causal association between these tumours and PFOA exposure.] Hepatocellular carcinomas occurred in one mouse per group at 0.3 and 5.0 mg/kg bw, and histiocytic sarcomas developed in one mouse per group at 0.1, 1.0, and 5.0 mg/kg bw, but the incidence did not reach statistical significance for either tumour type. There was a significant positive trend ($P < 0.01$, Cochran–Armitage trend test) in the incidence of liver haemangiosarcoma – 0/29, 0/29, 0/37, 1/26 (3.8%), 0/31, 2/21 (9.5%) in the groups at 0 (control group), 0.01, 0.1, 0.3, 1, and 5 mg/kg bw, respectively – with the incidence exceeding the upper bound of the range observed in historical controls – 3/897 (0.3%), range, 0–2% – reported by Giknis and Clifford (2010). In the control group, the only tumour found was a single malignant lymphoma. [The Working Group noted that lymphoma was a background lesion in historical controls for CD-1 females – 112/900 (12.4%); range, 0–6% (see Giknis and Clifford, 2010).] The overall incidence of malignant lymphoma was 3/144 (2.1%) after PFOA exposure versus 1/29 (3.4%) in the controls.

In the vehicle-treated 129/Sv wildtype mice, no tumours were found. The only tumour found in PFOA-treated 129/Sv wildtype mice was a histiocytic sarcoma in the group at 0.1 mg/kg bw. Hepatocellular adenomas developed in five PFOA-treated PPARα-knockout mice – one mouse in each of the three groups at the lower doses and two mice in the group at the highest dose (3 mg/kg bw) – leading to an overall incidence of 5/38 (13.2%) in PFOA-treated mice. An Ito cell tumour developed in one PPARα-knockout mouse treated with PFOA at 0.3 mg/kg bw. [The Working Group noted that the power to detect an effect was low for this study because of the low number of animals and that the knockout control group contained only six mice.]

Regarding non-neoplastic lesions, basophilic oreosinophilic foci were found in one CD-1 mouse in each PFOA-treated group at 0.01 mg/kg bw, 0.1 mg/kg bw (basophilic foci), and 0.3 mg/kg bw (eosinophilic foci). A significant positive trend in the incidence of oval cell hyperplasia, Ito cell hypertrophy, and centrilobular hepatocyte hypertrophy was observed in CD-1 mice after PFOA exposure, with the incidence being significantly increased for Ito cell hypertrophy and centrilobular hepatocyte hypertrophy in the group at 5 mg/kg bw. Chronic inflammation was common in CD-1 mice, and there was a dose-related increase in severity scores in PFOA-exposed livers; mean severity in the two groups at the highest dose was significantly higher than in the controls. In PPARα-knockout mice, clear cell focus developed in one mouse in the group

treated with PFOA at 0.1 mg/kg bw, and eosinophilic foci developed in one mouse in each group at 0 (control) and 0.3 mg/kg bw. In 129/Sv wildtype mice, eosinophilic foci developed in one mouse in at each group at 0.3 and 0.6 mg/kg bw.

Non-neoplastic changes were also numerous in the 129/Sv strain after PFOA exposure. Significant positive trends were observed in the incidence of both bile duct hyperplasia and bile duct inclusion bodies (hyaline droplets) in 129/Sv PPARα-knockout mice, but there was no increase in the incidence of either bile duct hyperplasia (although there was a decreasing trend in severity with dose) or hyaline droplet accumulation (although there was a decreasing trend in incidence) in 129/Sv wildtype mice. The incidence of Ito cell hypertrophy decreased with increasing PFOA dose in PPARα-knockout mice. There was a significant positive trend in the incidence of haematopoietic cell proliferation with increasing PFOA dose in PPARα-knockout mice, but not in the 129/Sv wildtype mice. There was a significant increase in the incidence of centrilobular hepatocyte hypertrophy with increasing PFOA dose in the PPARα-knockout mice. Although the incidence of centrilobular hepatocyte hypertrophy in 129/Sv wildtype mice did not significantly change with PFOA dose, the severity increased significantly with PFOA dose. A similar increase in mean severity was noted in PPARα-knockout mice, but that effect did not reach statistical significance.

[The Working Group noted that only females were studied. For all three mouse strains, when tumours were counted for mice killed before 18 months, only percentages (%) of mice born were stated. The initial numbers of F_1 females for each study were not provided. Thus, in Table 3.1 the numbers of mice given at start and surviving are both the numbers surviving at 18 months and included in the study. The number of tumours per mouse was not reported, thus, multiplicity was not known. The numbers of mice in the wildtype and knockout studies were low. No statistical comparisons between experimental blocks of mice treated with PFOA were reported. There was no statement that the dams were randomized to the treatment groups. It was not reported whether histopathology was done without knowledge of treatment.]

3.1.2 Rat

See Table 3.2.

(a) Oral administration (feed)

In a study by Biegel et al. (2001) that was designed to compare the carcinogenic effects of Wyeth-14,6431 (designated as WY group), a rodent peroxisome proliferator and carcinogen, with those of PFOA (specifically, ammonium salt; designated as the C8 group), an initial group of 156 male Sprague-Dawley rats [Crl:CD BR (CD)] (age, 6 weeks) were treated with feed containing PFOA (purity, 98–100%) at a dose of 300 ppm for 24 months. Two control groups (156 rats in each) were either fed ad libitum (designated as the control group) or received the same amount of feed as the PFOA-treated group (pair-fed control group, designated as the CP-C8 group), respectively. The average daily dose of PFOA was 13.6 mg/kg bw per day in the C8 group. There were initially 156 rats per group, and 6 rats per group were randomly selected and killed at eight interim time points (approximately 1, 3, 6, 9, 12, 15, 18, and 21 months) for histology evaluation (48 rats) and measurements of cell proliferation and peroxisome proliferation (48 rats), leaving 60 rats per group for the 2-year observation for carcinogenesis (Biegel et al., 2001). Hormone measurement was performed at the eight interim time points using 10 rats per group that were randomly selected and not killed. At study termination, survival rates were approximately 15%, 35%, 48%, and 16% for the control, CP-C8, C8, and WY groups, respectively. [The Working Group noted that, although not clearly documented, rats for hormone measurement were

Table 3.2 Studies of carcinogenicity in rats exposed to PFOA

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Crl:CD BR (M) 49 d [7 wk] 24 mo Biegel et al. (2001)	Oral administration (feed) PFOA (ammonium salt), 98–100% Feed 0 (controls fed ad libitum), 0 (pair-fed controls; CP-C8), 300 (C8) ppm (approximately 0, 0, and 13.6 mg/kg bw) 156, 156, 156 80, 79, 76	*Liver* Hepatocellular adenoma 2/80 (3%), 1/79 (1%), 10/76 (13%)* Hepatocellular carcinoma 0/80, 2/79 (3%), 0 /76 Hepatocellular adenoma or carcinoma (combined) 2/80 (3%), 3/79 (4%), 10/76 (13%)* *Testis* Leydig cell adenoma 0/80, 2/78 (3%), 8/76 (11%)* *Pancreas* Acinar cell adenoma 0/80, 1/79 (1%), 7/76 (9%)* Acinar cell carcinoma 0/80, 0/79, 1/76 (1%) Acinar cell adenoma or carcinoma (combined) 0/80, 1/79 (1%), 8/76 (11%)*	 *$P < 0.05$, Dunnett test; [$P = 0.0038$, Fisher exact test] NS *$P < 0.05$, Dunnett test; [$P = 0.0336$, Fisher exact test] *$P < 0.05$, Dunnett test; [$P = 0.0448$, Fisher exact test] *$P < 0.05$, Dunnett test; [$P = 0.0279$, Fisher exact test] NS *$P < 0.05$, Dunnett test; [$P = 0.0145$, Fisher exact test]	*Principal strengths*: long-term study; adequate number of rats per group; study covered most of the lifespan. *Principal limitations*: only one dose group; one sex used; age of rats when assessed for lesions (when killed) was not clearly documented; no results or data for trend test(s) were reported, despite large differences in survival rates among groups; scheduled and unscheduled deaths were not distinguishable and were shown as the denominator of the rat numbers in Table 2 of this paper. *Other comments*: only the liver, testes, epididymides, pancreas, and organs with gross lesions were examined microscopically; of 165 rats per group, 48 rats were designated for interim kill for measurement of cell proliferation, and another 48 rats for peroxisome proliferation (6 rats × 8 time points); hormone analysis was performed without killing; 60 rats were likely to be designated for pathological evaluation for the 2-yr time period; peer-review of the data on pancreatic lesions by a panel of pathologists (Caverly Rae et al., 2014) using the same diagnostic criteria as those applied in the study by Biegel et al. (2001) generated the following incidence data (which were statistically significant from those for pair-fed controls, *$P < 0.05$): pancreatic acinar hyperplasia: 14/80, 8/79, 30/76*

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley Crl: COBS (SD) BR (M) 39–41 d [6 wk] 104 wk Butenhoff et al. (2012a)	Oral administration (feed) PFOA (ammonium salt), 97.2% Feed 0, 30, 300 ppm (actual doses, 0, 1.3, and 14.2 mg/kg bw per day) 50, 50, 50 49, 50, 50	*Testis and epididymis* Leydig cell adenoma 0/49, 2/50 (4%), 7/50 (14%)*	[$P = 0.010$, Cochran–Armitage trend test] *$P \leq 0.05$, Fisher exact test (two-tailed) [*$P = 0.0067$, Fisher exact test (one-tailed)]	*Principal strengths*: adequate number of animals per group, males and females used, adequate duration; well-conducted study. *Other comments*: 65 rats in the control and higher-dose groups, 50 rats in the lower-dose group (15 rats from the control and higher-dose groups were killed at 1 yr); no neoplasms at 1-yr interim kill; peer review of the pancreatic lesion data by a panel of pathologists (Caverly Rae et al., 2014) using the same diagnostic criteria as those applied in the study by Biegel et al. (2001) generated the following incidence data: pancreatic acinar cell hyperplasia, 3/46, 1/46, 10/47* [*$P = 0.0382$, Fisher exact test (one-tailed)].
		Liver Hepatocellular carcinoma 3/49 (6%), 1/50 (2%), 5/50 (10%)	NS	
		Adrenal medulla Pheochromocytoma (benign) 2/49 (4%), 4/50 (8%), 4/50 (8%)	NS	
		Pheochromocytoma (malignant) 0/49, 1/50 (2%), 0/50	NS	
		Pituitary gland Adenoma 17/48 (35%), 17/47 (36%), 13/46 (28%)	NS	
		Thyroid gland, C-cell Adenoma 0/43, 2/47 (4%), 4/47 (9%)	NS	
		Carcinoma 2/43 (5%), 0/47, 0/47	NS	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley Crl: COBS (SD) BR (F) 39–41 d [6 wk] 104 wk Butenhoff et al. (2012a)	Oral administration (feed) Ammonium salt, 97.2% Feed 0, 30, 300 ppm Actual doses: 0, 1.6, 16.1 mg/kg bw per day 50, 50, 50 46, 45, 44	*Mammary gland* Fibroadenoma 10/46 (22%), 19/45 (42%)*, 21/44 (48%)** Adenocarcinoma 7/46 (15%), 14/45 (31%), 5/44 (11%) Lymphangiosarcoma 0/46, 0/45, 1/44 (2%) *Adrenal medulla* Pheochromocytoma (malignant) 0/50, 0/50, 1/49 (2%) *Liver* Hepatocellular carcinoma 0/50, 0/50, 1/50 (2%) *Pituitary gland* Adenoma 33/46 (72%), 39/47 (83%), 36/50 (72%) Adenocarcinoma 9/50 (18%), 16/50 (32%), 5/50 (10%)	 [*P* = 0.024, Cochran–Armitage trend test] [**P* = 0.0302, Fisher exact test (one-tailed)] [***P* = 0.0086, Fisher exact test (one-tailed)] NS NS NS NS NS NS	*Principal strengths*: adequate number of animals per group; males and females used; adequate duration. *Other comments*: 65 rats in the control and high-dose groups, 50 rats in the low-dose group (15 rats from the control and high-dose groups were killed at 1 yr); no neoplasms at 1-yr interim kill. Peer review of the mammary gland data by a panel of pathologists (Hardisty et al., 2010) using contemporary diagnostic criteria generated the following incidence data (with no statistical significance): adenocarcinoma of the mammary gland, 9/50 (18%), 16/50 (32%), 5/50 (10%); adenoma of the mammary gland: 1/50 (2%), 0/50, 0/50; fibroadenoma of the mammary gland: 16/50 (32%), 16/50 (32%), 20/50 (40%); fibroadenoma (multiple) of the mammary gland: 2/50 (4%), 6/50 (12%), 3/50 (6%) (not adjusted for survival).

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Hsd:Sprague-Dawley (M) Perinatal then PND 21–23 (study 2) 2 yr NTP (2020)	Oral administration (feed) PFOA, 98.8% NIH-07 (perinatal phase) and NTP-2000 (post-weaning phase) 0/0, 0/20, 0/40, 0/80, 300/0, 300/20, 300/40, 300/80 ppm Feed 50, 50, 50, 50, 50, 50, 50, 50 36, 42, 35, 37, 35, 38, 38, 39	*Liver* Hepatocellular adenoma (includes multiple) 0/50, 0/50, 7/50 (14%)*, 11/50 (22%)**, 0/50, 1/50 (2%), 5/50 (10%), 10/50 (20%)***	$P < 0.001$, Cochran–Armitage trend test *$P = 0.050$, Rao–Scott adjusted poly-3 test; [$P = 0.0062$, Fisher exact test] **$P = 0.010$, Rao–Scott adjusted poly-3 test; [$P = 0.0003$, Fisher exact test] ***$P = 0.006$, Rao–Scott adjusted poly-3 test; [$P = 0.0006$, Fisher exact test]	*Principal strengths*: adequate number of animals used; randomly allocated in groups; adequate duration; males and females used; multiple doses used; well-conducted GLP study. *Other comments*: historical controls: hepatocellular adenoma, all routes, 2/340 (0.67% ± 1.03%); range, 0–2%; hepatocellular carcinoma, 0/340; hepatocellular adenoma or carcinoma (combined), 2/340 (0.67% ± 1.03%); range, 0–2%; pancreatic acinar cell adenoma, all routes, 45/340 (12.33% ± 10.07%); range, 0–28%; pancreatic acinar cell adenocarcinoma, 2/340 (0.52% ± 0.85%); range, 0–2%; pancreatic acinar cell adenoma or adenocarcinoma (combined), 45/340 (12.33% ± 10.07%); range, 0–28%.
		Hepatocellular carcinoma 0/50, 0/50, 0/50, 0/50, 0/50, 0/50, 0/50, 4/50 (8%)	Positive trend for F_1 males with F_0 exposure only	
		Hepatocellular adenoma or carcinoma (combined) 0/50, 0/50, 7/50 (14%)*, 11/50 (22%)**, 0/50, 1/50 (2%), 5/50 (10)***, 12/50 (24%)****	$P < 0.001$, Cochran–Armitage trend test *$P = 0.050$, Rao–Scott adjusted poly-3 test; [$P = 0.0062$, Fisher exact test] **$P = 0.010$, Rao–Scott adjusted poly-3 test; [$P = 0.0003$, Fisher exact test] ***[$P = 0.0062$, Fisher exact test] ****$P = 0.003$, Rao–Scott adjusted poly-3 test; [$P = 0.0001$, Fisher exact test]	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Hsd:Sprague-Dawley (M) Perinatal then PND 21–23 (study 2) 2 yr NTP (2020) (cont.)		*Pancreas*		
		Acinar cell adenoma (includes multiple)		
		3/50 (6%), 28/50 (56%)**, 26/50 (52%)**, 32/50 (64%)**, 7/50 (14%), 18/50 (36%)*, 30/50 (60%)**, 30/50 (60%)**	$P < 0.001$, Cochran–Armitage trend test *$P = 0.016$, Rao–Scott adjusted poly-3 test; [$P = 0.0099$, Fisher exact test] **$P < 0.001$, Rao–Scott adjusted poly-3 test; [$P < 0.0001$, Fisher exact test]	
		Acinar cell adenocarcinoma		
		0/50, 3/50 (6), 1/50 (2%), 3/50 (6%), 0/50, 2/50 (4%), 1/50 (2%), 3/50 (6%)	NS	
		Acinar cell adenoma or adenocarcinoma (combined)		
		3/50 (6%), 29/50 (58%)**, 26/50 (52%)**, 32/50 (64%)**, 7/50 (14%), 20/50 (40%)*, 30/50 (60%)**, 30/50 (60%)**	$P < 0.001$, Cochran–Armitage trend test *$P = 0.006$, Rao–Scott adjusted poly-3 test; [$P < 0.0001$, Fisher exact test] **$P < 0.001$, Rao–Scott adjusted poly-3 test; [$P < 0.0001$, Fisher exact test]	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Hsd:Sprague-Dawley (F) Perinatal then PND 21–23 (study 1) 2 yr NTP (2020)	Oral administration (feed) PFOA, 98.8% NIH-07 (perinatal phase) and NTP-2000 (post-weaning phase) 0/0, 0/300, 0/1000, 150/300, 300/1000 ppm Feed 50, 50, 49, 50, 50 23, 28, 23, 32, 23	*Liver*		*Principal strengths*: adequate number of animals used, randomly allocated in groups; adequate duration; males and females used; multiple doses used; well-conducted GLP study. *Other comments*: historical controls: hepatocellular adenoma, all routes, 14/340 (3.63% ± 2.59%); range, 0–8%; hepatocellular carcinoma: 1/340 (0.33% ± 0.82%); range, 0–2%; hepatocellular adenoma or carcinoma (combined), 15/340 (3.96% ± 2.77%); range, 0–8%; pancreatic acinar cell adenoma, all routes, 0/340; pancreatic acinar cell adenocarcinoma, 0/340; pancreatic acinar cell adenoma or adenocarcinoma (combined), 0/340; adenoma of the uterus (standard evaluation), all routes, 1/150 (0.67% ± 1.15%); range, 0–2%; adenocarcinoma of the uterus (standard evaluation), 11/150 (7.33% ± 4.62%); range, 2–10%; adenoma or adenocarcinoma (combined) of the uterus, 12/150 (8% ± 3.46%); range (standard evaluation), 4–10%.
		Hepatocellular adenoma		
		2/50 (4%), 0/50, 1/49 (2%), 0/50, 3/50 (6%)	NS	
		Hepatocellular carcinoma		
		1/50 (2%), 1/50 (2%), 3/49 (6%), 0/50, 4/50 (8%)	NS	
		Hepatocellular adenoma or carcinoma (combined)		
		3/50 (6%), 1/50 (2%), 4/49 (8%), 0/50, 6/50 (12%)	NS	
		Pancreas		
		Acinar cell adenoma		
		0/50, 0/50, 1/49 (2%), 0/50, 3/50 (6%)	NS	
		Acinar cell adenocarcinoma		
		0/50, 0/50, 1/49 (2%), 0/50, 2/50 (4%)	NS	
		Acinar cell adenoma or adenocarcinoma (combined)		
		0/50, 0/50, 2/49 (4%), 0/50, 5/50 (10%)	(*P* = 0.018, Rao–Scott adjusted trend poly-3 test)	
		Uterus		
		Adenoma (extended evaluation)		
		1/50 (2%), 0/49, 0/48, 0/50, 0/48	NS	
		Acinar cell adenocarcinoma (extended evaluation)		
		1/50 (2%), 5/49 (10%), 7/48 (14%)*, 3/50 (6%), 5/48 (10%)	**P* = 0.005, Rao–Scott adjusted poly-3 test; [*P* = 0.0258, Fisher exact test]	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Hsd:Sprague-Dawley (F) Perinatal then PND 21–23 (study 1) 2 yr NTP (2020) (cont.)		Acinar cell adenoma or adenocarcinoma (combined) (extended evaluation)		
		2/50 (4%), 5/49 (10%), 7/48 (15%), 3/50 (6%), 5/48 (10%)	NS	
		Acinar cell adenoma (standard or extended evaluation combined)		
		1/50 (2%), 1/49 (2%), 0/49, 0/50, 0/50	NS	
		Acinar cell adenocarcinoma (standard or extended evaluation combined)		
		1/50 (2%), 5/49 (10%), 7/49 (14%)*, 3/50 (6%), 5/50 (10%)	*$P = 0.050$, Rao–Scott adjusted poly-3 test; [$P = 0.0277$, Fisher exact test]	
		Acinar cell adenoma or adenocarcinoma (combined) (standard or extended evaluation combined)		
		2/50 (4%), 5/49 (10%), 7/49 (14%), 3/50 (6%), 5/50 (10%)	NS	

bw, body weight; d, day(s); F, female; GLP, Good Laboratory Practice; M, male; mo, month(s); NIH, National Institutes of Health; NS, not significant; NTP, National Toxicology Program; PFOA, perfluorooctanoic acid; PND, postnatal day(s); ppm, parts per million; vs, versus; wk, week(s); yr, year(s).

not killed and were returned to the group for further treatment and observation. Accordingly, the Working Group estimated that the final number of rats for pathological evaluation at the 24-month time point was around 60 animals per group. Survival data were provided in graphic form only (the actual numbers were not reported) and therefore numbers were estimated by the Working Group from the graph presented in the original publication.] All surviving rats underwent complete necropsy with histopathological evaluation. The liver, testes, epididymis, pancreas, and organs with gross lesions were examined microscopically.

At 24 months, exposure to PFOA (C8 group) significantly increased the incidence of hepatocellular adenoma – 10/76 (13%) versus 1/79 (1%), ($P < 0.05$, Dunnett test; [$P = 0.0038$, Fisher exact test]) – and of hepatocellular adenoma or carcinoma (combined) – 10/76 (13%) versus 3/79 (4%), ($P < 0.05$, Dunnett test; [$P = 0.0336$, Fisher exact test]) – compared with the pair-fed control group CP-C8. There was a significant increase ($P < 0.05$, Dunnett test; [$P = 0.0448$, Fisher exact test]) in the incidence of testicular Leydig cell adenoma in the C8 group compared with the CP-C8 group – 8/76 (11%) versus 2/78 (3%). There were significant increases ($P < 0.05$, Dunnett test; [$P = 0.0279$, Fisher exact test]) in the incidence of pancreatic acinar cell adenoma in the C8 group compared with the CP-C8 group – 7/76 (9%) versus 1/79 (1%) – and in the incidence of pancreatic acinar cell adenoma or carcinoma (combined) in the C8 group ($P < 0.05$, Dunnett test; [$P = 0.0145$, Fisher exact test]) compared with the CP-C8 group – 8/76 (11%) versus 1/79 (1%). [The Working Group noted that the numbers of rats that were submitted for pathological diagnosis were indicated only in the footnote of Table 2 of the publication and are the sum of scheduled and unscheduled deaths.]

Regarding non-neoplastic lesions in the C8 group compared with the CP-C8 group, there was a significant increase in the incidence of pancreatic acinar cell hyperplasia.

[The Working Group noted that this was a long-term study using a large group size and that the duration was most of the lifespan, and that the stability of the test article was evaluated and the concentration in the diet measured. However, the study was limited by the use of a single dose; the use of one sex only; because the age of each animal at death was not clearly stated; and because no results or data for trend test(s) were reported, despite large differences in survival rates among groups.]

Butenhoff et al. (2012a) published a report of a well-conducted study carried out between 1981 and 1983. The original reports of this study (US EPA, 1987) were published in a peer-reviewed publication by Butenhoff et al. (2012a). In this study of chronic toxicity that complied with Good Laboratory Practice (GLP), groups of male and female Sprague-Dawley rats [Crl:COBS CD(SD)BR] (age, 39–41 days) were treated with feed containing PFOA (specifically, the ammonium salt; purity, 97.2%) at a concentration of 0 (control), 30, or 300 ppm, corresponding to an average daily dose of approximately 0, 1.3, and 14.2 mg/kg bw in males, and 0, 1.6, and 16.1 mg/kg bw in females. The control group and group at the higher dose contained 65 males and 65 females, and the group at the lower dose contained 50 males and 50 females. An interim kill at 1 year involved 15 males and 15 females from both the control group and the group at 300 ppm, and the remaining rats, 50 of each sex, were killed at 104 weeks (US EPA, 1987); also reported by Butenhoff et al., 2012a). At study termination at 104 weeks, survival was 70%, 72%, and 88% for male rats, and 50%, 48%, and 58% for female rats, for the groups at 0 (control), 30, and 300 ppm, respectively; the increased survival rate observed in males at the higher dose was statistically significant, compared with the males in the control group. For male rats at the higher dose, body-weight gains were decreased by more than 10% throughout the 66 weeks of the study, compared with controls. The largest

decrease was approximately 21% by week 6. In males at the lower dose, a slight but not significant (5%) decrease in body weight was observed at week 6, with little additional decrease subsequently. In female rats treated with PFOA, mean body weights did not change during the first 18 months of the study. After 18 months, there was a gradual decrease in mean body weight in females at 300 ppm; the maximum decrease was 11% lower than that of the controls at 22 months. Mean feed consumption relative to body weight was increased in all the PFOA-treated males throughout the study. In females, there was a trend towards lowered food consumption in both PFOA-treated groups.

In males, there was a significant positive trend [$P = 0.010$, Cochran–Armitage test] in the incidence of testicular Leydig cell adenoma, and the incidence – 0/49, 2/50 (4%), and 7/50 (14%) for the groups at 0 (control), 30, and 300 ppm, respectively – was significantly increased at 300 ppm ($P \leq 0.05$, Fisher exact test, two-tailed; [$P = 0.0067$, Fisher exact test, one-tailed]).

In females, there was a significant positive trend [$P = 0.024$, Cochran–Armitage test] in the incidence of fibroadenoma of the mammary gland, and the incidence – 10/46 (22%), 19/45 (42%), and 21/44 (48%) for the groups at 0 (control), 30, and 300 ppm, respectively – was significantly increased at 30 and 300 ppm ([$P = 0.0302$, $P = 0.0086$, Fisher exact test, respectively]).

In 2010, a pathology working group was convened to review the original slides of mammary glands from the study by US EPA (1987), to provide a consensus diagnosis for neoplasms of the mammary gland using contemporary diagnostic criteria (Hardisty et al., 2010). The pathology working group concluded that some lesions originally diagnosed as lobular hyperplasia had features consistent with fibroadenoma of the mammary gland (mainly in slides from the control group), and that, consequently, PFOA did not induce neoplasms of the mammary gland. Both the initial data on mammary pathology (US EPA, 1987) and the reviewed data (Hardisty et al., 2010) were reported by Butenhoff et al. (2012a).

There was an increase in the incidence of hepatocellular hypertrophy in males and females at the higher dose, and an increase in the incidence of liver cystoid degeneration, vascular mineralization of the testis, and portal mononuclear cell infiltrate in males at the higher dose (Butenhoff et al., 2012a). Increases in the incidence of pancreatic acinar cell hyperplasia in male rats – 0/46 (0%), 2/46 (4%), and 2/49 (4%) in the groups at 0 (control), 30, and 300 ppm, respectively – were not statistically significant. Pancreatic acinar hyperplasia was not reported in female rats in this study. [The Working Group noted that this study used an adequate number of rats per group, both sexes, and an adequate duration of exposure. Discrepancy between the original study pathology and the review pathology (Hardisty et al., 2010) regarding the diagnosis of mammary lesions was noted. The Working Group also noted that increases in the incidence of Leydig cell adenoma were the only positive finding when using contemporary diagnostic criteria. In addition, the Working Group noted that faster elimination occurs in female rats than in males, as outlined in Section 4.1 of the present monograph, which may explain why minimal effects were observed in females.]

In a review of the pancreatic lesions observed in male rats in US EPA (1987), also reported by Butenhoff et al. (2012a), using the same diagnostic criteria as those applied in the study by Biegel et al. (2001), a significant positive trend ($P < 0.05$, Cochran–Armitage trend test) in the incidence of pancreatic acinar cell hyperplasia was observed in males, and the incidence was significantly increased [$P = 0.0382$, Fisher exact test] at the higher dose – 3/46 (7%), 1/46 (2%), and 10/47 (21%) (Caverly Rae et al., 2014). There were no statistically significant or test-related increases in the incidence of acinar cell adenoma or in acinar cell carcinoma separately with either

PFOA dose, but there was a significant positive trend ($P < 0.05$, Cochran–Armitage trend test) in the incidence of all three lesions combined (hyperplasia, adenoma, and carcinoma) – 3/46 (7%), 1/46 (2%), and 11/47 (23%). [The Working Group noted that only one neoplasm was observed, which was a carcinoma in the group at the higher dose. Both pancreatic acinar cell hyperplasia and pancreatic acinar adenoma were considered to be proliferative lesions, and this review supported the conclusion that the pancreas is a target of PFOA in male rats. In addition, the Working Group noted that hyperplasia, adenoma, and carcinoma were combined under the assumption that they are sequential pathological lesions.]

In a well-conducted study of chronic toxicity that complied with GLP and in which early-life exposure to PFOA on carcinogenicity outcomes was investigated, PFOA (purity, 98.8%) was administered to groups of 36 Hsd:Sprague-Dawley pregnant rats from day 6 of gestation through lactation, and subsequently to their pups for 2 years (NTP, 2020, revised in 2023). The control group comprised 103 pregnant females. F_0 females received feed containing PFOA at a concentration of 0, 150, or 300 ppm and were housed individually during gestation and together with their respective litters during lactation. The pups (F_1) were weaned on postnatal days 21–23. All F_1 exposure groups comprised 50 males and 50 females and were treated with feed containing PFOA at a concentration of 150 or 300 ppm for males and 300 or 1000 ppm for females. The initial dose setting, i.e. 150 ppm and 300 ppm during the mating and preweaning period (F_0) combined with 300 ppm and 1000 ppm (females) or 150 ppm and 300 ppm (males) for 2-year dietary exposure to the offspring (F_1) was tolerated only by female offspring (designated as study 1 for females only). [The Working Group noted that elimination is faster in female rats than in males (as outlined in Section 4.1 in the present monograph), which was used to explain the higher post-weaning doses used in females.] Therefore, a second study was started that was focused entirely on males, and post-weaning concentrations were lowered (designated as study 2 for males only). A single perinatal exposure concentration was used, i.e. 300 ppm for the F_0 rats, and 20, 40 and 80 ppm for the F_1 rats. Total and live litter sizes and survival of the F_1 rats during lactation were not affected by exposure.

The treatment groups are indicated by the given doses in parts per million for the F_0 (gestation/lactation) and F_1 (post-weaning) as F_0/F_1, such as 0/1000.

At termination of study 2 (2 years, males only), group mean body weights for the groups at 0/20, 0/40, 0/80, 300/0, 300/20, and 300/40 ppm (males) were within 10% of those for the respective control groups (0/0 ppm or 300/0 ppm). The terminal mean body weight of the group at 300/80 ppm was 13% less than that of the control group at 0/0 ppm. Post-weaning consumption of PFOA in males was 1.1/1.0, 2.2/2.1 and 4.6/4.6 mg/kg per day for the groups at 20, 40, and 80 ppm, with or without perinatal exposure. At termination of study 1 (2 years, females only), group mean body weights for the groups at 0/1000 and 300/1000 ppm were lower (19% and 27%, respectively) than those in the 0/0 ppm control group (females). Group mean feed consumption in females over the course of the study averaged 93%, 99%, 96%, and 88% of that in the 0/0 ppm control group for the groups at 0/300, 150/300, 0/1000, and 300/1000 ppm, respectively. After weaning, PFOA consumption for females in the groups at 0/300 and 150/300 ppm averaged 18.2 and 18.4 mg/kg per day, respectively. PFOA consumption averaged 63.4 and 63.5 mg/kg per day for the groups at 0/1000 and 300/1000 ppm, respectively (NTP, 2020).

In F_1 male rats (2 years, study 2), there was a significant positive trend in the incidence of hepatocellular adenoma (includes multiple) ($P < 0.001$, Cochran–Armitage trend test) in

both the F_0 exposed and the F_0 unexposed groups, with the incidence being significantly increased ($P = 0.050$, Rao–Scott adjusted poly-3 test, [$P = 0.0062$, Fisher exact test]; $P = 0.010$, Rao–Scott adjusted poly-3 test, [$P = 0.0003$, Fisher exact test]; $P = 0.006$, Rao–Scott adjusted poly-3 test, [$P = 0.0006$, Fisher exact test]) at 0/40, 0/80, and 300/80 ppm, respectively) in both the F_0 exposed and the F_0 unexposed groups, i.e. 0/50, 0/50, 7/50 (14%), 11/50 (22%) at 0/0, 0/20, 0/40, 0/80 ppm, and 0/50, 1/50 (2%), 5/50 (10%), 10/50 (20%) at 300/0, 300/20, 300/40, and 300/80 ppm. In addition, the incidence in all treated groups, except the group at 300/20 ppm, exceeded the upper bound of the range observed in historical controls from this laboratory – 2/340 (0.067% ± 1.03%); range, 0–2%. There was a significant positive trend in the incidence of hepatocellular carcinoma ($P = 0.049$, Cochran–Armitage trend test) in male rats with perinatal exposure. No carcinomas were observed in the male rats with only post-weaning exposure. There was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) ($P < 0.001$, Cochran–Armitage trend test) in both the F_0 exposed and the F_0 unexposed groups, with the incidence being significantly increased ($P = 0.050$, Rao–Scott adjusted poly-3 test, [$P = 0.0062$, Fisher exact test]; $P = 0.010$, Rao–Scott adjusted poly-3 test, [$P = 0.0003$, Fisher exact test]; $P = 0.003$, Rao–Scott adjusted poly-3 test, [$P = 0.0001$, Fisher exact test] at 0/40, 0/80, and 300/80 ppm, respectively, in both the F_0 exposed and the F_0 unexposed groups, i.e. 0/50, 0/50, 7/50 (14%), and 11/50 (22%) for the groups at 0/0, 0/20, 0/40, and 0/80 ppm, and 0/50, 1/50 (2%), 5/50 (10%), and 12/50 (24%) for the groups at 300/0, 300/20, 300/40, 300/80 ppm. In addition, the incidence in all treated groups, except in the group at 300/20 ppm, exceeded the upper bound of the range observed in historical controls from this laboratory – 2/340 (0.067% ± 1.03%); range, 0–2%.

There was a significant positive trend in the incidence of acinar cell adenoma of the pancreas (includes multiple) ($P < 0.001$, Cochran–Armitage trend test) in both the F_0 exposed and the F_0 unexposed groups, with the incidence being significantly increased ($P < 0.0001$, Rao–Scott adjusted poly-3 test, [$P > 0.0001$, Fisher exact test] at 0/20, 0/40, 0/80, and 300/40 ppm; and $P = 0.016$, Rao–Scott adjusted poly-3 test, [$P = 0.0002$, Fisher exact test] at 300/20 ppm) in both the F_0 exposed and the F_0 unexposed groups – 3/50 (6%), 28/50 (56%), 26/50 (52%), and 32/50 (64%) for the groups at 0/0, 0/20, 0/40, and 0/80 ppm, and 7/50 (14%), 18/50 (36%), 30/50 (60%), and 30/50 (60%) for the groups at 300/0, 300/20, 300/40, and 300/80 ppm, respectively). In addition, the incidence in all treated groups exceeded the upper bound of the range observed in historical controls from this laboratory – all routes, 45/340 (12.33% ± 10.07%); range, 0–28%. The incidence of pancreatic acinar cell adenocarcinoma was not statistically significant in any of the treated groups versus controls and exceeded the upper bound of the range observed in historical controls from this laboratory – all routes, 2/340 (0.52% ± 0.85%); range, 0–2% – for the groups at 0/20, 0/80, 300/20, and 300/80 ppm. There was a significant positive trend in the incidence of acinar cell adenoma or adenocarcinoma (combined) of the pancreas ($P < 0.001$, Cochran–Armitage trend test) in both the F_0 exposed and the F_0 unexposed groups, with the incidence being significantly increased ($P < 0.0001$, Rao–Scott adjusted poly-3 test, [$P < 0.0001$, Fisher exact test] for all treated groups) in both the F_0 exposed and the F_0 unexposed groups – 3/50 (6%), 29/50 (58%), 26/50 (52%), and 32/50 (64%) for the groups at 0/0, 0/20, 0/40, and 0/80 ppm, and 7/50 (14%), 20/50 (40%), 30/50 (60%), and 30/50 (60%) for the groups at 300/0, 300/20, 300/40, and 300/80 ppm, respectively). In addition, the incidence in all treated groups exceeded the upper bound of the range observed in historical

controls from this laboratory – all routes, 45/340 (12.33% ± 10.07%); range, 0–28%.

The effect of perinatal exposure (F_0) over the effect of postnatal exposure (F_1) was not apparent for hepatocellular adenoma and acinar cell adenoma of the pancreas. There was a suggestive but not statistically significant effect of perinatal exposure on the incidence of hepatocellular carcinoma in male rats – 0/50, 0/50, 0/50, and 0/50 versus 0/50, 0/50, 0/50, 4/50 ($P = 0.049$ by Rao–Scott adjusted poly-3 test). [The Working Group noted that hepatocellular carcinoma is a rare neoplasm (0/340 in historical controls).]

In female rats (2 years, study 1), there was a significant positive trend ($P = 0.018$, Rao–Scott adjusted poly-3 test) in the incidence of pancreatic acinar cell adenoma or adenocarcinoma (combined) with perinatal exposure – 0/50, 0/50, and 5/50 (10%) for the groups at 0/0, 150/300, and 300/1000 ppm, respectively). There was a significant increase in the incidence of adenocarcinoma of the uterus (standard or extended evaluation combined) ($P = 0.050$, Rao–Scott adjusted poly-3 test, [$P = 0.0227$, Fisher exact test] for the group at 0/1000 ppm) in F_0 exposed groups – 1/50 (2%), 5/49 (10%), 7/49 (14%), 3/50 (6%), and 5/48 (10%) for the groups at 0/0, 0/300, 0/1000, 150/300, and 300/1000 ppm, respectively). [The Working Group noted that the new and extended evaluation used a combination of two sectioning methods. Because of this change in methods, the historical controls were of limited utility for the results obtained by the new method. The Working Group also noted that the new data reflected the 2023 revision.]

In 2023, a revision was made due to the identification of an error in the combining process for the uterine adenocarcinomas: "One animal with a squamous cell carcinoma in the 0/1000 ppm group was inadvertently combined in the adenocarcinoma analysis of the extended evaluation. The number of animals examined during the standard, extended and standard or extended (combined) evaluations was also corrected in the 0/300, 0/1000, and 300/1000 ppm groups" (NTP, 2020; revised in 2023). [The Working Group noted that the squamous cell carcinoma is of the same origin as the endometrial epithelium and can be combined with the adenoma and carcinoma. The Working Group also noted that the significant difference in the incidence of adenocarcinoma of the uterus in the group with the highest exposure without perinatal exposure was still statistically significant.]

Regarding non-neoplastic lesions, exposure to PFOA resulted in increases in the incidence of non-neoplastic lesions in the liver (hepatocyte cytoplasmic alteration; hepatocyte hypertrophy; hepatocyte single cell death; necrosis; and pigment in males and females) in males; hepatocyte cytoplasmic alteration; hepatocyte hypertrophy; hepatocyte single cell death; necrosis; pigment; bile duct hyperplasia; hepatocyte increased mitoses, in females; pancreatic acinus hyperplasia in male rats; and follicular cell hypertrophy of the thyroid gland of female rats. [The Working Group noted that pancreatic acinus adenoma and adenocarcinoma are rare lesions in females of this rat species and that pancreatic acinus hyperplasia was also considered to be rare; although these effects were of low incidence, they were consistent with the increased incidence of pancreatic acinar cell lesions reported in male rats.]

[The Working Group noted that this was a well-conducted study that complied with GLP and that used an adequate number of rats per group, both sexes, and an adequate duration of exposure. The Working Group also noted that internal exposure was measured in male and female rats, and that the stability of the test article was tested.]

(b) *Initiation–promotion studies*

See Table 3.3.

In an initiation–selection–promotion study, adult male Wistar rats [age not reported] were initiated with diethylnitrosamine (DEN)

Table 3.3 Initiation–promotion studies in rats and fish exposed to PFOA

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Initiation–promotion (tested as promoter) Rat, Wistar (ICO:WI IOPS AF:Han) (M) Age NR ("adult") 7 mo after initiation Abdellatif et al. (1990)	Oral administration (feed) PFOA, NR (analytical grade) Feed Basal feed (control), PB (positive control), PFOA (% feed) NDEA (single i.p. injection), 2-AAF (feed), carbon tetrachloride (gavage), PFOA and PB (feed) – Initiation: NDEA, 200 mg/kg bw (all three groups) – Selection: 2 wk after initiation, 0.03% 2-AAF for 2 wk; carbon tetrachloride, 2 mL/kg bw in 1:1 corn oil (after 1 wk of 2-AAF) – Promotion: 0% (control), 0.05% PB or 0.15% PFOA for 7 mo 7, 8, 12 7, 8, 12	*Liver* Total tumours Tumour incidence: 0/7, 6/8 (75%)**, 4/12 (33%)* Tumour multiplicity: 0, 3.4, 1.2	 *$P < 0.05$, Student *t*-test **$P < 0.02$, Student *t*-test	*Principal strengths*: the only report on the promoting activity of PFOA identified in rats via an initiation–selection–promotion protocol; PFOA concentrations measured in serum; end-points were measure at two time points. *Principal limitations*: only a limited number of rats were used in the experiment; histopathological examination of the liver only; only one sex used; average daily dose of PFOA was not reported.
Initiation–promotion (tested as promoter) Rat, Wistar (ICO:WI IOPS AF:Han) (M) Age NR ("adult") 12 mo Abdellatif et al. (1991)	Oral administration (feed) PFOA, NR (analytical grade) Feed Basal feed (control), PB (positive control), PFOA (0.005%), PFOA (0.02%) % in feed NDEA (single i.p. injection), PB and PFOA (feed) – Initiation: NDEA, 200 mg/kg bw (all three groups) – Promotion: basal feed (control), 0.05% PB, or 0.005% or 0.02% PFOA for 12 mo 10, 10, 10, 10 7, 7, 7, 9	*Liver* Hepatocellular carcinoma Tumour incidence: 0/7, 2/7 (28%), 1/7 (14.3%), 5/9 (55.5%)*	 *$P < 0.05$, Scheffé test NR	*Principal strengths*: the only report on promoting activity of PFOA identified in rats via an initiation–selection–promotion protocol; PFOA concentrations measured in serum; end-points were measured at two time points. *Principal limitations*: only a limited number of rats were included in the experiment; average daily dose of PFOA was not reported; histopathological examination of the liver only; only one sex used.

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Initiation–promotion (tested as promoter) Rat, Sprague-Dawley (F) 3 wk 7 mo Su et al. (2022)	Oral administration (gavage) PFOA, 95% Sesame oil 0, 0.01 mg/kg bw PFOA, zeranol, or PFOA + zeranol for 3 wk; at the age of 50 d, all rats received a single dose of DMBA 37, 37 37, 37	*Mammary gland* Papillary adenocarcinoma or cribriform carcinoma		*Principal limitations*: dose of the initiation agent DMBA was not optimal: extremely high tumour incidence in control (DMBA-treated group) did not allow investigation of potential enhancement of tumour incidence in the DMBA/PFOA-treated group; only one sex used; histological examination of the mammary gland only; only one dose. *Other comments*: the mixture of invasive papillary adenocarcinoma type 2 (prevalent) and invasive cribriform carcinoma was the most frequent mixed type for the PFOA group; histologically identified mammary tumours were also investigated by RNA-seq and qRT-PCR analyses; immunohistochemical analysis of selected receptors and effects on the endocrine system.
		Tumour incidence: 35/37 (94.6%), 35/37 (94.6%)	NS	
		Tumour multiplicity: 3.5 ± 2.2, 3.7 ± 2.2		
		Total tumours: 121, 129		
Initiation–promotion (tested as promoter) Rainbow trout (*Oncorhynchus mykiss*), Mount Shasta strain (M, F) (combined) 10 wk post-hatch 6 mo Tilton et al. (2008)	Oral administration (feed) PFOA, NR Feed 0, 200, 1800 ppm Initiation: aqueous exposure to AFB_1 or to vehicle (ethanol) for 30 min; after 3 mo, fed experimental diets containing lower or higher dose of PFOA 140, 140, 140 NR, NR, NR	*Liver* Mixed tumour, malignant		*Principal strengths*: adequate number of fish; two doses used. *Principal limitations*: data for males and females combined; no survival data; histopathological examination of the liver only. *Other comments*: no tumours were observed in non-initiated fish treated with feed containing PFOA; liver tumour enhancement after AFB_1/PFOA treatment might be related to induced estrogen-like signalling; the historical incidence of spontaneous liver tumours in trout (age 9 mo) fed control feed was 0.1%.
		36%, 34%, 71%*	*$P < 0.05$, logistic regression analysis (compared with AFB_1/control)	
		Hepatocellular adenoma		
		3%, 0%, 8%	NR	
		Hepatocellular carcinoma		
		10%, 11%, 46%	NR	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Initiation–promotion (tested as promoter) Rainbow trout (*Oncorhynchus mykiss*), Mount Shasta strain (M, F) (combined) 10 wk post-spawn 12 mo Benninghoff et al. (2012)	Oral administration (feed) PFOA, "analytical grade" aqueous exposure Sham/control, Sham/PFOA, AFB_1/control, AFB_1/PFOA ppm Initiation: aqueous exposure to AFB_1 (10 ppb [0.01 ppm]) for 30 min; promotion: after 1 mo, fed experimental diets containing PFOA (2000 ppm) 5 d/wk for 6 mo 250, 250, 250, 250 NR, NR, NR, NR	*Liver* Total tumours 0, 0, 13%, 62%*	*$P < 0.01$, logistic regression analysis (compared with AFB_1/control)	*Principal strengths*: the experiment was supplemented with hepatic gene expression analysis, adequate number of fish per group. *Principal limitations*: a short-term exposure was used in the global gene-expression experiment; males and females combined; only one dose used; no survival data. *Other comments*: no liver tumours were observed in non-initiated fish treated with PFOA; increased multiplicity and size of liver tumours, but statistical analysis not provided.
		Hepatocellular adenoma 0, 0, 26%, 10%	NS	
		Hepatocellular carcinoma 0, 0, 0, 27%	NS	
		Mixed carcinoma 0, 0, 47%, 54%	NS	
Initiation–promotion (tested as promoter) Rainbow trout, Mount Shasta strain (M, F) (combined) 10 wk post-spawn 12 mo Benninghoff et al. (2012)	Oral administration (feed) PFOA, "analytical grade" Aqueous exposure Sham/control, sham/PFOA, MNNG/control, MNNG/PFOA Initiation: aqueous exposure to MNNG (35 ppm) for 30 min; promotion: after 1 mo, fed experimental diets containing PFOA (2000 ppm) 5 d/wk for 6 mo 250, 250, 250, 250 NR, NR, NR, NR	*Liver* Total tumours 0, 0, 51%, 81%*	*$P < 0.0001$, logistic regression analysis (compared with MNNG/control)	*Principal strengths*: the use of MNNG as the initiation agent allowed investigation of whether tumorigenesis in other organs (kidney and swim bladder) was affected by PFOA treatment; adequate number of fish per group. *Principal limitations*: the MNNG dose was too high for estimation of effects of PFOA on stomach carcinogenesis (stomach tumour incidence in control fish was 99%); males and females combined; only one dose used; no survival data. *Other comments*: significant increase in liver tumour multiplicity ($P < 0.005$, Kruskal–Wallis test with Dunnett post hoc test for multiple comparisons).
		Hepatocellular adenoma 0, 0, 25%, 26%	NS	
		Hepatocellular carcinoma 0, 0, 28%, 11%	NS	
		Hepatocellular carcinoma [mixed] 0, 0, 39%, 55%	NS	
		Stomach, kidney, swim bladder No significant increase in the incidence of tumours		

2-AAF, 2-acetylaminofluorene; AFB_1, aflatoxin B_1; bw, body weight; d, day(s); DMBA, 7,12-dimethylbenz[*a*]anthracene; F, female; i.p., intraperitoneal; M, male; min, minute(s); MNNG, *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine; mo, month(s); NDEA, *N*-nitrosodiethylamine; NR, not reported; NS, not significant; PB, phenobarbital; PFOA, perfluorooctanoic acid; ppb, parts per billion; ppm, parts per million; RNA-seq, RNA sequencing; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; vs, versus; wk, week(s); yr, year(s).

(200 mg/kg bw, intraperitoneally), followed 2 weeks later by a selection procedure – feed containing 0.03% 2-acetylaminofluorene (2-AAF) for 2 weeks and in the middle of this treatment, after week 1, a treatment with a single dose of carbon tetrachloride at 2 mL/kg bw by gavage, 1:1 in corn oil (Abdellatif et al., 1990, also reported by Abdellatif et al., 1991 and Nilsson et al., 1991). One week after the selection procedure, the rats were divided into three groups receiving a basal diet (control), or a diet supplemented with either 0.05% phenobarbital (positive control) or 0.015% PFOA (analytical grade) for 23 weeks. [The average daily doses of 2-AAF, phenobarbital, and PFOA were not reported.] There were 7 rats in the control group, 8 rats in the phenobarbital-treated group, and 12 rats in the PFOA-treated group. Body weight was slightly but non-significantly decreased in the PFOA-treated group compared with the control group. [No data on survival at study termination were reported.] Liver samples were collected, and histological and histochemical evaluations were performed.

There was a significant increase ($P < 0.05$, Student *t*-test) in the incidence of total liver tumours in the phenobarbital-treated and PFOA-treated groups compared with the control group: 0/7, 6/8 (75%), and 4/12 (33%) for the control group, and groups receiving phenobarbital and PFOA, respectively. In the PFOA-treated group, 25% of the liver tumours were hepatocellular carcinomas (type I) and 8% were other tumours. In the phenobarbital-treated group, 63% were hepatocellular carcinomas (type I), and 12% were hepatocellular carcinomas (type IV). The tumour multiplicity was 3.4 and 1.2 for the phenobarbital- and PFOA-treated rats, respectively (Abdellatif et al., 1990).

Regarding pre-neoplastic lesions, some eosinophilic, basophilic, or mixed cell type foci and a few nodules were detected (Abdellatif et al., 1991). According to Nilsson et al. (1991), there were 8 nodules in the PFOA-treated group.

In the initiation–promotion study performed by the same research team (Abdellatif et al., 1991), groups of 15 adult male Wistar rats [age not reported] received a single intraperitoneal dose of DEN at 200 mg/kg bw for initiation. Control groups did not receive initiation treatment with DEN. After 2 weeks, the rats were fed basal feed (control), or feed containing 0.05% phenobarbital (positive control), or 0.005% or 0.02% PFOA (analytical grade) until termination at 12 months. The average daily doses of phenobarbital and PFOA were not reported. From each group, 5 rats were killed at the interim time of 3 months, and 10 rats were killed 12 months after the start of the experiment. Survival in the initiated groups was 7/10 (total in the control, phenobarbital-treated, and 0.005% PFOA-treated groups) and 9/10 in 0.02% PFOA-treated rats. Liver samples were collected, and histological and histochemical evaluations were performed.

There was a significant increase ($P < 0.05$, Sheffe test) in the incidence of hepatocellular carcinoma in the group at the higher dose of PFOA (DEN-initiated) – 0/7, 1/7 (14.3%), and 5/9 (55.5%), for the control group and the groups at 0.005% PFOA and 0.02% PFOA, respectively – at 12 months. For the positive control, phenobarbital, the result was 2/7 (28.6%) (Abdellatif et al., 1991). All the malignant tumours were well-differentiated type I hepatocellular carcinoma in rats treated with 0.005% PFOA. In the rats treated with 0.02% PFOA, four out of nine rats had moderately differentiated type II hepatocellular carcinoma, and one rat had a poorly differentiated type III hepatocellular carcinoma. No tumours were identified in rats that died at an early stage of the experiment, all within the first 8 months of the study, with the cause of death reported to be pneumonia in all cases. Tumour multiplicity was not reported for any treatment groups.

Regarding pre-neoplastic lesions, the rats with or without malignant tumours had foci and nodules containing a mainly eosinophilic, but

also basophilic, clear cell population or a mixed cell pattern (Abdellatif et al., 1991). No foci, nodules or malignant tumours were observed in non-initiated control rats either after 3 or 12 months, or in the initiated rats killed after 3 months. [The Working Group noted that PFOA concentrations were measured in the serum, and end-points were measured at two time points. However, both studies (Abdellatif et al., 1990, 1991) used only one sex; the purity of PFOA was not reported for either of these two protocols, only that it was of the purest available analytical grade; the average daily doses of PFOA, phenobarbital, and 2-AAF, and survival at study termination were not reported; and the histopathological examination was limited to the liver.]

In a study by Su et al. (2022), the effect of pubertal exposure to an environmentally relevant dose of PFOA was investigated, using a model of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced tumorigenesis in the rat mammary gland. The aim of the study was to investigate whether exposure to PFOA during puberty might alter susceptibility to breast cancer. Female Sprague-Dawley rats (age, 21 days) were randomized into 36 or 37 rats per group and exposed via gavage to sesame oil (controls), or to PFOA (purity, 95%) at a dose of 0.01 mg/kg bw, or to a combination of PFOA and zeranol (a metabolite of the mycotoxin zearalenone) (0.01 mg/kg bw), 5 days per week from age 21 to 42 days. At age 50 days, all rats were challenged with a single dose of DMBA (30 mg/kg bw) via gavage. There was no significant difference in body weight between treated and control groups. Survival was not significantly affected by PFOA treatment. The rats were monitored for the development of mammary gland tumours for 7 months.

There were no significant differences in tumour incidence or the number of tumours per rat in the groups treated with PFOA or with PFOA plus zeranol compared with the DMBA control group. Overall, tumour latency, based on tumour-free survival, was not significantly affected with PFOA alone.

Regarding pre-neoplastic lesions, none were reported. [The Working Group noted that this study used only one sex and one dose, and histological examination was performed on the mammary glands only. The Working Group also noted that tumour incidence in both the control group (DMBA-treated) and in groups treated with PFOA and DMBA was extremely high – 35/37 (94.6%) – therefore, it may have been very difficult to detect any promotion effects.]

In an initiation–promotion study in rainbow trout (*Oncorhynchus mykiss*), approximately 1000 fry were initiated at 10 weeks post-hatch with aqueous exposure to aflatoxin B_1 (AFB_1) at 0.01 ppm for 30 minutes. The non-initiated controls were sham-exposed trout, exposed to vehicle alone (0.01% ethanol). After 3 months, initiated trout were randomly divided among experimental treatment groups (140 animals per group) and fed a semi-purified casein-based diet containing PFOA [purity not reported] at a concentration of 200 or 1800 ppm (equivalent to doses of 5 and 50 mg/kg per day, respectively) for 5 days per week. [The Working Group noted that the concentration of PFOA in the water tank was not reported.] At 9 months post-initiation, juvenile fish were killed and sampled for liver tumour histological identification and examination using haematoxylin and eosin (Tilton et al., 2008).

No tumours were observed in non-initiated fish fed with control or PFOA diets, indicating lack of carcinogenic potential by themselves in this model. There was a significant increase ($P < 0.05$, logistic regression analysis) in the incidence of total liver tumours (cholangiocellular carcinoma, hepatocellular adenoma, hepatocellular carcinoma, and mixed adenoma and mixed carcinoma) – 36%, 34%, and 71% for the AFB_1/0 (control group), and the groups treated with AFB_1/200 ppm PFOA, and AFB_1/1800 ppm PFOA, respectively. [The Working Group noted

that mixed adenoma and carcinoma comprised both cholangiocellular and hepatocellular cell types that are considered to be originated from a common progenitor cell of bile duct cells and liver cells.] Specifically, there was a significant increase in overall tumour incidence (71%) in the group treated with AFB_1/1800 ppm PFOA compared with the control group ($P < 0.05$, logistic regression analysis). There was also an increase in the incidence of hepatocellular carcinoma – 10%, 11%, and 46% for the groups treated with AFB_1/0 (control), AFB_1/200, and AFB_1/1800 ppm, respectively – and in the incidence expressed as a percentage of hepatocellular adenoma – 3%, 0%, and 8% for the groups treated with AFB_1/0 (control), AFB_1/200, and AFB_1/1800 ppm, respectively – although no statistical testing for the individual tumour types was reported. In addition, the multiplicity of the induced tumours per animal was also increased.

Regarding non-neoplastic lesions, PFOA exposure produced hepatomegaly and basophilic foci (Tilton et al., 2008). [The Working Group noted that this study used an adequate number of animals per group and tested two doses of PFOA. However, data were combined for males and females, the purity of PFOA was not reported, no information on survival was provided, and histopathological examination was performed on the liver only.]

In another initiation–promotion study in Mount Shasta rainbow trout (*Oncorhynchus mykiss*), PFOA was evaluated by initiating about 3500 fry (age, 10 weeks) with AFB_1 at 10 ppb [0.01 ppm] or about 1000 fry (age, 10 weeks) with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) at 35 ppm for 30 minutes (Benninghoff et al., 2012). Since MNNG is a multiorgan carcinogen, MNNG initiation was used to determine whether the tumour-promoting effects of dietary PFOA were specific to hepatocarcinogenesis or dependent on the initiating carcinogen. Non-initiated sham controls in the two experiments were treated with the vehicle (0.01% ethanol or 0.01% dimethyl sulfoxide, DMSO, respectively). After initiation, the fry were given untreated feed (a semi-purified casein-based diet) for 1 month. Then, within each initiation cohort, trout were randomly divided into the treatment groups. In the first (AFB_1) cohort, fish were fed experimental diets containing PFOA at 2000 ppm (equivalent to 50 mg/kg per day, analytical grade), ad libitum, 5 days per week for 6 months. Untreated feed was used for controls. There were four exposure groups, each containing 250 fish: sham/control, sham/+PFOA, AFB_1/control, and AFB_1/+PFOA. The MNNG-initiated trout were exposed to the vehicle or PFOA at 2000 ppm. There were four exposure groups, each containing 250 fish: sham/−PFOA, sham/+PFOA, MNNG/−PFOA, and MNNG/+PFOA. The diet was prepared on a monthly basis and kept frozen at −20°C before use. The fish were terminated at 12.5 months post-spawn and examination of tumours was performed. Neoplasms were classified according to the criteria described in Hendricks et al. (1984).

A total of cholangiocellular adenomas or cholangiocellular carcinomas, hepatocellular adenomas or hepatocellular carcinomas, and mixed adenomas, and mixed carcinomas that consist of both cholangiocellular and hepatocellular cell types were counted as liver tumours. Initiation with AFB_1 at 10 ppb resulted in a moderate increase in liver tumour incidence (13%) compared with the control group. PFOA exposure significantly enhanced the incidence of liver tumours (62%) ($P < 0.01$, logistic regression analysis), and increased liver tumour multiplicity and size (both $P < 0.05$, Kruskal–Wallis test with Dunnett post hoc test for multiple comparisons). There was a significant increase ($P < 0.0001$, logistic regression analysis) in liver tumour incidence in the MNNG/PFOA group (86%) compared with the MNNG/control group (51%). Tumour multiplicity and size were also significantly increased by PFOA treatment (both $P < 0.001$, Kruskal–Wallis test with Dunnett post

hoc test for multiple comparisons). After MNNG initiation, kidney and stomach carcinogenesis was not significantly affected by PFOA exposure. Mixed carcinoma followed by hepatocellular adenoma and hepatocellular carcinoma were the most prevalent liver tumour types in both experiments, with the prevalence being lower than that seen in groups treated with AFB_1 alone and MNNG alone (individual tumour types were not statistically analysed). [The Working Group noted that this study used an adequate number of animals per group; however, data were combined for males and females, only one dose of PFOA was tested, and the purity for PFOA was reported only as "analytical grade". It was noted that the results of these studies indicated that PFOA can act as a promoter in this fish model. Furthermore, the data reflect chemical-specific responses in the liver with both AFB_1 (liver-specific) and MNNG (multiorgan) initiators.]

3.2 Perfluorooctanesulfonic acid (PFOS)

See Table 3.4.

3.2.1 Mouse

Transplacental exposure

The tumorigenic effects of gestational exposure to PFOS were evaluated in C57BL/6J-*Apc*$^{Min/+}$ mice, a mouse model that develops intestinal tumours because of a mutation in the tumour suppressor gene, adenomatous polyposis cell (*Apc*) (Ngo et al., 2014). The wildtype (*Apc*$^{+/+}$) females were mated to heterozygotic males (*Apc*$^{Min/+}$). Wildtype dams were then treated by gavage with PFOS (purity, ≥ 98%) at a dose of 0 (water vehicle), 0.01, 0.1, or 3.0 mg/kg per day on days 1–17 of gestation. Insufficient rates of pregnancy and littering rates and low F_1 survival were observed in the first experimental block – block 1, 0 (water vehicle), PFOS at 0.1 and 3.0 mg/kg bw per day; 104 mice (age, 7–8 weeks) – thus a second block was added that had a lower PFOS exposure (block 2, 0 (water vehicle), PFOS at 0.01 and 0.1 mg/kg bw per day; 100 dams (age, 9–10 weeks). The numbers of mice (*Min*/+) obtained in each dose group (both blocks combined) were 15, 10, 12, and 7 for males and 23, 6, 13, and 5 for females exposed in utero to vehicle (water), 0.01, 0.1, and 3.0 mg/kg bw of PFOS, respectively. Because of difficulty in ascertaining pregnancy status, exposure varied on days 14 to 17 of gestation. F_1 offspring were genotyped via polymerase chain reaction (PCR) using DNA collected from ear punches. Offspring were genotyped as wildtype (*Apc*$^{+/+}$) and heterozygous (*Apc*$^{Min/+}$), and only the *Min*/+ mice were used for the carcinogenesis study. Efforts were made to verify dosing level by measuring PFOS stability in the dosing solution. Furthermore, PFOS concentrations in tap water and feed used for the study were quantified, and levels of PFOS in the serum of mice in the vehicle control group were below the LOQ. Internal exposure was quantified in the serum (2 mice per time point) in dams on day 18 of gestation, and postnatal day 23 (block 1) or postnatal days 26–28 (block 2), and F_1 pups on postnatal days 25–27 (depending on block). The LOQ in serum was 0.05 ng/mL. Although minimal data were generated, they confirmed that there was internal exposure within dams and pups and that it increased with dose and decreased with time after dosing (day 18 of gestation versus postnatal day 23 in dams). Evaluation of intestinal tumorigenesis occurred at age 11 weeks in the F_1 *Min*/+ mice. The number, diameter, and localization of tumours in the small intestine and colon were measured by transillumination in an inverse light microscope. The reviewer scored lesions at 20× magnification and was blinded to treatment. Statistical analysis of incidence was conducted on both an individual and litter basis; furthermore, blocks were combined in the analysis if no consistent differences were found between blocks 1 and 2. None of the PFOS doses

Table 3.4 Studies of carcinogenicity in experimental animals exposed to PFOS

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J-*Apc*$^{Min/+}$ (M) Day 1 of gestation 11 wk Ngo et al. (2014)	Oral administration (gavage) PFOS, ≥ 98% Water 0, 0.01, 0.1, 3.0 mg/kg bw per day 15, 10, 12, 7 15, 10, 12, 7	*Small intestine (duodenum, jejunum or ileum)* No significant increase in tumour incidence 15/15, 10/10, 12/12, 7/7 *Colon* No significant increase in tumour incidence 15/15, 3/3, 19/19, 0	 NS NS	*Principal strengths*: males and females studied; multiple doses; analysed background levels of PFOS in feed and drinking-water; analysed internal doses of PFOS; tested stability of PFOS; blocks of PFOS administration were compared statistically. *Principal limitations*: small number of mice per group; short duration of study; histopathological examination not conducted; batch number of PFOS was not stated; varied exposure (14–17 d). *Other comments*: study of transplacental exposure; increase in the incidence of spontaneous tumours was studied in this mouse model; tumours of the small intestine were observed in all experimental groups of *Min*/+ mice, demonstrating 100% incidence for this end-point, as is usual in this mouse model; multiplicity was not increased by PFOS exposure.

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J-*Apc*[Min/+] (F) Day 1 of gestation 11 wk Ngo et al. (2014)	Oral administration (gavage) PFOS, ≥ 98% Water 0, 0.01, 0.1, 3.0 mg/kg bw per day 23, 6, 13, 5 23, 6, 13, 5	*Small intestine (duodenum, jejunum or ileum)* No significant increase in tumour incidence 23/23, 6/6, 13/13, 5/5 *Colon* No significant increase in tumour incidence	 NS	*Principal strengths*: males and females studied; multiple doses; analysed background concentrations of PFOS in feed and drinking-water; analysed internal doses of PFOS; tested stability of PFOS; blocks of PFOS administration were compared statistically. *Principal limitations*: small and unbalanced number of mice per group; short duration of study; histopathological examination not conducted; batch number of PFOS was not reported. *Other comments*: study of transplacental exposure; increase in incidence of spontaneous tumours was studied in this mouse model; small intestinal tumours were observed in all experimental groups of *Min*/+ mice, demonstrating 100% incidence for this end-point, as is usual in this mouse model; multiplicity was not increased by PFOS exposure.
Full carcinogenicity Rat, Crl:CD (SD)IGS BR (M) Approx. 41 d 104 wk Butenhoff et al. (2012b)	Oral administration (feed) PFOS (potassium salt), 86.9% Acetone 0, 0.5, 2, 5, 20 ppm 7 d/wk 50, 50, 50, 50, 51 11, 11, 17, 25, 23	*Liver* Hepatocellular adenoma 0/60, 3/50, 3/50, 1/50, 7/60* *Thyroid gland* Follicular cell adenoma 3/60, 5/49, 4/50, 4/49, 4/59 Follicular cell carcinoma 3/60, 1/49, 1/50, 2/49, 1/59 Follicular cell adenoma or carcinoma (combined) 6/60, 6/49, 5/50, 5/49, 5/59	 $P = 0.0276$, Cochran–Armitage trend test *$P = 0.046$, Dunnett *t*-test NS NS NS	*Principal strengths*: adequate number of rats used; randomly allocated in groups; males and females used; adequate duration. *Other comments*: "N at Start" removed rats from 4, 14, and 52 wk interim necropsy; note: Laboratory Report and Butenhoff include 52-wk interim animals in "*n*" for tumour incidence in the 0 and 20 ppm groups (e.g. 20 ppm *n* = 7/60 including 52 interim weeks vs *n* = 7/50 using only ≥ 53 wk); survival data in laboratory report were calculated using *n* = 50.

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Rat, Crl:CD (SD)IGS BR (M) Approx. 41 d 104 wk Butenhoff et al. (2012b) (cont.)		*Pancreas*		
		Islet cell adenoma		
		4/60, 3/49, 4/50, 4/50, 4/60	NS	
		Islet cell carcinoma		
		1/60, 2/49, 2/50, 5/50, 5/60	[*P* = 0.02, Cochran–Armitage trend test (not survival adjusted)] [*P* = 0.13, poly-3 trend test (survival adjusted, with interim animals)] [*P* = 0.117, poly-3 trend test (survival adjusted, without interim animals)]	
		Islet cell adenoma or carcinoma (combined)		
		5/60, 5/49, 6/50, 8/50, 9/60	NS	
Full carcinogenicity Rat, Crl:CD (SD)IGS (F) Approx. 41 d 104 wk Butenhoff et al. (2012b)	Oral administration (feed) PFOS (potassium salt), 86.9% Acetone 0, 0.5, 2, 5, 20 ppm 7 d/wk 50, 50, 50, 50, 50 25, 15, 10, 17, 26	*Liver*		*Principal strengths*: adequate number of rats used; randomly allocated in groups; males and females used; adequate duration. *Other comments*: inclusion of animals killed at the 52-wk interim time point in statistical analysis; experiment terminated at 103 wk for the group at 2 ppm; "N at Start" removed rats from 4, 14, and 52 wk interim necropsy; note: US EPA (2002) and Butenhoff included 52-wk interim animals in "*n*" for tumour incidence in the groups at 0 and 20 ppm (e.g. 20 ppm, *n* = 7/60 including 52 interim weeks vs *n* = 7/50 using only ≥ 53 wk); survival data in this laboratory report were calculated using *n* = 50.
		Hepatocellular adenoma		
		0/60, 1/50, 1/49, 1/50, 5/60*	*P* = 0.0153, Cochran–Armitage trend test **P* = 0.0386, Dunnett *t*-test	
		Hepatocellular carcinoma		
		0/60, 0/50, 0/49, 0/50, 1/60	NS	
		Hepatocellular adenoma or carcinoma (combined)		
		0/60, 1/50, 1/49, 1/50, 6/60*	*P* = 0.0057, Cochran–Armitage trend test **P* = 0.0204, Dunnett *t*-test	
		Thyroid gland		
		Follicular cell adenoma		
		0/60, 0/50, 0/49, 2/50, 1/60	NS	

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Rat, Crl:CD (SD)IGS (F) Approx. 41 d 104 wk Butenhoff et al. (2012b) (cont.)		Follicular cell carcinoma 0/60, 0/50, 0/49, 1/50, 0/60	NS	
		Follicular cell adenoma or carcinoma (combined) 0/60, 0/50, 0/49, 3/50*, 1/60	*P = 0.047, Dunnett t-test	
		Mammary gland Fibroadenoma 20/60, 27/50*, 19/48, 24/50, 11/60	*P = 0.0337, Dunnett t-test	
		Adenoma 7/60, 6/50, 5/48, 7/50, 4/60	NS	
		Carcinoma 11/60, 12/50, 15/48, 11/50, 14/60	NS	
		Fibroadenoma or adenoma (combined) 23/60, 30/50*, 22/48, 26/50, 15/60	*P = 0.318, Dunnett t-test	
		Fibroadenoma, adenoma, or carcinoma (combined) 29/60, 36/50*, 31/48**, 29/50, 24/60	P = 0.0482, Cochran–Armitage trend test *P = 0.0474, Dunnett t-test **P = 0.0066, Dunnett t-test	

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Rat, Crl:CD (SD)IGS BR rats (M) Approx. 41 d 52 wk Butenhoff et al. (2012b)	Oral administration (feed) PFOS (potassium salt), 86.9% Acetone 0, 20 (recovery) ppm 0, 20 ppm, 7 d/wk for 52 wk followed by basal feed with acetone control for 52 wk 50, 40 11, 11	*Liver*		*Principal strengths*: adequate number of rats used; randomly allocated in groups; males and females used; adequate duration. *Other comments*: “N at Start” removed rats from the interim necropsy at 4, 14, and 52 wk; note: US EPA (2002) and Butenhoff included 52-wk interim rats in “*n*” for tumour incidence in the groups at 0 and 20 ppm (e.g. 20 ppm $n = 7/60$ including 52 interim weeks vs $n = 7/50$ using only ≥ 53 wk); survival data in this laboratory report were calculated using $n = 50$.
		Hepatocellular adenoma		
		0/60, 0/40		
		Thyroid gland		
		Follicular cell adenoma		
		3/60, 9/39*	*$P = 0.0280$, Dunnett *t*-test	
		Follicular cell carcinoma		
		3/60, 1/39	NS	
		Follicular cell adenoma or carcinoma (combined)		
		6/60, 10/39	NS	
		Pancreas		
		Islet cell adenoma		
		4/60, 11/40	NS	
		Islet cell carcinoma		
		1/60, 3/40	NS	
		Islet cell adenoma or carcinoma (combined)		
		5/60, 4/40	NS	
Full carcinogenicity Rat, Crl:CD (SD)IGS (F) Approx. 41 d 52 wk Butenhoff et al. (2012b)	Oral administration (feed) PFOS (potassium salt), 86.9% Acetone 0, 20 (recovery) ppm 0, 20 ppm, 7 d/wk for 52 wk followed by basal feed with acetone control for 52 wk 50, 40 25, 19	*Liver*		*Principal strengths*: adequate number of rats used, randomly allocated in groups, males and females used, adequate duration; well-conducted GLP study. *Other comments*: inclusion of 52-wk interim rats in statistical analysis; experiment terminated at 103 wk for the group at 2 ppm; “N at Start” removed rats from interim necropsy at 4, 14, and 52 wk; note: US EPA (2002) and Butenhoff included 52-wk interim rats in “*n*” for tumour incidence in the groups at 0 and 20 ppm (e.g. 20 ppm, $n = 7/60$ including 52 interim weeks vs $n = 7/50$ using only ≥ 53 wk); survival data in this laboratory report were calculated using $n = 50$.
		Hepatocellular adenoma		
		0/60, 2/40	NS	
		Hepatocellular carcinoma		
		0/60, 0/40	NS	
		Hepatocellular adenoma or carcinoma (combined)		
		0/60, 2/40	NS	
		Thyroid gland		
		Follicular cell adenoma		
		0/60, 1/40	NS	
		Follicular cell carcinoma		
		0/60, 0/40	NS	
		Follicular cell adenoma or carcinoma (combined)		
		0/60, 1/40		

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Rat, Crl:CD (SD)IGS (F) Approx. 41 d 52 wk Butenhoff et al. (2012b) (cont.)		*Mammary gland* Fibroadenoma 20/60, 15/40 Adenoma 7/60, 4/40 Carcinoma 11/60, 4/40 Fibroadenoma or adenoma (combined) 23/60, 16/40 Fibroadenoma, adenoma, or carcinoma (combined) 29/60, 17/40	 NS NS NS NS	
Initiation–promotion (tested as promoter) Rainbow trout, (*Oncorhynchus mykiss*) (M, F) (combined) 15 wks (at initiation) 6 mo Benninghoff et al. (2012)	Oral administration (feed) PFOS, unspecified 0.01% EtOH Sham/control, Sham/PFOS, AFB_1/control, AFB_1/PFOS ppm 5d/wk 250, 250, 250, 250 NR, NR, NR, NR	*Liver* Total tumours 0, 0, 1%, 13%*	*$P < 0.01$, logistic regression analysis (compared with AFB_1/control)	*Principal strengths*: adequate number of animals used; randomly allocated in groups. *Principal limitations*: males and females combined; only one dose; purity not reported. *Other comments*: survival and incidence number not reported, just the percentage incidence in each group; appeared that multiplicity increased somewhat, but numbers not provided; liver tumour diameter not increased.
Initiation–promotion (tested as promoter) Zebrafish (*Danio rerio*), *Kras*^{V12} transgenic (M, F) (combined) 90 d post fertilization 10 d Zhu et al. (2021)	Aqueous exposure PFOS (potassium salt), > 98% 0.1% DMSO (v/v) DMSO, DOX, PFOS, DOX + PFOS µg/L 7 d/wk 24, 24, 24, 24 NR, NR, NR, NR	*Liver* Hepatocellular adenoma 0/6, 3/6, 0/6, 1/6 Hepatocellular carcinoma 0/6, 2/6, 0/6, 5/6*	 [NS] [*$P = 0.0076$, Fisher exact test]	*Principal strengths*: liver histology conducted. *Principal limitations*: neoplasm incidences not reported; short duration; small number of animals per group; data combined for males and females; limited histopathological description. *Other comments*: incidence derived from a graph; histology conducted on 6 fish per treatment.

AFB_1, aflatoxin B_1; bw, body weight; d, day(s); DMSO, dimethyl sulfoxide; DOX, doxycycline; EtOH, ethanol; F, female; M, male; mo, month(s); NR, not reported; NS, not significant; PFOS, perfluorooctanesulfonic acid; ppm, part per million; v/v, volume per volume; wk, week(s).

affected body weight, compared with dams and F_1 offspring in the vehicle control group.

There was a 100% incidence of tumours of the small intestine in heterozygous *Min*/+ mice, as is usual in this mouse model. Exposure to PFOS did not cause a significant increase in the number or multiplicity of small intestine tumours among male and female heterozygous mice. Tumour diameters were not significantly increased with increasing PFOS exposure within male mice, but there was an increase in tumour size in females in the groups treated with PFOS at 0.01 and 3.0 mg/kg bw compared with the control group (mice at the intermediate dose of 0.1 mg/kg bw were unaffected). Fewer tumours were observed in the colon than in the small intestine, as usual in this model, and no statistical differences were observed between PFOS-exposed groups and the control group with regard to incidence, number per mouse (multiplicity), or size of colon tumours. The localization of tumours along the small intestine and colon was not affected by treatment with PFOS. [The Working Group noted that this study used both sexes and multiple doses; PFOS stability was tested and the internal dose was analysed, as were background levels of PFOS in feed and drinking-water. However, no histological examination was performed.]

3.2.2 Rat

Oral administration (feed)

There was only one study on the carcinogenicity of PFOS in rodents. For this well-conducted study that complied with GLP, the data were available in the original laboratory report (US EPA, 2002) and as a manuscript published at a later date (Butenhoff et al., 2012b). In this study, PFOS (T-6295; purity, 86.9%) was administered in the feed at a concentration of 0 (control), 0.5, 2, 5, or 20 ppm to initial groups of 70, 60, 60, 60, 70, and 40 (20 ppm recovery group) Sprague-Dawley (Crl:CD(SD)IGS BR) male and female rats, respectively. Of these rats, 50 rats per group received control feed or PFOS for the full 2-year exposure (groups at 0–20 ppm). A recovery group of 40 males and 40 females was also included; rats in this group were treated with feed containing PFOS at 20 ppm for 52 weeks, and then allowed a recovery of 52 weeks. Controls received the control feed (basal feed with acetone as vehicle). Interim necropsies were carried out at 4, 14, and 53 weeks, when clinical chemistry, PFOS concentrations, and liver end-points (palmitoyl-CoA oxidase activity for PPARα activity, cell proliferation) were evaluated. PFOS consumption was 0.024, 0.098, 0.242, and 0.984 mg/kg bw per day for males and 0.029, 0.120, 0.299, and 1.251 mg/kg bw per day for females over the 104-week period, for the groups at 0.5, 2, 5, and 20 ppm, respectively. PFOS consumption for the recovery groups at 20 ppm was 1.144 and 1.385 mg/kg bw per day for males and females, respectively. Body weight was lower in the recovery groups of males and females at 20 ppm compared with that in the control group at the end of the dosing period, but body weights in the recovery group post-exposure tended back towards control values. At 104 weeks, survival of rats selected for the 2-year evaluation was: 11/50, 11/50, 17/50, 25/50, 23/51, and 11/40 in males and 25/50, 15/50, 10/50 (at 102 weeks), 17/50, 26/50, and 19/40 in females, for the groups at 0 (control), 0.5, 2, 5, 20 ppm, and 20 ppm (recovery), respectively. There was some indication of longer survival among male rats at 5 and 20 ppm compared with controls, possibly because of lower survival in the controls. [The Working Group noted that survival was lower in the male control groups compared with the treated groups, and the Peto statistical test was performed, taking differences in the survival into account.] One group of treated females (2 ppm) had decreased survival compared with controls (US EPA, 2002; also reported by Butenhoff et al., 2012b).

Rats from the 53-week evaluation of the control group and the group at 20 ppm (highest dose) and from the 104-week terminal necropsy were

included to determine tumour incidence within the groups. For the evaluation of the incidence of non-neoplastic lesions, rats were included from the 14-, 53-, and 104-week necropsies.

In males, there was a significant positive trend (P = 0.0276, Cochran–Armitage trend test) in the incidence of hepatocellular adenoma, and the incidence – 0/60, 3/50, 3/50, 1/50, and 7/60 for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively – was significantly increased at 20 ppm (P = 0.0456, Dunnett t-test), compared with controls. Incidence in the recovery group at the highest dose (20 ppm) was similar to that in controls (0/60 versus 0/40). There was no significant increase in the incidence of thyroid follicular cell adenoma in continuously exposed male rats – 3/60, 5/49, 4/50, 4/49, and 4/59 for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively – however, there was a significant increase (P = 0.0280, Dunnett t-test) in the incidence of thyroid follicular cell adenoma in the recovery group at 20 ppm (9/39) compared with the control group (3/60).

In females, there was a significant positive trend (P = 0.0153, Cochran–Armitage trend test) in the incidence of hepatocellular adenoma – 0/60, 1/50, 1/49, 1/50, and 5/60, for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively – with the incidence being significantly increased (P = 0.0386, Dunnett t-test) in the group at 20 ppm compared with controls. There was a significant positive trend (P = 0.0057, Cochran–Armitage trend test) in the incidence of hepatocellular adenoma or carcinoma (combined), with the incidence – 0/60, 1/50, 1/49, 1/50, and 6/60 for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively – being significantly increased (P = 0.0204, Dunnett t-test) in the groups at 20 ppm compared with controls. In the group at 20 ppm, the incidence of hepatocellular carcinoma was 2% (1/60). For females, there was no difference between the recovery group (20 ppm) and the controls. There was a significant increase (P = 0.047, Dunnett t-test) in the incidence of thyroid follicular cell adenoma or carcinoma (combined) in females in the group at 5 ppm – 0/60, 0/50, 0/49, 3/50, and 1/60 for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively. The incidence of mammary gland fibroadenoma – 20/60, 27/50, 19/48, 24/50, and 11/60 for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively – was significantly increased in the group at 0.5 ppm compared with controls (P = 0.0337, Dunnett t-test). The incidence of mammary gland fibroadenoma or adenoma (combined) was increased in the group at 0.5 ppm (P = 0.0318, Dunnett t-test). Not reported in Butenhoff et al. (2012b), but present in the laboratory report (US EPA, 2002), the incidence of mammary gland fibroadenoma, adenoma, or carcinoma (combined) – 29/60, 36/50, 31/48, 29/50, and 24/60 for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively – was significantly increased in the groups at 0.5 and 2.0 ppm (P = 0.0474 and P = 0.0066, Dunnett t-test, respectively). The increases in the incidence of combined mammary neoplasms within these groups were mostly because of increases in the incidence of fibroadenoma. There was no increase in the incidence of tumours in females in the recovery group (20 ppm) compared with the controls. [The Working Group noted that there was a tumour response for cancer of the thyroid gland in male (recovery group at 20 ppm) and female (continuous exposure at 5 ppm) rats compared with controls. However, the response was not dose-dependent in females, and no increase was observed in males at the highest dose and continuous exposure. Furthermore, mammary gland tumours were only observed at the lowest exposures and the association with PFOS was uncertain. The positive findings from this study were liver tumours in males and females.]

Regarding non-neoplastic lesions, these were observed in the liver in both males and females, primarily in the group at 20 ppm, and included hepatocellular hypertrophy, eosinophilic granular cytoplasm, hepatocellular pigmentation,

individual hepatocyte necrosis, hepatocellular vacuolation, and cystic degeneration (males only). In females, there was an increase in the incidence of lymphohistiocytic infiltrate and pigmented macrophage infiltrate within the liver. Increased liver weight (absolute and relative) in males and hepatocyte hypertrophy in both males and females in the group at 20 ppm were observed at weeks 14 and 53. However, for both sexes, no significant increases in the incidence of liver cell proliferation were observed at weeks 4 and 14 (proliferating cell nuclear antigen, PCNA) or at week 53 (bromodeoxyuridine).

[The Working Group noted that this study used an adequate number of animals per group, both sexes, and an adequate duration of exposure. The reason for the inclusion of interim animals (control group and 20 ppm) from week 52 with animals from week 104 in the report by US EPA (2002) and Butenhoff et al. (2012b) was unclear, as the exposure was significantly different. A review of the histopathology results for males and females from week 52 showed that many of the rats had no neoplasms (26/39) or only had pituitary adenomas (11/39). The Working Group also noted the nearly significant positive trend in the incidence of pancreatic islet carcinoma, but there were no significant changes by pairwise comparison. The Working Group was uncertain of this finding and the association with PFOS exposure because hyperplasia, adenomas, and the combination of adenoma or carcinoma were also not significant in males, and no pancreatic islet cell effect in female rats was observed. The Working Group also noted that the pancreatic islet cell tumours were not reported in Butenhoff et al. (2012b) but were reported in US EPA (2002). The Working Group conducted survival-adjusted statistical analyses on the data for pancreatic islet cell carcinoma, because it was noted that survival in controls was low, using the poly-3 test method. There was no significant difference in the trend test results in analyses including the 53-week interim animals ($P = 0.130$) or excluding the 53-week interim animals ($P = 0.117$), and, similarly to in the report by US EPA (2002), no significant pairwise comparisons with incidence in the controls were found.]

3.2.3 Fish

Initiation–promotion studies

Benninghoff et al. (2012) used Mount Shasta rainbow trout (*Oncorhynchus mykiss*) to evaluate PFOS promoter activity by initiating 1000 fry (age, 15 weeks) for 30 minutes with either aflatoxin B_1 (AFB_1) at a concentration of 10 ppb or a sham control of 0.01% ethanol. These initiated fry were then treated with a diet containing PFOS (purity not reported; analytical grade) at a concentration of 100 ppm (equivalent to 2.5 mg/kg bw per day). In all, there were four exposure groups, each containing 250 fish: sham/control, sham/PFOS, AFB_1/control, and AFB_1/PFOS. The dietary concentration of PFOS was selected on the basis of a pilot study in which the concentration used, 2000 ppm, resulted in high mortality. The PFOS diet was provided for 6 months; during this time, the diet was prepared on a monthly basis and kept frozen at –20 °C before use. Body weight was decreased after AFB_1/PFOS exposure compared with sham/control ($P < 0.05$, logistic regression analysis), whereas body weight in sham/control and sham/PFOS exposure groups was not significantly different. Liver weights (absolute and relative) were increased after treatment with PFOS, with and without initiation. The fish were killed at 12.5 months post-spawn, and examination of tumours was performed.

After initiation with AFB_1 at 10 ppb and in the absence of subsequent PFOS exposure, there was a 1% induction of liver tumours. In the sham controls with and without PFOS exposure, there was no induction of liver tumours. After initiation followed by PFOS exposure, there was a significant increase in the incidence of liver tumours, compared with controls ($P < 0.01$, logistic regression analysis; 13% compared with

1%). Tumour multiplicity and tumour size after PFOS exposure were not different from those for the controls. Mixed carcinoma was the most prevalent tumour type after AFB_1/PFOS exposure and AFB_1/control. [The Working Group noted that an adequate number of fish per group was used in this study; however, data were combined for males and females, only one dose of PFOS was tested, and PFOS purity was not reported.]

In a study by Zhu et al. (2021), male *Kras*^{V12} transgenic zebrafish (*Danio rerio*) (age, 90 days post-fertilization), in which hepatocellular carcinomas can be initiated via doxycycline (DOX)-induced expression of the *Kras* G12V oncogene in the liver (see Chew et al., 2014), were used to determine whether PFOS alone or DOX + PFOS could initiate or promote hepatocellular carcinoma, respectively. Adult transgenic zebrafish were immersed in PFOS (purity, > 98%) with or without DOX, in the dark for 10 days, to avoid photodegradation of the DOX. Four exposure groups were evaluated: 0.01% DMSO (control), DOX (20 mg/L), PFOS (500 µg/L), and DOX + PFOS (20 mg/L plus 500 µg/L, respectively). The selection of PFOS exposures was made on the basis of a short-term (4 day) study in zebrafish larvae exposed to PFOS at concentrations of 50, 100, 200, 500, and 1000 µg/L. After the 10-day exposure, the fish were killed, and livers were evaluated. Three exposure replicates were conducted, with 8 fish per replicate. From these 24 fish, livers from 6 fish were used for histological analysis and livers from 3 fish were used for transcriptomics analysis. [The Working Group noted that PFOS concentrations were not verified either in the aqueous exposure or in the internal dose.]

The hepatosomatic index (liver weight relative to body weight) in adult zebrafish was increased after treatment with DOX or DOX + PFOS, compared with DMSO (both $P < 0.05$), but not with PFOS alone, and was increased after treatment with DOX + PFOS versus DOX ($P < 0.05$). Liver size (mm^2), and fluorescence intensity (resulting from expression of a liver-specific enhanced green fluorescent protein) were increased in the group exposed to DOX + PFOS compared with the group exposed to DOX alone (both $P < 0.05$). The incidence of hepatocellular carcinoma was higher in the group exposed to DOX + PFOS (5/6) than in the group exposed to DOX alone (2/6), and no hepatocellular carcinomas were observed in the groups exposed to PFOS alone (0/6) or DMSO (0/6). [The Working Group noted that a small number of fish were used (n = 6) for histopathological evaluation of liver tumours. Statistical analysis by the Working Group showed no significant difference between the DOX group (2/6; 40%) and the DOX + PFOS group (5/6; 83%) [P = 0.1212, Fisher exact test]. The duration of the study appeared to be based on a 3-month study in which the establishing of this transgenic line was reported, and in which increasing mortality was observed shortly after induction or initiation in a 3-month study.]

3.3 Evidence synthesis for cancer in experimental animals

3.3.1 PFOA

The carcinogenicity of PFOA has been assessed in two well-conducted GLP studies, one in male and female Sprague-Dawley rats treated by oral administration (in the feed) in a combination of F_0 (in utero and lactation) and F_1 (post-weaning) exposure (NTP, 2020) and the other in male and female Sprague-Dawley rats treated by oral administration (in the feed) (US EPA, 1987); also reported by Butenhoff et al., 2012a) [histological re-analysis by Hardisty et al. (2010) (mammary gland) and Caverly Rae et al., 2014 (pancreas)]. The carcinogenicity of PFOA was also evaluated in studies that did not comply with GLP. Specifically, these were studies of oral administration (feed) in male Sprague-Dawley rats (Biegel et al., 2001); oral administration (gavage) in male and female C57BL/6J-*Apc*$^{Min/+}$

mice (Ngo et al., 2014); and studies in female CD-1 mice, female 129/Sv wildtype, and 129/Sv PPARα-knockout mice (Filgo et al., 2015). In addition, there were six initiation–promotion studies of oral administration (feed) in male Wistar rats (Abdellatif et al., 1990, 1991; also reported by Nilsson et al., 1991), in male and female rainbow trout (Tilton et al., 2008; Benninghoff et al., 2012); of oral administration (drinking-water) in male and female *LSL-Kras^{G12D};Pdx-1 Cre* (KC) transgenic mice (Kamendulis et al., 2022); and of oral administration (gavage) in female Sprague-Dawley rats (Su et al., 2022).

In the dietary study that complied with GLP in F_1 male and female Sprague-Dawley rats (NTP, 2020), a significant positive trend in the incidence of hepatocellular adenoma (includes multiple) was observed in males, and the incidence was significantly increased in both the F_0 exposed and F_0 unexposed groups. There was a positive trend in the incidence of hepatocellular carcinoma only in F_1 males with F_0 exposure. There was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) in F_1 males in both the F_0 exposed and F_0 unexposed groups, and the incidence was significantly increased in both groups. There was a significant positive trend in the incidence of acinar cell adenoma of the pancreas (includes multiple) in F_1 males in both the F_0 exposed and F_0 unexposed groups, and the incidence was significantly increased in both groups. There was a significant positive trend in the incidence of acinar cell adenoma or adenocarcinoma (combined) of the pancreas in F_1 males in both the F_0 exposed and F_0 unexposed groups, and the incidence was significantly increased in both groups. In female rats, there was a significant increase in the incidence of adenocarcinoma of the uterus in the group with F_1 exposure at the highest dose without F_0 exposure. In female rats, there was a significant positive trend in the incidence of pancreatic acinar cell adenoma or adenocarcinoma (combined) in F_1 rats with F_0 exposure. [The Working Group noted that a low incidence of pancreatic acinar cell adenoma or carcinoma (combined) and of pancreatic acinus hyperplasia was observed in females; these rare lesions in female rats were considered to be associated with PFOA exposure.]

In another dietary study that complied with GLP in male and female Sprague-Dawley rats (Butenhoff et al., 2012a), there was a significant positive trend in the incidence of testicular Leydig cell adenoma, and the incidence was significantly increased at the highest dose in males. In females, there was a significant positive trend in the incidence of fibroadenoma of the mammary gland, and the incidence was significantly increased at both doses. A pathology working group was convened by the study sponsor(s) to review the original slides of the mammary glands from the study by US EPA (1987), a study that was also reported by Butenhoff et al. (2012a), and concluded that PFOA did not induce neoplasms of the mammary gland in those studies (Hardisty et al., 2010; Butenhoff et al., 2012a). [The Working Group agreed with the conclusion of Hardisty et al. (2010) that PFOA did not induce neoplasms of the mammary gland.]

In the single-dose dietary study in male Sprague-Dawley rats (Biegel et al., 2001), there was a significant increase in the incidence of hepatocellular adenoma, and of hepatocellular adenoma or carcinoma (combined). There was a significant increase in the incidence of testicular Leydig cell adenoma. There were significant increases in the incidence of pancreatic acinar cell adenoma, and in the incidence of pancreatic acinar cell adenoma or carcinoma (combined).

In a study of oral administration (gavage) in female CD-1 mice, there was a significant increase in the incidence of hepatocellular adenoma at the intermediate dose only, and a significant positive trend in the incidence of liver haemangiosarcoma (Filgo et al., 2015). No increase in the incidence of hepatic neoplasms was observed in treated female 129/Sv wildtype and PPARα-knockout

mice. [The Working Group considered the liver haemangiosarcomas to be possibly associated with PFOA exposure; however, the Working Group acknowledged that 16–28% of unscheduled deaths in all groups were not examined. The Working Group was uncertain regarding the biological significance of the hepatocellular adenoma results.]

In studies of oral administration (feed) and initiation–promotion in rats (Abdellatif et al., 1990, 1991; also reported by Nilsson et al., 1991) and fish (Tilton et al., 2008; Benninghoff et al., 2012), the promoting activity of PFOA was investigated. There was a significant increase in the incidence of total tumours of the liver at the intermediate and higher doses (Abdellatif et al., 1990) and a significant increase in the incidence of hepatocellular carcinoma at the highest dose (Abdellatif et al., 1991) in male Wistar rats. There was a significant increase in the incidence of total tumours of the liver (malignant and benign) (Tilton et al., 2008) and of total tumours of the liver Benninghoff et al. (2012) in male and female rainbow trout. [The Working Group noted that the proportion of malignant tumours (as a percentage of the total liver tumours) was higher than that of benign tumours. The Working Group also noted that PFOA acted as a cancer promoter in these studies.]

In a study of oral administration (gavage) in male and female C57BL/6J-*Apc*$^{Min/+}$ mice (Ngo et al., 2014), and a promotion study of oral administration (drinking-water) in male and female *LSL-Kras*^{G12D}*;Pdx-1 Cre* (KC) transgenic mice (Kamendulis et al., 2022) there was no significant increase in the incidence of tumours. In a promotion study of oral administration (gavage) in female Sprague-Dawley rats, no significant increase in tumour incidence was found (Su et al., 2022). [The Working Group noted that this negative result may have been due to the high initiating dose.] [The Working Group noted that an effect in the liver and pancreas in male rats was observed consistently throughout the studies, while an effect in the pancreas in female rats was only observed in the NTP study. This effect in the female pancreas is possibly a result of the higher exposure to PFOA in females than in males, compensating for the faster elimination in females than in males.]

3.3.2 PFOS

The carcinogenicity of PFOS has been assessed in one well-conducted study that complied with GLP in male and female Sprague-Dawley rats treated by oral administration (feed) (US EPA, 2002; also reported by Butenhoff et al., 2012b). The carcinogenicity of PFOS was also evaluated in three studies that did not comply with GLP. One study was of oral administration (gavage) in male and female C57BL/6J-*Apc*$^{Min/+}$ mice (Ngo et al., 2014). Two initiation–promotion studies were of oral administration (feed) in male and female rainbow trout (*Oncorhynchus mykiss*) (Benninghoff et al., 2012) and of aqueous exposure in male and female *Kras*^{V12} transgenic zebrafish (Zhu et al., 2021).

In a dietary study in male and female Sprague-Dawley rats (Butenhoff et al., 2012b), there was a significant positive trend in the incidence of hepatocellular adenoma in males, and the incidence was significantly increased at the highest dose. In females, there was a significant positive trend in the incidence of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined), with the incidence being significantly increased at the highest dose. There was a significant increase in the incidence of thyroid follicular cell adenoma or carcinoma (combined) at the higher intermediate dose in females. There was a significant increase in the incidence of fibroadenoma of the mammary gland and of fibroadenoma or adenoma (combined) at the lowest dose. There was significant positive trend in the incidence of fibroadenoma, adenoma or carcinoma (combined) of the mammary gland, with the incidence being significantly increased

at the two lower doses. In the recovery group of males, there was a significant increase in the incidence of thyroid follicular cell adenoma. [The Working Group noted that the liver was a target organ for PFOS in both male and female rats. The Working Group also noted that the association between PFOS exposure and the incidence of thyroid follicular cell tumours and mammary gland tumours was uncertain.]

In an initiation–promotion study of oral administration (in the feed) of PFOS, there was a significant increase in the incidence of total liver tumours in male and female rainbow trout (*Oncorhynchus mykiss*) at the highest dose (Benninghoff et al., 2012). [The Working Group noted that this study provided evidence that PFOS can be a cancer promoter in a rainbow trout model.]

In a study of oral administration (gavage) in male and female C57BL/6J-*Apc*$^{Min/+}$ mice (Ngo et al., 2014) and of aqueous exposure in male and female *Kras*^{V12} transgenic zebrafish (Zhu et al., 2021), there was no significant increase in the incidence of tumours.

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4. MECHANISTIC EVIDENCE

4.1 Absorption, distribution, metabolism, and excretion

Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are characterized by long half-lives in humans (years) and much shorter half-lives in experimental animals (days or weeks). Numerous toxicokinetic (TK) studies in laboratory animals were available, and several physiologically based pharmacokinetic (PBPK) models of PFOA and PFOS have been developed. However, human TK data are still scarce in comparison with those from experimental animals, and the mechanistic bases for the observed TK differences among species remain to be fully elucidated.

4.1.1 Humans

(a) Exposed humans

(i) Absorption

Exposure to PFOA and PFOS in non-occupational settings occurs mainly via the oral route. After a single oral dose of ammonium perfluorooctanoate (APFO, 50–1200 mg, as a gelatin capsule) was administered during a clinical trial of patients with cancer, the serum concentrations reached a plateau after approximately 600 hours for the highest dose, but they were still increasing after 864 hours, the latest time point at which they were assessed, for lower doses (Convertino et al., 2018). [The Working Group noted that these doses were much higher than estimated human environmental exposures and that there were no healthy controls in the study. The Working Group also noted that the salt and protonated forms used in this study were comparable to those used in other studies described in this section.] No direct estimates of the oral absorption efficiency of PFOA or PFOS were available for humans. The oral absorption efficiency is assumed to be close to 100%, according to TK models that simulate the human serum concentrations of PFOA, on the basis of comparisons with data obtained from experimental animals. PFOA and PFOS are excreted in the bile (Fujii et al., 2015) and are reabsorbed from the gastrointestinal tract into the enterohepatic circulation; a high absorption efficiency would be required to be consistent with the observed long half-lives of these compounds (Harada et al., 2007).

The inhalation and dermal routes can significantly contribute to PFOA and PFOS exposure in occupational settings, such as in firefighters (Christensen and Calkins, 2023; Mazumder et al., 2023). In a transdermal absorption study of a single volunteer, whole-skin application of $[^{13}C]$PFOA mixed into sunscreen resulted in the maximum serum concentration 22 days post-application, and the absorption efficiency was estimated to be 1.6% of the administered dose (Abraham and Monien, 2022). No data regarding

the absorption of PFOA or PFOS by inhalation were available to the Working Group.

(ii) Distribution

Few data were available regarding the distribution of PFOA and PFOS, and most of the existing data were obtained from postmortem samples. The liver, blood, and lungs appear to be important sites of accumulation for both compounds, and there are generally lower levels of accumulation in the kidneys, bone, muscle, brain, and fat (Olsen et al., 2003a; Maestri et al., 2006; Pérez et al., 2013; Yeung et al., 2013a; Fàbrega et al., 2014; Mamsen et al., 2019; Di Nisio et al., 2022). In one study, PFOA concentrations in the liver were found to be similar to those in the blood, whereas PFOS concentrations in the liver were higher by approximately 2.7-fold (Maestri et al., 2006). In contrast, accumulation in the liver of laboratory animals appeared to be more marked, as discussed below.

In two other studies, PFOA and PFOS concentrations in human follicular fluid were found to be comparable to those in the serum (Petro et al., 2014; McCoy et al., 2017). PFOA and PFOS have been detected in the thyroid at concentrations just below those in the serum (Pirali et al., 2009). PFOA and PFOS have also been found in semen and cerebrospinal fluid, but at much lower levels than in serum (Raymer et al., 2012; Wang et al., 2018a; Di Nisio et al., 2019). In addition, PFOA and PFOS have been detected in the amniotic fluid, placenta, cord blood, and embryonic tissues at lower concentrations than those in the maternal serum (Zhang et al., 2013a; Mamsen et al., 2019).

The elimination of PFOA and PFOS from human serum has been demonstrated to follow first-order kinetics, and therefore the overall distribution can be characterized using the volume of distribution (V_d) (Harada et al., 2005; Olsen et al., 2007; Russell et al., 2015). Based on a Bayesian TK analysis of data from 13 studies in which PFOA and PFOS serum levels, PFOA and PFOS concentrations in drinking-water, and background PFOA and PFOS exposures were considered, the V_d for a one-compartment TK model was estimated to be 0.43 L/kg and 0.32 L/kg for PFOA and PFOS, respectively (Chiu et al., 2022). [The Working Group noted that these values are higher than some of the previously proposed PFOA and PFOS V_d estimates, but they appeared to be based on the largest dataset, analysed using the best available methods.] The previously widely used PFOA V_d estimate of 0.17 L/kg was derived from a single dataset using a similar approach (Thompson et al., 2010). This value was used as the initial estimate for the PFOA V_d in the Bayesian analysis performed by Chiu et al. (2022).

On the basis of their structure and physicochemical properties, PFOA and PFOS are unlikely to cross cellular membranes directly. Various membrane transporters are thought to mediate their transmembrane transport, as described in more detail in the section regarding in vitro studies below.

Blood is an important compartment for the accumulation of PFOA and PFOS. PFOA and PFOS in the blood primarily distribute to the serum, but a significant fraction also partitions to blood cells, indicating that the use of a default factor of two to account for the volume of plasma in whole blood (a common practice when no prior knowledge about chemical distribution between blood fractions is available) may not be an appropriate method for the blood-to-serum concentration conversion (Jin et al., 2016; Poothong et al., 2017; Liu et al., 2023a). Indeed, in two studies in which the mass fraction in plasma (F_p) was measured, it was 0.7–0.8 for PFOA and 0.8–0.85 for PFOS (Jin et al., 2016; Liu et al., 2023a). [The Working Group noted that F_p would equal 0.5 if the compound distributed to plasma.] In fractionated plasma collected from healthy volunteers ($n = 4$), PFOA and PFOS were bound to albumin, with little affinity for lipoproteins (Forsthuber et al., 2020).

An analysis of biomonitoring data using PBPK modelling showed that serum concentrations of PFOA and PFOS were lower in women of reproductive age than in age-matched men. This was thought to be the result of menstrual blood loss and placental and lactational transfer associated with pregnancy and childbirth (Wong et al., 2014; Gomis et al., 2017).

PFOA and PFOS cross the placenta and can also be transferred to infants via breast milk. In a review of the available studies, it was calculated that the placental transfer (median and range), defined as the ratio of PFAS fetal (cord blood) concentration to the maternal serum concentration, was 0.79 (0.60–1.5) for PFOA and 0.37 (0.29–0.56) for PFOS (Pizzurro et al., 2019). PFOS concentrations in maternal serum during the first trimester were significantly higher than those during the second and third trimesters, but there were no significant differences in PFOA concentration between trimesters (Mamsen et al., 2019). In another study, PFOA and PFOS concentrations both significantly decreased by approximately 40% between 16 weeks of gestation and time of delivery (Kato et al., 2014). [The Working Group noted that decreases in PFOA and PFOS levels in pregnancy were probably because of blood volume expansion and increase in the glomerular filtration rate (GFR) and, to a minor extent, transfer to the fetus. Differences among studies could be caused by differences in PFOA levels and experimental variation.]

Lactational transfer (median and range of the values obtained from the available studies), defined as the ratio of the breast milk to the maternal serum concentration, was calculated to be 0.04 (0.03–0.12) for PFOA and 0.01 (0.01–0.03) for PFOS (Pizzurro et al., 2019). Lactational PFOA transfer probably accounts for the higher (2.7–4.6-fold) PFOA serum concentrations in infants aged 2–6 months than in mothers. However, PFOS concentrations were similar in infants and mothers (Fromme et al., 2010; Gyllenhammar et al., 2018). By age 18 months, the mean PFOA concentration in the infants' serum was similar to that in the mothers' serum (Højsager et al., 2022), probably because of the cessation of breastfeeding and the growth-dependent dilution of PFOA according to body burden. There was very high inter-individual variability in the time-course profiles for PFOA and PFOS concentrations in infants (Mogensen et al., 2015a). Serum PFOA and PFOS concentrations in children were similar at age 2, 4, and 6 years (Kim et al., 2020). On the basis of the available data, Goeden et al. (2019) developed a TK model that predicts serum PFOA concentrations in people of all ages, including the very young.

The available evidence suggested that PFOA and PFOS undergo enterohepatic circulation. Although these compounds were not detected (< 0.5 ng/g) in the faeces of a single participant (serum concentrations 6.8 and 26.0 ng/g for PFOA and PFOS, respectively), the addition of cholestyramine, which inhibits the reabsorption of bile acids and thereby increases their excretion, to the diet for 1 week (4 g, three times per day) resulted in detectable faecal PFOA (0.96 ng/g) and PFOS (9.06 ng/g) (Genuis et al., 2010). Harada et al. (2007) measured the PFOA and PFOS concentrations in bile (4 participants) and, using the total and urinary clearances of these compounds, calculated their biliary reabsorption rates (the proportions reabsorbed) to be 0.89 and 0.97, respectively. The biliary reabsorption rate of PFOA was found to be 0.98 in another study (n = 5) in which similar methodology was used (Fujii et al., 2015).

PFOA and PFOS are present in the environment as a mixture of linear and branched isomers. PFOA is primarily present as a linear isomer in various exposure matrices, whereas the PFOS isomer composition is more variable (Shan et al., 2016). In human blood and serum, the fraction of the linear PFOA isomer (n-PFOA) is 96–100% (Zhang et al., 2014a, 2017a), which is consistent with the high proportion of n-PFOA in food and

drinking-water and its expected slower elimination compared with branched isomers (Zhang et al., 2013b; Zhou et al., 2014). In contrast, the proportion of *n*-PFOS in the blood or serum (35–80% of total blood PFOS) is lower than would be expected in most exposure scenarios (Zhang et al., 2014a, 2017a; Gebbink et al., 2015; Salihović et al., 2015; Nilsson et al., 2022). This apparent relative enrichment of branched isomers could be the result of the metabolism of unknown PFOS precursor(s) to form branched isomers (Gebbink et al., 2015; Shan et al., 2016).

The proportion of *n*-PFOA in cord serum has been reported to be 98–99% (Beesoon et al., 2011; Zhang et al., 2017a), and that of *n*-PFOS to be 52–75% of the total, which was lower than that in the maternal serum in studies in which such data were available (Hamm et al., 2010; Beesoon et al., 2011; Zhang and Qin, 2014; Zhang et al., 2017a).

(iii) Metabolism

No in vivo data on the metabolism of PFOA and PFOS were available to the Working Group. On the basis of their chemical structure and data demonstrating a lack of metabolism in experimental animals, PFOA and PFOS are not expected to be metabolized in humans.

The metabolism of precursor compounds, including fluorotelomer alcohols, perfluoroalkyl sulfonamides, and amidoalcohols, to form PFOA and PFOS was estimated to contribute to 2–8% and 2–5% of the internal doses of PFOA and PFOS, respectively, in an intermediate-level exposure scenario, and to 28–55% and 60–80%, respectively, in a high-level exposure scenario (Vestergren et al., 2008). By using exposure and biomonitoring data in a TK model, Gomis et al. (2016) estimated that the metabolism of 8:2 fluorotelomer alcohol contributed to 45% of the serum concentration of PFOA in ski-waxers who have a high level of occupational exposure.

(iv) Excretion

PFOA and PFOS are characterized by very long half-lives in humans, with estimates of 2–5 years reported in most studies (Harada et al., 2005; Olsen et al., 2007; Spliethoff et al., 2008; Costa et al., 2009; Bartell et al., 2010; Brede et al., 2010; D'eon and Mabury, 2011; Seals et al., 2011; Glynn et al., 2012; Olsen et al., 2012; Yeung et al., 2013b; Zhang et al., 2013b; Russell et al., 2015; Fu et al., 2016; Gomis et al., 2016, 2017; Worley et al., 2017a; Li et al., 2018a; Xu et al., 2020a). [The Working Group noted that these were observational population kinetic studies, in which participants may have been subject to unspecified background exposures, in addition to known contamination sources.] Recently, Chiu et al. (2022) applied a Bayesian TK analysis to data from 13 studies of the association between exposure and serum concentration; they reported serum half-life ($T_{1/2}$) estimates for the population geometric mean of 3.14 years for PFOA and 3.36 years for PFOS.

[The Working Group noted that several studies have investigated the serum half-lives of PFOS isomers (Xu et al., 2020a; Li et al., 2022a; Nilsson et al., 2022), but the results were inconsistent among studies.]

Xu et al. (2020a) reported a longer $T_{1/2}$ for *n*-PFOS than for the sum of branched-chain forms of PFOS, but the other two studies conducted by the same research group reported shorter $T_{1/2}$ estimates for *n*-PFOS than for the branched isomers (Li et al., 2022a; Nilsson et al., 2022).

In humans, PFOA and PFOS are primarily eliminated in the urine and faeces. In women of reproductive age, blood loss during menstruation, fetal transfer during pregnancy, and lactational transfer during breastfeeding are also thought to reduce the body burden of PFOA and PFOS (Mondal et al., 2014; Wong et al., 2014; Gomis et al., 2017; Pizzurro et al., 2019).

Multiple studies reported renal clearance of PFOA and PFOS, with estimates of 0.03–0.8 mL/(kg-day) and 0.01–0.03 mL/(kg-day), respectively, calculated in a 24-hour period and assuming an average human body weight of 50–70 kg (Harada et al., 2005; Zhang et al., 2013b; Zhou et al., 2014; Fujii et al., 2015; Gao et al., 2015a; Zhang et al., 2015a; Fu et al., 2016).

Linear PFOA and PFOS isomers appeared to be eliminated more slowly than branched isomers in the urine (Zhang et al., 2013b; Gao et al., 2015a). In only one study, PFOA and PFOS levels were reported to be above the limit of detection in the faeces, and only after addition of the bile acid sequestrant cholestyramine to the diet (Genuis et al., 2010).

A comparison of the urinary and total elimination rates by Fujii et al. (2015) showed that the faecal and urinary PFOA clearances contribute approximately 54% (0.052 mL/(kg-day) and 46% (0.044 mL/(kg-day), respectively, to the overall (serum) clearance, calculated in a 24-hour period and assuming a human body weight of 50 kg.

Using the same approach, Harada et al. (2007) found that urinary PFOA clearance constitutes approximately 20% of the total serum clearance – 0.03 mL/(kg-day) to 0.15 mL/(kg-day) – and urinary PFOS clearance constitutes approximately 14% – 0.015 mL/(kg-day) to 0.106 mL/(kg-day).

[The Working Group noted that the urinary clearance appears to be of less importance as an excretion route for PFOA and PFOS in humans than the estimated faecal clearance. The limits of detection for PFOA and PFOS in faeces appear to be higher than for other physiological matrices, which may explain the scarcity of published data.]

The body burden of PFOA and PFOS, indicated by serum concentrations, increases in response to exposure to these chemicals. Their excretion increases with increasing serum concentration until, if the intake is constant, an equilibrium is reached. The relation between intake and the serum concentration at steady state can be estimated, assuming first-order kinetics. One-compartment TK models are widely used in the literature to back-calculate PFOA and PFOS exposure (ng/kg per day) from serum levels (ng/mL). The outcome in these models depends on selected V_d and $T_{1/2}$ values. Several two-compartment human TK models have also been developed for PFOA and PFOS, to investigate gestational and lactational transfer or to fit TK data from a clinical trial with PFOA (Verner et al., 2016; Convertino et al., 2018; Goeden et al., 2019). Several multicompartment PBPK human models have been developed for PFOA and PFOS that included renal reabsorption, to account for the long retention times in humans (Loccisano et al., 2011, 2013; Fàbrega et al., 2014; Worley et al., 2017b; Chou and Lin, 2019). Of note, these models do not include either the faecal elimination route (Loccisano et al., 2011, 2013; Fàbrega et al., 2014) or the complete enterohepatic loop (Worley et al., 2017b; Chou and Lin, 2019).

[The Working Group noted that not fully accounting for faecal elimination would probably cause an overestimation of the effect of renal elimination relative to total elimination, with possible implications for PBPK studies of confounders dependent on renal elimination. The Working Group noted that a recently published PBPK model for PFOA (Husøy et al., 2023) appeared to include the faecal elimination route; however, this model was not evaluated in the present monograph.]

(b) Human cells in vitro

Approximately 24% of the applied dose of PFOA was found to penetrate human skin in vitro, and the skin permeability coefficient for ionized PFOA (a physiologically relevant form) was 6.0×10^{-5} cm/hour (Franko et al., 2012).

Cell-free and cell culture in vitro experimental systems have been used to characterize the interactions of PFOA and PFOS with membrane transporters, serum albumin, liver fatty acid-binding protein (L-FABP), thyroid receptor,

and transthyretin. Interactions with membrane transporters probably mediate the absorption and reabsorption of PFOA and PFOS from the gastrointestinal tract, their reabsorption in the kidney, and their ability to cross the placenta. High-affinity binding to serum albumin and L-FABP may underly the accumulation of PFOA and PFOS in serum and the liver.

Most available data regarding the interactions of PFOA and PFOS with transporters concern organic anion transporting polypeptides (OATPs), a family of proteins that are expressed in the gut, kidney, and placenta.

In uptake studies of cells that were stably transfected with human membrane transporters, PFOA was found to be transported by (human) OAT1 (K_m = 48 μM), OAT3 (K_m = 49.1 μM), OAT4 (K_m = 310 μM), URAT1 (K_m = 64.1 μM) and Na^+/taurocholate cotransporting polypeptide (NTCP) (K_m = 1.8 mM) (Nakagawa et al., 2008, 2009; Yang et al., 2010; Kummu et al., 2015), but not by OAT2, OATP1A2, or ABCG2 (Nakagawa et al., 2008; Yang et al., 2010; Kummu et al., 2015). In uptake studies of cells that were transiently transfected with human membrane transporters, PFOS was transported by (human) OSTα/β (no reported K_m) and NTCP (K_m = 130 μM), but not by ABCG2 (Zhao et al., 2015).

Kummu et al. (2015) proposed that OAT4 is involved in the transport of PFOA across the placenta, on the basis of a positive correlation between protein expression and the transport of PFOA by isolated placenta cells. Kimura et al. (2017) showed a decrease in the uptake of PFOA across the apical membrane of human intestinal Caco-2 cells when they were co-incubated with PFOA and inhibitors of OATPs or with the OATP substrate sulfobromophthalein. Furthermore, PFOA inhibited the uptake of sulfobromophthalein at this location (Kimura et al., 2020).

HEK293 cells stably transfected with NTCP exhibited PFOA uptake (K_m = 1.8 mM) and the inhibition of taurocholate uptake by PFOA (K_i = 7.5–28 μM) (Ruggiero et al., 2021).

PFOA and PFOS bind to several human proteins in cell-free systems, including to serum albumin, with dissociation constants K_d = 3.7 × 10^{-6}–4 × 10^{-4} M for PFOA, and 4.5–20 × 10^{-5} M for PFOS, and to L-FABP with K_d = 2.4–50.4 × 10^{-6} M for PFOA and 18.5 × 10^{-6} M for PFOS (Han et al., 2003; Messina et al., 2005a; Chen and Guo, 2009; Li et al., 2009; Wu et al., 2009; Luo et al., 2012; Zhang et al., 2013c; Sheng et al., 2016; Maso et al., 2021). [Where appropriate, the Working Group converted association or absorption constants to dissociation constants for the purposes of comparison; see Supplementary Table S4.1, in Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636.] PFOA was able to displace the endogenous ligand oleic acid from human serum albumin indicating the potential to interfere in fatty acid transport in the blood (D'Eon et al., 2010).

The linear isomers *n*-PFOA and *n*-PFOS bind much more tightly to human serum albumin than do the branched isomers, with dissociation constants that are several orders of magnitude lower (Beesoon and Martin, 2015). As described in more detail in Section 4.2.8, PFOA and PFOS were able to displace thyroid hormones (T3, T4) from the human thyroid receptor and the thyroid hormone transporter protein transthyretin (Weiss et al., 2009; Ren et al., 2015, 2016).

(c) Considerations regarding exposure metrics

Cross-sectional measurements of serum concentrations of PFOA and PFOS are often used to study associations in epidemiological investigations, because of cost constraints, and with the assumption that such metrics would represent quantitative indicators of exposure over an appropriate time frame for the chronic

effects studied (see Section 1.6.2, and Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). In support of this approach, consecutive measurements of the serum concentrations of PFOA and PFOS, measured in the same non-occupationally exposed individuals in 1979, 1986, 1994, 2001, and 2007, were closely correlated (Spearman ρ coefficient, > 0.6), even though the mean levels increased 3.66-fold, peaking in 2001, and then decreased in 2007 (Nøst et al., 2014). In another investigation of the applicability of the use of a single serum PFOA measurement in human studies, retrospective PFOA exposure was reconstructed for participants in the C8 cohort, using geocoding, documented air and water concentrations, and a pharmacokinetic model with an approximate Bayesian computation (Zhu et al., 2022). These data were combined with the measured PFOA serum concentrations to produce a better longitudinal estimate of PFOA exposure. Associations between PFOA and pre-eclampsia based on reconstructed historical PFOA exposure estimates in this population were found to be similar to those reported, based on one-time PFOA measurements (Savitz et al., 2012), suggesting that the rank order of exposure estimates of study participants was important for statistically significant associations, and not the exact exposure amount (Zhu et al., 2022).

On the basis of current understanding of PFOA and PFOS kinetics, various physiological factors can affect their serum concentrations and lead to possible reverse causation effects in epidemiological analyses of associations. This type of bias has been termed pharmacokinetic bias, and several examples regarding PFOA and PFOS have been described in a recent review (Andersen et al., 2021). These examples are detailed below.

Dhingra et al. (2017) examined associations of menopause and estimated glomerular filtration rate (eGFR) with the measured or modelled serum concentrations of PFOA in a highly exposed cohort. Serum PFOA concentrations were from single sample collections, and the modelled PFOA concentrations were based on the retrospective exposure to PFOA and a one-compartment TK model (Shin et al., 2011). The modelled PFOA concentrations were generated independently of the measured concentrations and had a Spearman correlation of $\rho = 0.71$ (Winquist et al., 2013; Dhingra et al., 2017). Menopause and eGFR were found to be significantly associated with the measured, but not modelled, serum PFOA concentration (Winquist et al., 2013; Dhingra et al., 2017). Specifically, there was an inverse relation (negative trend) of eGFR with the quintile of measured serum PFOA concentration, but neither the modelled serum PFOA concentration nor the modelled cumulative exposure showed an association with eGFR (Dhingra et al., 2017). In the same study, a significant increasing trend for reported menopause was identified alongside an increase in the measured serum PFOA categories, but not in the modelled metrics (Dhingra et al., 2017). [The Working Group noted that these findings can be interpreted as an instance of reverse causation, in which changes in eGFR, which also occur as a consequence of menopause, would directly affect PFOA excretion and serum PFOA concentration.]

The same reverse causation mechanism, in which changes in GFR would affect concentrations of PFOA and/or PFOS and the outcome of interest, has been investigated for potential associations with respect to low birth weight (Verner et al., 2015). A human pregnancy PBPK model of PFOA/PFOS was updated and parametrized by Loccisano et al. (2013) to incorporate the relation between GFR and birth weight, based on the results of previous studies of substances other than perfluoroalkyl and polyfluoroalkyl substances (PFAS). A PBPK model-driven meta-analysis was performed, investigating associations of serum PFOA and PFOS concentrations with birth weight, using data obtained from seven

studies. The use of the modified PBPK model was intended to “remove” the confounding effect of low birth weight on GFR, and to simulate direct effects of increases in serum PFOA and/or PFOS concentrations on birth weight. The resulting levels were approximately 50% of those reported on the basis of measured PFOA and PFOS concentrations. The authors concluded that a substantial proportion of the reported associations of prenatal PFOA and PFOS concentrations with birth weight was attributable to confounding by GFR (Verner et al., 2015). [The Working Group noted that the TK adjustment for GFR confounding in the described examples relied on models that assumed urinary excretion to be the only excretion route. However, faecal elimination in humans also appeared to be equally, if not more, important. Thus, such adjustments may overestimate the effects of GFR and urinary elimination on serum PFOA/PFOS levels.]

Hypotheses regarding reverse causation have been also investigated with respect to associations of serum PFOA and PFOS concentrations with serum cholesterol (EFSA, 2018; Steenland et al., 2020). Two possible mechanisms were proposed. Firstly, PFOA and PFOS might preferentially distribute to cholesterol-containing lipoprotein particles in the blood, resulting in greater accumulation in the presence of higher cholesterol concentrations, therefore explaining the reverse causation. The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain provided a detailed analysis of this hypothetical mechanism and concluded that it was not supported by the available mechanistic data (EFSA, 2018). Secondly, the concentrations of serum PFOA and PFOS might be influenced by inter-individual variability in bile acid transporters, for which they are substrates, in enterohepatic resorption and would correlate with bile acid concentrations by virtue of sharing the same retention mechanism. However, an increase in bile acid concentrations would inhibit the metabolism of cholesterol to form bile acids, resulting in increases in cholesterol concentrations and an indirect relationship of the PFOA and PFOS concentrations with those of cholesterol. This mechanism was also briefly discussed in the updated EFSA report of 2020, in light of the available findings (EFSA, 2020).

One of the proposed reasons against this mechanism was a study that found significant associations for serum cholesterol based on estimated PFOA/PFOS external exposures, and not just serum levels (Li et al., 2020a). Additionally, in a study that reported associations between 20 measured PFAS (including PFOA and PFOS) and 19 bile acids in samples from healthy individuals (n = 20), the overall trend was that the concentrations of the majority of bile acids were negatively associated with those of PFAS (Salihović et al., 2020a), also indicating a lack of support for the shared retention mechanism. [The Working Group noted that the existing evidence does not support the proposed mechanisms of reverse causality between the measured PFAS and serum cholesterol.]

The effects of advanced cancers on the TKs of PFOA and PFOS remain unclear. PFOA has been previously tested in a phase 1 therapeutic trial of 43 patients with cancers at various stages, and the TK data obtained were made publicly available in a patent application (Elcombe et al., 2013). [The Working Group noted that these data have not been curated or peer-reviewed.] In this trial, up to 1200 mg of PFOA was administered to the patients once per week for 6 weeks, and their serum PFOA concentrations were monitored. The experimental set-up and the results have been described and discussed in separate publications (Convertino et al., 2018; Dourson et al., 2019), in which the authors noted that secondary to this very high level of exposure, which exceeded the typical level of environmental exposure by several orders of magnitude, the serum PFOA concentration appeared to reach steady state within weeks, which would correspond to a much

shorter half-life estimate than the 2–5 years that has generally been recorded in the literature (see above). [The Working Group noted that it was not clear whether the apparently faster elimination was because of higher PFOA doses or altered physiological parameters in the study participants, all of whom were patients with cancer. The study did not appear to include healthy controls.] [The Working Group noted that there was no information available on the kinetic effects of cancer or cancer treatment on PFOA or PFOS levels in patients with cancer.]

4.1.2 Experimental systems

(a) Experimental systems in vivo

The TK mechanisms associated with PFOA and PFOS have been extensively investigated in the mouse and rat, and a brief review of the findings obtained using these two species follows. In addition, information obtained from primates is included, where available.

The oral absorption efficiencies of PFOA and PFOS have been estimated in animal studies by comparing the area under the curve (AUC) of the serum/plasma concentration, with the dose-adjusted AUC obtained using data collected after an intravenous (i.v.) dose (an oral/i.v. ratio of 1 indicates 100% absorption efficiency), or alternatively by estimating the amount of the administered compound that remained in the faeces during the 24 hours after administration. [The Working Group noted that the second method could underestimate absorption efficiency, because some excretion via the faecal route would be expected within the observation period.] Using the first method, a comparison of AUCs yielded an oral absorption efficiency for PFOA of close to 100% in rats (Kim et al., 2016a; Dzierlenga et al., 2020) and mice (Fujii et al., 2015), and also for PFOS in rats (Kim et al., 2016a; Huang et al., 2019a, 2021). There were no studies available measuring PFOS in mice. With the second method (subtraction of the excretion via faecal route from 100%), the PFOA oral absorption efficiency was ≥ 93% in rats (Cui et al., 2010), and the PFOS oral absorption efficiency in mice was ≥ 98% (Jandacek et al., 2010).

PFOA exposure via inhalation increased the plasma PFOA concentrations of male and female rats in a dose-dependent manner (Hinderliter et al., 2006), and these authors concluded that a route-to-route extrapolation would be possible using the plasma PFOA concentration as an index of the internal dose metric. [No other TK studies of inhalation were available to the Working Group.]

In rats, the dermal application of APFO increased blood organofluorine concentration (a surrogate for PFOA concentration) in a dose-dependent manner, indicating that PFOA is absorbed through the skin (Kennedy, 1985). Similarly, the topical application of PFOA increased serum PFOA concentration of mice in a dose-dependent manner (Franko et al., 2012).

The Working Group identified more than 50 studies on PFOA and PFOS distribution in rats and mice, including studies of single and multiple dosing; studies of oral administration by gavage, in the drinking-water or in the diet; i.v. and intraperitoneal (i.p.) administration; and studies in pregnant animals (e.g. Ylinen et al., 1990; Vanden Heuvel et al., 1991; Austin et al., 2003; Luebker et al., 2005; Hundley et al., 2006; Kudo et al., 2007; Benskin et al., 2009; Cui et al., 2009; Bogdanska et al., 2011; Fujii et al., 2015; Iwabuchi et al., 2017; Dzierlenga et al., 2020). The data on organ distributions identified for PFOA and PFOS were generally consistent across the studies, with liver being the primary site of accumulation of both compounds, followed by the kidney and the blood/serum. Lower levels of accumulation were detected in the brain, fat, muscle, thyroid, testes, thymus, and skin than in the serum. PFOS was found to preferentially accumulate in the lungs in both mice and rats, but PFOA was not. The data available for monkeys were limited to the liver and serum, and showed preferential

accumulation of PFOS in the liver (Seacat et al., 2002), but much lower levels of PFOA in the liver than in the serum (Griffith and Long, 1980; Butenhoff et al., 2004). Most animal studies were conducted using low to intermediate milligram per kilogram doses (either single or repeated administration). Several of the studies included doses in the microgram per kilogram range (e.g. Seacat et al., 2002; Bogdanska et al., 2011; and Li et al., 2017a), and these generated organ distribution data that were comparable to the results obtained using higher doses.

PFOA and PFOS are efficiently transferred to fetuses during pregnancy and to pups via lactation (Lau et al., 2003; Chang et al., 2009; Fenton et al., 2009; Macon et al., 2011; Lai et al., 2017a). Similar to humans, PFOA and PFOS are involved in enterohepatic resorption, because cholestyramine treatment in the diet for 14 days in rats substantially increased the levels of [^{14}C]PFOA and [^{14}C]PFOS in the faeces after a single i.v. dose of PFOA at 13.3 or PFOS at 3.4 mg/kg (Johnson et al., 1984). PFOA and PFOS isomers showed minor differences in their organ distributions in male and female rats (Benskin et al., 2009; De Silva et al., 2009).

In the literature, there were several estimates of V_d in rats, mice, and monkeys. For comparison purposes, the following examples are from studies in which a one-compartment TK model was assumed or in which a non-parametric analysis was applied.

In rats treated with a single oral dose of PFOA (1 mg/kg) or PFOS (2 mg/kg), the PFOA V_d estimates were approximately 106 and 154 mL/kg for males and females, respectively, and the PFOS V_d estimates were approximately 280 and 289 mL/kg for males and females, respectively (Kim et al., 2016a).

In mice treated with a single i.v. dose of PFOA (0.13 mg/kg), V_d estimates were approximately 180 mL/kg in males and 150 mL/kg in females (Fujii et al., 2015). In mice treated with a single oral dose of PFOS (1 or 20 mg/kg), the V_d estimates were approximately 263–290 mL/kg in males and 258–261 mL/kg in females (Chang et al., 2012).

In monkeys treated with a single i.v. dose of PFOA (10 mg/kg), the V_d estimates were 181 and 198 mL/kg for males and females, respectively (Butenhoff et al., 2004). In monkeys treated with a single oral dose of PFOS (9 or 14 mg/kg), the V_d estimates were 127–135 mL/kg and 127–141 mL/kg in males and females, respectively (Chang et al., 2017).

[The Working Group noted that the magnitude of the V_d estimates for rats, mice, and monkeys (150–250 mL/kg) is consistent with the current understanding of the PFOA and PFOS distribution mechanism, with strong binding to proteins in the blood and accumulation in several organs.]

Two detailed studies of PFOA metabolism in rats were available. Vanden Heuvel et al. (1992) treated male and female rats with a single i.p. dose of [1-^{14}C]PFOA (9.4 μmol/kg or 3.9 mg/kg) and investigated the tissue distribution and tissue-specific concentrations at various time points up to 28 days. They did not find PFOA metabolites or conjugates in many tissues (liver, kidney, heart, muscle, fat, or testes) or in the plasma, urine, bile, or faeces, based on the similarity of the high-performance liquid chromatography (HPLC) chromatograms of the sampled extracts to the chromatogram of the parent compound. In addition, they found no increases in the serum or urine concentrations of fluoride, which would have indicated PFOA defluorination (Vanden Heuvel et al., 1991). In a study by Goecke et al. (1992), a single i.p. dose of PFOA (50 mg/kg) was administered to male rats, and ^{19}F-nuclear magnetic resonance spectra were acquired from blood, urine, bile, and liver homogenates 3 days after dosing. Only resonances referable to the parent compound were identified in all the analyses performed, which implies a lack of covalent modification of the PFOA molecule, i.e. absence of metabolites or

conjugates (Goecke et al., 1992). Other studies in rats confirmed the lack of phase I or phase II metabolism of PFOA (Ophaug and Singer, 1980; Ylinen et al., 1989; Kuslikis et al., 1992).

[The Working Group noted that, on the basis of the structural similarities between PFOA and PFOS and similarities in their TK mechanisms among species, PFOA and PFOS are not expected to be metabolized in mammals.]

There are species- and sex-specific differences with respect to the elimination of PFOA and PFOS. Estimates of the serum $T_{1/2}$ for PFOA in male rats after oral administration ranged from 6.4 days to 13.4 days on the basis of five studies that included a total of 12 dose groups (Kudo et al., 2002; US EPA, 2003; Benskin et al., 2009; Iwabuchi et al., 2017; Dzierlenga et al., 2020). Only one study with a single dose group produced a lower estimate of oral PFOA serum $T_{1/2}$ of 1.64 days (Kim et al., 2016a). However, the serum $T_{1/2}$ estimates for PFOA for female rats ranged from 2.75 to 13.9 hours, based on the results of three studies comprising a total of 10 dose groups (US EPA, 2003; Kim et al., 2016a; Dzierlenga et al., 2020). It was hypothesized that the sex-specific differences in rats with respect to the elimination of PFOA are the consequence of differential expression of specific transporter proteins in the kidneys, resulting in more effective reabsorption in males (Weaver et al., 2010; Pizzurro et al., 2019).

After a single oral dose by gavage, the serum $T_{1/2}$ for PFOA was 21.7 days in male mice and 15.6 days in female mice (Lou et al., 2009). The only available estimates for primates were obtained in monkeys (*Simia cynomolgus* or *Macaca fascicularis*). Butenhoff et al. treated monkeys with PFOA as an i.v. dose of 10 mg/kg, yielding a serum $T_{1/2}$ estimate for PFOA of 13.6–35.3 days in males and 26.8–41.7 days in females (Butenhoff et al., 2004).

In rats, the serum $T_{1/2}$ estimates for PFOS after gavage were 8.23–41.19 days for males and 23.5–71.13 days for females in three studies of a total of 5 dosage groups (Chang et al., 2012; Kim et al., 2016a; Huang et al., 2019a, 2021).

Longer $T_{1/2}$ estimates were obtained for female rats than for males in one of these studies (Chang et al., 2012), but no sex-specific differences were identified in the other two.

In mice, the serum $T_{1/2}$ estimates for PFOS after oral administration were 36–43 days for males and 30–38 days for females, with no significant sex-specific differences (Chang et al., 2012). In monkeys, the serum $T_{1/2}$ estimates for PFOS after oral administration were 117–200 days for males and 102–200 days for females, also with no significant sex-specific differences (Seacat et al., 2002; Chang et al., 2017).

Linear *n*-PFOA and *n*-PFOS isomers appear to be eliminated more slowly than the branched isomers. When mixtures of PFOA or PFOS isomers were administered to male rats by oral gavage, isomer-specific blood elimination $T_{1/2}$ was determined (Benskin et al., 2009). In this study, *n*-PFOA was found to have the longest blood elimination $T_{1/2}$, 13.4 days, compared with 1.28–9.10 days for the branched isomers; the $T_{1/2}$ for *n*-PFOS (33.7 days) was at the top end of the range obtained (the geometric mean for all the PFOS isomers was 23.8 days) (Benskin et al., 2009).

In rats and mice, PFOA and PFOS are eliminated in the urine and faeces. Vanden Heuvel et al. (1991) measured [^{14}C]PFOA elimination in male and female Sprague-Dawley rats. They found that, in females, 91% of the i.p. dose (9.4 µmol/kg or 3.9 mg/kg) was eliminated in the urine during the first 24 hours, with negligible amounts being present in the faeces, whereas in males, over the 28-day collection period, 36.4% and 35.1% of the administered dose was recovered in the urine and faeces, respectively (Vanden Heuvel et al., 1991). However, Cui et al. (2010) found greater excretion (approximately two-fold) of PFOA in the urine than in the faeces during the daily administration of PFOA by gavage at 5 or 20 mg/kg to male Sprague-Dawley rats. In this

study in male Sprague-Dawley rats, the rate of PFOS excretion in urine exceeded the excretion rate in faeces, at doses of either 5 or 20 mg/kg (Cui et al., 2010). Another study in male and female Sprague-Dawley rats also showed that urinary excretion was the primary means of eliminating PFOS after a single oral dose of 2 or 15 mg/kg of potassium perfluorooctanesulfonate (Chang et al., 2012). Furthermore, the urinary PFOA clearance, in male or female FVB/NJc1 mice, was approximately twice as high as the faecal clearance after either i.v. or oral gavage administration (0.313 µmol/kg and 3.13 µmol/kg, respectively, equal to 0.13 and 1.3 mg/kg) (Fujii et al., 2015).

Similarly, the clearance rate of PFOS in CD-1 mice after a single oral dose (1 or 20 mg/kg of the potassium salt, potassium perfluorooctanesulfonate) exceeded the faecal clearance rate (Chang et al., 2012). [The Working Group noted that urinary excretion appears to be the primary route of elimination for PFOA and PFOS in rats and mice.]

In monkeys, larger amounts of PFOA were also excreted in the urine than in the faeces at steady state (Butenhoff et al., 2004). [The Working Group noted that urinary excretion is more relevant than faecal excretion in laboratory animals, while in humans the opposite is true, at least for PFOS.]

Several TK and PBPK models were available regarding PFOA and PFOS in rats, mice, and monkeys (reviewed in Bernstein et al., 2021). [The Working Group noted that most of these models included the assumption that urinary elimination is the primary route, on the basis of the experimental evidence. Accordingly, the kidney reabsorption loop has been extensively studied using PBPK models. However, the enterohepatic reabsorption component in rodents, despite the evidence for its presence (Johnson et al., 1984), has not received much attention, and it has not been fully incorporated into the existing rodent PBPK models.]

Organic anion transporters are thought to be involved in the renal elimination and reabsorption of PFOA also in non-human mammals. The expression levels of OATP1a1, OAT2, and OAT3 were found to be sex-dependent and responsive to changes in steroid hormone concentrations in rats (Kudo et al., 2002; Cheng et al., 2006). In rats, multiple regression analysis suggested that the clearance of PFOA was at least in part dependent on the renal expression of OAT2 and OAT3 (Kudo et al., 2002).

The renal clearance of PFOA was increased by mannitol infusion (which increases urine flow) but was reduced by a low-phosphate diet in both male and female rats (Katakura et al., 2007).

The renal clearance of PFOA did not change in the rat knockout model for multidrug resistance protein (MRP2) compared with wildtype rats (Katakura et al., 2007), indicating that MRP2 is not involved in PFOA transport.

(b) Experimental systems in vitro

Approximately 39% of the applied concentration of PFOA was found to penetrate mouse skin in vitro (Franko et al., 2012). [The Working Group noted that the PFOA penetration of mouse skin samples in vitro was higher than that for human skin samples in this study, which was 24% of the dose administered.]

The transport of PFOA by rat transporters has been reported for OAT1 (K_m = 43.2–51 µM), OAT3 (K_m = 65.7–80.2 µM), and OATP1a1 (K_m = 126.5–162 µM), using transiently transfected oocytes of *Xenopus laevis* and human embryonic kidney HEK293 cells in vitro (Katakura et al., 2007; Nakagawa et al., 2009; Weaver et al., 2010). No transport was observed in vitro for rat OAT2 or URAT1 (Nakagawa et al., 2009; Weaver et al., 2010).

Woodcroft et al. (2010) investigated the binding of PFOA to bacterially expressed rat L-FABP and, depending on the method used (direct displacement or isothermal titration calorimetry), predicted the existence of two

Table 4.2 Serum half-life ($T_{1/2}$) estimates for key species

Substance	Human[a] Population GM (95% CI)	Monkey Range	Mouse Range or mean	Rat Range
PFOA	3.14 yr (2.69–3.73 yr)	M, 13.6–35.3 d F, 26.8–41.7 d	M, 21.7 d F, 15.6 d	M, 6.4–13.4 d F, 2.75–13.9 h
PFOS	3.36 yr (2.52–4.42 yr)	M, 117–200 d F, 102–200 d	M, 36–43 d F, 30–38 d	M, 8.23–41.19 d F, 23.5–71.13 d

CI, confidence interval; d, day(s); F, female; GM, geometric mean; h, hour(s); M, male; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; yr, year(s).

[a] Estimates for humans are for both men and women.

Data were obtained from Kudo et al. (2002); Seacat et al. (2002); US EPA (2003); Butenhoff et al. (2004); Benskin et al. (2009); Lou et al. (2009); Kim et al. (2016a); Chang et al. (2017); Iwabuchi et al. (2017); Huang et al. (2019a, 2021); Dzierlenga et al. (2020); Chiu et al. (2022).

or three binding sites, with K_m estimates of $3.1–52.6 \times 10^{-6}$ M. Furthermore, PFOA and PFOS displaced a fluorescent fatty acid analogue from its binding site on rat L-FABP in vitro, indicating their potential to interfere with fatty-acid binding in vivo (Luebker et al., 2002). PFOA bound to rat and human serum albumin with similar affinities in vitro ($K_m = 4 \times 10^{-4}$ M) (Han et al., 2003).

(c) Interspecies differences

There are marked interspecies differences in the $T_{1/2}$ of PFOA and PFOS (summarized in Table 4.2). The rat demonstrates sex-specific differences with respect to PFOA but not PFOS, and the female rat has the shortest PFOA $T_{1/2}$ overall. However, no sex-specific differences have been identified in other species with respect to either PFOA or PFOS. The mechanisms responsible for the long $T_{1/2}$ in humans have not been elucidated. It has been hypothesized that tighter binding to endogenous proteins, such as albumin in the serum or L-FABP in the liver, or more efficient reabsorption by OATs in the kidney may be responsible. However, in vitro studies of these molecular targets did not report differences in the affinities of the rat and human proteins for PFOA (Nakagawa et al., 2008). Of note, owing to a shorter $T_{1/2}$ for PFOA, implying more rapid excretion, female rats appear to represent a more complex combination of laboratory species and sex, such that extrapolation of data on PFOA to humans is more difficult than from male rats or mice. [The Working Group noted that longer human PFOA and PFOS $T_{1/2}$ appear to correlate with relatively greater faecal excretion, whereas the shorter half-lives in monkeys, mice, and rats appear to favour the urinary excretion of PFOA and PFOS.]

Most previous research has focused on OATs and the involvement of bile acid transporters in the intestinal reabsorption of PFOA and PFOS; therefore data regarding possible interspecies differences in this mechanism are scarce. The human kidney reabsorbs bile acids during filtration (Stiehl, 1974), suggesting that that bile acid transporters may be present, and these may also play a role in the renal reabsorption of PFOA and PFOS.

Although PFOA and PFOS accumulate in the liver in humans, the liver/serum and liver/blood concentration ratios for each agent are much lower than those reported in animal studies. For example, in postmortem samples collected from adults, Maestri et al. (2006) found liver/blood ratios of 1 for PFOA and 2.7 for PFOS (Maestri et al., 2006). However, a study of human fetal postmortem tissues showed that liver levels of PFOA and PFOS were lower than those in maternal serum (Mamsen et al., 2019). Furthermore, in repeated oral dosing studies, PFOA and PFOS were found to accumulate in the rat and mouse liver and yield much higher concentration ratios versus blood (e.g. liver/blood ratios for PFOA of

3.3–5.6 and for PFOS of 4.8; Cui et al., 2009). Moreover, PFOA and PFOS have been shown to accumulate in the livers of fetal mice and rats (Luebker et al., 2005; Chang et al., 2009; Macon et al., 2011; Ishida et al., 2017; Lai et al., 2017a).

The kidney is another organ that shows interspecies differences with respect to PFOA accumulation. In postmortem kidney samples obtained from adult humans, PFOA and PFOS concentrations were slightly higher than those in the blood, with kidney/blood ratios of 1.2 for both compounds (Maestri et al., 2006). In rats, the kidney/blood ratio was approximately 0.7 for PFOA and approximately 1.0 for PFOS in 3-month oral multiple dose studies (Gao et al., 2015b; Iwabuchi et al., 2017), and similar values were obtained using other study designs. In studies conducted in mice exposed to PFOS, the kidney/blood ratio was estimated to be 0.5–0.9 (Bogdanska et al., 2011; Chang et al., 2012), whereas studies in mice exposed to PFOA yielded an estimated kidney/blood ratio of 0.15–0.2 (Lou et al., 2009; Fujii et al., 2015). Thus, the kidney/blood ratio appears to be lower by 6–8-fold in mice than in humans, indicating that these agents accumulate to a lesser extent in this organ in mice. [The Working Group acknowledged that limited data were available and noted that differences in the site-specific preferential accumulation of PFOA and PFOS may contribute to species differences in adverse effects.]

Synopsis

[In summary, the Working Group noted that there were few available data regarding the absorption and distribution of PFOA and PFOS in humans. PFOA and PFOS are absorbed after oral, inhalation, or dermal exposure. On the basis of their structures, PFOA and PFOS are not likely to cross cellular membranes directly, and various membrane transporters are thought to mediate transmembrane transport. The liver, blood, and lungs are important sites of accumulation for both agents. The distribution of PFOA and PFOS appears to be driven by binding to specific proteins, such as albumin in the blood and L-FABP in the liver. The available evidence suggests that enterohepatic circulation of PFOA and PFOS and bile acid transporters in the gut could be responsible for this effect. PFOA and PFOS cross the placenta and are transferred to infants via breast milk. Neither agent is metabolized. PFOA and PFOS are excreted in the faeces, which is an important route in humans, and the urine. In women of reproductive age, blood loss during menstruation, fetal transfer during pregnancy, and transfer during breastfeeding also contribute to the loss of PFOA and PFOS. The half-lives of PFOA and PFOS in humans are approximately 3.14 and 3.36 years, respectively, whereas those in monkeys, mice, and rats are in the order of hours to months. Rats and mice also have PFOA and PFOS distribution patterns that are distinct to those in humans. The mechanisms underlying the interspecies TK differences in PFOA and PFOS are not well understood.]

4.2 Evidence relevant to key characteristics of carcinogens

This section reviews the mechanistic data for the 10 key characteristics (KCs) of carcinogens (Smith et al., 2016) encompassed by the agents PFOA and PFOS. The studies in exposed humans used to support the mechanistic evidence were evaluated for the quality of the study design, exposure assessment, and assay accuracy and precision, and were found to reflect suitable methods for human environmental epidemiological studies. The evaluated human studies also accounted for important confounding and modifying variables. The determination of the Working Group may also have been buttressed by mechanistic evidence from human primary cells and tissues or from experimental systems.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) Humans

No studies in exposed humans or human cells in vitro were available to the Working Group.

(b) Experimental systems

The fluoro-carbon chain of PFOA and PFOS forms hydrophobic bonds with DNA, which may cause strand unwinding and steric hindrance for covalent binding (Lu et al., 2012, 2016a; Qin et al., 2022a). Several studies have demonstrated that PFOA binds to proteins in plasma (i.e. albumin), liver (especially L-FABP), kidney (i.e. alpha2u-globulin) and testes of rats (Vanden Heuvel et al., 1992; Luebker et al., 2002; Han et al., 2003, 2004, 2005). In addition, it has been shown that PFOA binds to cysteine residues in murine acetyl-coenzyme A (CoA) carboxylase A and B (Shao et al., 2018). PFOA has been shown to bind to rat and human albumin with similar strengths (Han et al., 2003; Messina et al., 2005b). Studies on bovine serum albumin have indicated that PFOA mainly binds by Van der Waals forces and hydrogen bonds at the Sudlow site 1 on albumin (Yang et al., 2023). PFOA binds to haemoglobin (Perera et al., 2023). [The Working Group noted that while the binding affinity to haemoglobin in this study was comparable to the binding affinity to albumin, as found in multiple studies, this finding probably had little biological relevance, because PFOA preferentially distributed to plasma proteins in vivo (see Section 4.1).] It has been shown that PFOS binds more strongly than PFOA to rat liver L-FABP (Luebker et al., 2002).

Synopsis

[The Working Group noted that PFOA and PFOS do not appear to have electrophilic properties or to be metabolized to electrophilic compounds (see also Section 4.1.1). PFOA and PFOS form non-covalent bonds with DNA. In addition, studies have shown that PFOA can interact with proteins, possibly through non-covalent hydrogen bonds and Van der Waals forces.]

4.2.2 Is genotoxic

See Tables 4.3 to 4.10.

(a) Humans

(i) Exposed humans

Genotoxic effects in humans were reported in the Flemish Environment and Health Study, a cross-sectional environmental study of adolescents from the Flanders region, Belgium, exposed to various hazardous compounds, including heavy metals, polycyclic aromatic hydrocarbons (PAHs), benzene, phthalates, and organophosphate pesticides (n = 606, in the entire cohort) (Franken et al., 2017). PFOA concentrations in serum were measured in a subpopulation from Menen (Flanders, Belgium), that was the location of a shredding factory (geometric mean, 2.55 ng/mL; 95% CI, 2.44–2.65 ng/mL; n = 197). The study participants were recruited between May 2010 and February 2011. [The Working Group noted that the PFOA concentrations in

serum were consistent with background levels of PFOA, indicating that the participants from Menen were not highly exposed.] The study showed a positive association between serum levels of PFOA and DNA strand breaks in leukocytes, measured using the alkaline comet assay (9% interquartile range, IQR; 95% CI, 1.5–17%, adjusted for sex, age, smoking status and maximum temperature, 7 days before sample collection). However, the positive association was not statistically significant after controlling for multiple comparisons using the method of Benjamini and Hochberg, which controlled the false discovery rate (FDR) at $P = 0.05$ (Franken et al., 2017). [The Working Group noted that PFOA serum concentrations were not correlated (Pearson correlation test) with marker levels of exposure to heavy metals, PAHs, benzene, phthalate, and organophosphate pesticides.]

[The Working Group noted that the study by Franken et al. (2017) had reliable measurements of exposure biomarkers and DNA damage end-points, and the positive association between PFOA and DNA strand breaks did not appear to be confounded by heavy metals, PAHs, benzene, phthalate, or organophosphate pesticides, and it was not statistically significant after controlling for multiple comparisons. The DNA damage was not mediated by oxidative stress, because no association between PFOA exposure and damaged DNA was observed using the formamidopyrimidine-DNA glycosylase (Fpg)-modified comet assay in leukocytes (see also Section 4.2.5).]

DNA fragmentation in spermatozoa has been assessed in two studies that aimed to evaluate semen quality and male fertility. One study recruited male partners of pregnant women at their first antenatal care visit to hospitals in Greenland ($n = 199$), Poland ($n = 197$), and Ukraine ($n = 208$) (Specht et al., 2012). The exposure levels differed between the populations, as reflected in the serum concentrations of PFOA (mean concentrations, 4.8, 5.1, and 1.8 ng/mL) and PFOS (51.9, 18.6, and 8.1 ng/mL in the populations from Greenland, Poland, and Ukraine, respectively). There was no association of the PFOA and PFOS serum concentrations with DNA fragmentation index in spermatozoa of this population of fertile men (Specht et al., 2012).

Another study recruited male partners of couples at their first visit to a reproductive medical clinic in Nanjing, China (Pan et al., 2019). The population was described as heterogeneous because it included both men with fertility issues and fertile men who were partners to infertile women ($n = 664$). The study included PFOA and PFOS measurements in serum and semen. There were similar serum concentrations of PFOA (median, 8.6 ng/mL; lower and upper cut-off values, 6.8 ng/mL and 11.0 ng/mL) and PFOS (median, 8.4 ng/mL; lower and upper cut-off values, 5.6 ng/mL and 13.1 ng/mL). Likewise, semen concentrations were similar for PFOA (median, 0.23 ng/mL; lower and upper cut-off values, 0.15 ng/mL and 0.36 ng/mL) and PFOS (median, 0.10 ng/mL; lower and upper cut-off values, 0.06 ng/mL and 0.18 ng/mL). There were strong correlations between serum and semen concentrations of PFOA ($r = 0.70$; $P < 0.001$) and PFOS ($r = 0.8$; $P < 0.001$). Serum concentrations of PFOA and PFOS or the increase per 1-unit increase in the log-transformed concentration were not associated with the DNA fragmentation index (PFOA: $\beta = 0.046$; 95% CI, −0.052 to 0.144; PFOS: $\beta = 0.040$; 95% CI, −0.037 to 0.116), adjusted for age, body mass index (BMI), BMI^2, smoking, alcohol intake, and the duration of abstinence from both, whereas there were positive associations between the DNA fragmentation index and concentrations of PFOA ($\beta = 0.136$; 95% CI, 0.064–0.209) and PFOS ($\beta = 0.087$; 95% CI, 0.033–0.142) in seminal fluid (Pan et al., 2019). [The Working Group considered that the two studies were less relevant than the findings reported in the study by Franken et al. (2017) described above, because DNA fragmentation was measured using the sperm

Table 4.3 End-points relevant to genotoxicity in human cells in vitro exposed to PFOA

End-point (assay)	Cells	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks (comet assay)	HepG2	+	1.9 µM	Positive control included (H_2O_2)	Ojo et al. (2022a)
DNA strand breaks (comet assay)	HepG2	+	10 µM	Positive control included (H_2O_2)	Wielsøe et al. (2015)
DNA strand breaks (comet assay)	TK6	+	125 µg/mL [0.3 µM]	No positive control included	Yahia et al. (2016)
DNA strand breaks (comet assay)	HepG2	+	50 µM	No positive control included	Yao and Zhong (2005)
DNA strand breaks (comet assay)	Sperm (primary cells)	–	1000 µM	Positive control included (H_2O_2)	Emerce and Çetin (2018)
DNA strand breaks (comet assay)	HepG2	–	400 µM	Positive control included (Ro19-8022 + light)	Eriksen et al. (2010)
DNA strand breaks (comet assay)	HepG2	–	400 µM	Positive control included (H_2O_2)	Florentin et al. (2011)
DNA double-strand breaks (γH2AX assay)	HaCaT	(+)	50 µM	Single concentration; no positive control included	Peropadre et al. (2018)
Chromosomal aberrations	Primary lymphocytes (from males)	–	2010 µg/mL	Increased frequency of endoreduplication in the cells observed at an exposure level that also caused a significant decrease in mitotic index; positive controls included (mitomycin C and cyclophosphamide for assays without or with metabolic activation, respectively)	US EPA (1996a)
Chromosomal aberrations	Primary lymphocytes (from males)	–	100 µg/mL	Positive controls included (mitomycin C and cyclophosphamide for assays without or with metabolic activation, respectively)	Butenhoff et al. (2014)
Micronuclei (CBMN assay)	HepG2	–	400 µM	Positive control included (mitomycin C)	Florentin et al. (2011)
Micronuclei (CBMN assay)	HepG2	+	100 µM	No positive control included	Yao and Zhong (2005)

CBMN, cytokinesis-block micronucleus assay; H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; LEC, lowest effective concentration; PFOA, perfluorooctanoic acid; γH2AX, γ-H2A histone family member X.

[a] –, negative; +, positive; (+), positive in a study of limited quality.

chromatin structure assay, which can be considered an indirect indicator of DNA damage.]

(ii) Human cells in vitro

See Table 4.3 and Table 4.4.

No studies conducted in human tissues were available. Four studies aiming to assess genotoxic effects induced by PFOA or PFOS treatment were conducted in human primary cells. The amount of DNA strand breaks (represented by DNA % tail as measured by comet assay) was not altered in human sperm cells by exposure to PFOA or PFOS (Emerce and Çetin, 2018). Exposure to PFOA or PFOS did not affect the frequency of chromosomal aberrations in human lymphocytes (US EPA, 1996a, 1999b; Butenhoff et al., 2014).

Six studies have assessed the formation of DNA strand breaks using the comet assay in human hepatoma (HepG2) and in human lymphoblastoid permanent TK6 cells; four of these studies have shown high levels of

Table 4.4 End-points relevant to genotoxicity in human cells in vitro exposed to PFOS

End-point (assay)	Cells	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks (comet assay)	HepG2	+	0.8 μM	Positive control included (H_2O_2)	Ojo et al. (2022a)
DNA strand breaks (comet assay)	HepG2	+	0.2 μM	Positive control included (H_2O_2)	Wielsøe et al. (2015)
DNA strand breaks (comet assay)	Sperm	–	1000 μM	Positive control included (H_2O_2)	Emerce and Çetin (2018)
DNA strand breaks (comet assay)	HepG2	–	400 μM	Positive assay control included (THP-1 cells treated with Ro19-8022 + light)	Eriksen et al. (2010)
DNA strand breaks (comet assay)	HepG2	–	300 μM	Positive controls included (H_2O_2 and benzo[*a*]pyrene)	Florentin et al. (2011)
Chromosomal aberrations	Primary lymphocytes (from males)	–	349 μg/mL	Negative with and without metabolic activation; positive controls included (mitomycin C and cyclophosphamide); one experiment with technical replicates	US EPA (1999b)
Micronuclei (CBMN assay)	HepG2	–	300 μM	Positive control included (mitomycin C)	Florentin et al. (2011)

CBMN, cytokinesis-block micronucleus assay; H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; LEC, lowest effective concentration; PFOS, perfluorooctanesulfonic acid.
[a] –, negative; +, positive.

genotoxicity (Yao and Zhong, 2005; Wielsøe et al., 2015; Yahia et al., 2016; Ojo et al., 2022a). The other two studies showed unaltered levels of DNA strand breaks in human HepG2 cells after exposure to both PFOA and PFOS when compared with a positive control group (Eriksen et al., 2010; Florentin et al., 2011). No change in the frequencies of chromosome aberrations and micronuclei in the HepG2 cells was observed after exposure to PFOA and PFOS in one of these studies (Florentin et al., 2011). Yao and Zhong instead reported an increase in the frequency of micronuclei in HepG2 cells after PFOA exposure (Yao and Zhong, 2005).

Exposure to PFOA induced an increase in the frequency of phosphorylated H2A histone family member X (γH2AX) foci in human HaCaT keratinocytes, which is an indicator of either DNA double-strand breaks or stalled replication forks (Peropadre et al., 2018).

(b) Experimental systems

(i) Non-human mammalian systems

See Table 4.5 (PFOA in vivo), Table 4.6 (PFOS in vivo), Table 4.7 (PFOA in vitro), Table 4.8 (PFOS in vitro).

DNA strand breaks (measured using the comet assay) were not induced in the liver and testes of mice exposed to PFOA in drinking-water (Crebelli et al., 2019). However, in the oocytes of mice that were exposed orally to PFOA there was an increased frequency of γH2AX foci, which is an indicator of either DNA double-strand breaks or stalled replication forks (Zhang et al., 2022a). No increases in the frequency of micronuclei in blood reticulocytes or spleen lymphocytes were observed in mice (Crebelli et al., 2019). In addition, oral exposure to PFOA did not alter the frequency of micronucleated bone marrow cells in mice (US EPA, 1995b, 1996d; Butenhoff et al., 2014) (see Table 4.5). [The Working Group considered that the negative results from the micronucleus assays were reliable, because the

Table 4.5 End-points relevant to genotoxicity in non-human mammals in vivo exposed to PFOA

End-point (assay)	Species, strain (sex)	Tissue(s)	Result[a]	Dose (LED, HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (comet assay)	Mouse, C57BL/6 (M)	Liver, testis	–	5 mg/kg	Oral, 5 wk, drinking-water	Positive control included (MMS)	Crebelli et al. (2019)
DNA double strand breaks (γH2AX assay)	Mouse, ICR (F)	Oocytes	(+)	5 mg/kg per day	Oral, 28 days, drinking-water	Method for the detection of γH2AX foci was not described; no positive control group	Zhang et al. (2022a)
Micronuclei	Mouse, C57BL/6 (M)	Blood (reticulocytes) and spleen (lymphocytes)	–	5 mg/kg	Oral, 5 wk, drinking-water	Positive control included (MMS)	Crebelli et al. (2019)
Micronuclei	Mouse, ICR (F and M)	Bone marrow	–	1000 mg/kg	Oral, 24–72 h, single dose	Positive control included (cyclophosphamide)	Butenhoff et al. (2014)
Micronuclei	Mouse, Crl:CD-1 (ICR) BR (F and M)	Bone marrow	–	1990 mg/kg	Oral, single dose, killed 24, 48 or 72 h post-exposure	Positive control included (cyclophosphamide)	US EPA (1996d)
Micronuclei	Mouse, Crl:CD-1 (ICR) BR (F and M)	Bone marrow	–	5000 mg/kg	Oral, single dose, killed 24, 48 or 72 h post-exposure	Positive control included (cyclophosphamide)	US EPA (1995b)

F, female; h, hour(s); γH2AX, γ-H2A histone family member X; HIC, highest ineffective concentration; HPLC-ECD, high-performance liquid chromatography-electrochemical detection; LEC, lowest effective concentration; M, male; MMS, methyl methanesulfonate; PFOA, perfluorooctanoic acid; wk, week(s).

[a] –, negative; +, positive; (+), positive in a study of limited quality.

Table 4.6 End-points relevant to genotoxicity in non-human mammals in vivo exposed to PFOS

End-point (assay)	Species, strain (sex)	Tissue	Result[a]	Dose (LED, HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (comet assay)	Rat, Wistar (M)	Bone marrow	+	0.6 mg/kg	Oral, 30 d, gavage at 48-h intervals	Positive control group included (mitomycin C)	Çelik et al. (2013)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Leukocytes (whole blood)	+	0.6 mg/kg	Oral, 30 d, gavage at 48-h intervals	Positive control group included (mitomycin C)	Eke and Çelik (2016)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Liver	+	0.6 mg/kg	Oral, 30 d, gavage at 48-h intervals	Positive control group included (mitomycin C)	Eke et al. (2017)
Mutations (*Spi*⁻ assay)	Mice, *gpt* delta transgenic (M)	Liver	–	10 mg/kg per day	Oral, 28 d, gavage at 24-h intervals	No positive control group	Wang et al. (2015a)
Micronuclei	Mice, *gpt* delta transgenic (M)	Bone marrow	–	10 mg/kg per day	Oral, 28 d, gavage at 24-h intervals	No positive control group	Wang et al. (2015a)
Micronuclei	Rat, Sprague-Dawley (F and M)	Erythrocytes (blood)	–	5 mg/kg per day	Oral, 28 d, gavage once daily	A slightly increased percentage of micronucleated cells in female rats was related to PFOS-induced bone-marrow toxicity	NTP (2019)
Micronuclei	Rat, Wistar (M)	Bone marrow	+	1.25 mg/kg	Oral, 30 d, gavage at 48-h intervals	Positive control group included (mitomycin C)	Çelik et al. (2013)
Micronuclei	Rat, Wistar (M)	Leukocytes (whole blood)	+	0.6 mg/kg	Oral, 30 d, gavage at 48-h intervals	Positive control group included (mitomycin C)	Eke and Çelik (2016)
Micronuclei	Rat, Wistar (M)	Liver	+	0.6 mg/kg	Oral, 30 d, gavage at 48-h intervals	Positive control group included (mitomycin C)	Eke et al. (2017)
Micronuclei	Mouse, Crl:CD-1 (ICR) BR (F and M)	Bone marrow	–	950 mg/kg	Oral, single dose, killed 24, 48 or 72 h post-exposure	Positive control group included (cyclophosphamide)	US EPA (1996e)

d, day(s); F, female; h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; M, male; PFOS, perfluorooctanesulfonic acid.

[a] –, negative; +, positive.

Table 4.7 End-points relevant to genotoxicity in non-human mammalian cells in vitro exposed to PFOA

End-point (assay)	Cells	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks (comet assay)	Oocytes (porcine)	(+)	40 µM	Increased comet tail length, although changes in tail intensity and tail moment were not statistically significant; it was uncertain whether the statistical analysis was based on all comets (*n* = 250) or independent experiments; no positive control included	Mario et al. (2022)
DNA strand breaks (comet assay)	SHE cells	–	300 µM	Positive control included (H_2O_2)	Jacquet et al. (2012a)
DNA strand breaks (comet assay)	Rat testicular cells	–	300 µM	Positive control included (1,2-dibromo-3-chloropropane)	Lindeman et al. (2012)
DNA double-strand breaks (γH2AX assay)	Mouse oocytes	(+)	300 µM	No positive control included in the experiments; toxicity at the highest concentration (approximately 30% cell death and decreased oocyte maturation at lowest concentration)	Guo et al. (2021a)
DNA double-strand breaks (γH2AX assay)	Mouse oocytes	(+)	200 µM	Single concentration; statistical analysis was based on all oocytes (*n* = 50–58), rather than independent experiments; no positive control group included	Zhou et al. (2022)
HGPRT locus mutations	CHO-K1	–	39 µg/mL [90 µM]	Positive control included (ethylmethanesulfoxide and dimethylbenzanthracene for the test conditions without and with metabolic activation, respectively)	US EPA (2002)
CD59 locus mutations	Human–hamster hybrid (A_L) cells	+	200 µM	Mutagenic effect observed after extended period of exposure (16 days, but not after 1, 4 or 8 days of exposure); no positive control group included	Zhao et al. (2011a)
Micronuclei (assay not specified)	CHL V79 fibroblasts	–	10 µM	Single concentration; positive controls included (ethylmethylsulfonate and cyclophosphamide)	Buhrke et al. (2013)
Chromosomal aberrations	CHO cells	–	996 µg/mL	Positive control included (mitomycin C and cyclophosphamide for the test conditions without and with metabolic activation, respectively)	Butenhoff et al. (2014)
Chromosomal aberrations	CHO cells	–	2250 µg/mL	Negative without metabolic activation; increased frequency of endoreduplication in cells and polyploidy in cells with metabolic activation; positive controls included (mitomycin C and cyclophosphamide)	US EPA (1996b)
Chromosomal aberrations	CHO cells	+ (with metabolic activation)	3740 µg/mL	Induction of chromosomal aberrations in cells with metabolic activation; negative without metabolic activation; positive controls included (mitomycin C and cyclophosphamide)	US EPA (1996c)

CHL, Chinese hamster lung; CHO, Chinese hamster ovary; γH2AX, γ-H2A histone family member X; H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; LEC, lowest effective concentration; PFOA, perfluorooctanoic acid; SHE, Syrian hamster embryo.

[a] –, negative; +, positive; (+), positive in a study of limited quality.

Table 4.8 End-points relevant to genotoxicity in non-human mammalian cells in vitro exposed to PFOS

End-point (assay)	Cells	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA double-strand breaks (γH2AX assay)	Porcine ovary	(+)	100 µM	Single concentration; no positive control included; the statistical analysis was based on all the oocytes (*n* = 27–30), rather than independent experiments	Chen et al. (2021)
DNA strand breaks (comet assay)	Sperm (boar)	(+)	461 µM	Single concentration; no positive control included	Oseguera-López et al. (2020)
DNA double-strand breaks (γH2AX assay; western blot)	MEF	(+)	20 µM	Genotoxic response occurred at concentrations with < 50% viability; no positive control included	Wang et al. (2015a)
DNA strand breaks (comet assay)	SHE cells	–	93 µM	Positive control included (H_2O_2)	Jacquet et al. (2012b)
Unscheduled DNA synthesis	Rat hepatocytes	–	25 µg/mL	Positive control included (2-acetylaminofluorene)	US EPA (1999c)
Mutations (*Spi*$^-$ mutation assay; *redBA/gam* locus)	MEF	+	10 µM	No positive control included	Wang et al. (2015a)
Mutations (CD59$^-$ mutants)	Human–hamster (A_L) hybrid cells	–	200 µM	No positive control included	Wang et al. (2013)
Micronuclei	CHL fibroblasts (V79)	–	12.5 µg/mL	Single concentration with metabolic activation (S9 mix); positive control included (cyclophosphamide)	Jernbro et al. (2007)
Aneuploidy	Mouse oocytes	(+)	600 µM	Single concentration; aneuploidy caused by dysfunction of spindle assembly and chromosome alignment in mitosis; no positive control included	Wei et al. (2021)

CHL, Chinese hamster lung; H_2O_2, hydrogen peroxide; γH2AX, γ-H2A histone family member X; HIC, highest ineffective concentration; LEC, lowest effective concentration; MEF, mouse embryonic fibroblasts; PFOS, perfluorooctanesulfonic acid; S9, 9000 × *g* supernatant; SHE, Syrian hamster embryo.

[a] –, negative; +, positive; (+), positive, in a study of limited quality.

studies included positive controls for genotoxic effects (Butenhoff et al., 2014; Crebelli et al., 2019).]

In three consecutive studies performed in rats by the same research group, oral exposure to PFOS was shown to induce an increase in DNA strand breaks, measured using the comet assay, and in the frequency of micronuclei in bone marrow cells (Çelik et al., 2013), leukocytes (whole blood) (Eke and Çelik, 2016), and liver (Eke et al., 2017).

One study assessed genotoxic effects in *gpt* transgenic mice, which were exposed by oral gavage to PFOS at 1.5, 4, or 10 mg/kg once daily for 28 days (Wang et al., 2015a). Compared with the control group, there were higher frequencies of mutations in the liver (2.2 and 6.8 λ mutants/10^6 plaques) and of micronucleated polychromatic erythrocytes in the bone marrow (3.0% and 2.9%, equal to 0.52- and 0.43-fold) at the two highest doses, although the increase was not statistically significant (Wang et al., 2015a). [The Working Group noted that the authors considered the fold increases as evidence that PFOS is mutagenic in vivo. However, the greater mutagenic effect in the highest dose group may have been because of an outlier, and genotoxic effects occurred only at doses that caused increases in the serum levels of alkaline phosphatase and alanine aminotransferase, suggesting genotoxicity may have been because of tissue toxicity.]

The study presented in a report by the National Toxicology Program (NTP) showed no effect on micronucleated cells in the peripheral blood of rats exposed orally (NTP, 2019). Lastly, one study showed unaltered frequency of micronuclei in bone marrow cells after a single oral administration of PFOS in mice (US EPA, 1996e) (see Table 4.6).

Two in vitro studies showed that PFOA did not induce the formation of DNA strand breaks, assessed using the comet assay, in rodent cells (Jacquet et al., 2012a; Lindeman et al., 2012).

In contrast, more recently, Mario et al. (2022) reported that PFOA exposure generated DNA strand breaks, assessed using the comet assay in porcine oocytes. [The Working Group noted that the study was of limited relevance because the statistical analysis seemed to be based on individual comets, rather than the mean results from independent experiments, and the study did not include a positive control group.]

In two different studies in mouse oocytes, PFOA treatment induced an increase in γH2AX foci in comparison with the negative control (Guo et al., 2021a; Zhou et al., 2022). [The Working Group noted that the statistical analysis in the study of Zhou et al. (2022) also seemed to be based on individual comets, rather than the mean results from independent experiments, and the two studies did not include a positive control group.]

US EPA (2002) reported no increase in mutation frequency in Chinese hamster ovary CHO-K1 cells, using the hypoxanthine-guanine phosphoribosyl transferase assay. An increase in mutation frequency was observed at the CD59 locus in human–hamster hybrid (A_L) cells exposed to PFOA for 16 days, whereas a shorter exposure to PFOA for 1, 4, or 8 days was not associated with increases in mutation (Zhao et al., 2011a). No increases of chromosome aberrations or in the frequency of micronuclei were observed in PFOA-exposed rodent cells (US EPA, 1996b; Buhrke et al., 2013; Butenhoff et al., 2014). An increase of chromosome aberrations was observed in another study in CHO cells in the presence of metabolic activation (US EPA, 1996c) (see Table 4.7).

In three in vitro studies, exposure to PFOS induced DNA strand breaks (assessed using the comet assay) and γH2AX foci in porcine ovary cells, sperm of boar, and mouse embryonic fibroblasts (Wang et al., 2015a; Oseguera-López et al., 2020; Chen et al., 2021), whereas, in a fourth study, exposure to the potassium salt of PFOS did

not alter the level of DNA strand breaks in Syrian hamster embryo cells (Jacquet et al., 2012b).

PFOS did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes (US EPA, 1999c). One study showed an increase in mutation frequency (*spi* mutation assay) in mouse embryonic fibroblasts (Wang et al., 2015a). In human–hamster (A_L) hybrid cells, exposure to PFOS did not alter the frequency of $CD59^-$ mutants (Wang et al., 2013). [The Working Group noted that a positive control group was not included in the study.]

No change in the frequency of micronuclei was reported in Chinese hamster lung fibroblasts (V79) (Jernbro et al., 2007). Lastly, Wei and colleagues observed aneuploidy in oocytes from mice after exposure to the potassium salt of PFOS, which was speculated to be caused by dysfunctions of spindle assembly and chromosome alignment in mitosis (Wei et al., 2021) [The Working Group noted that aneuploidy encompasses both gain and loss of chromosomes. However, the study had some limitations: the statistical analysis of aneuploidy appeared to be based on the number of oocytes (group sizes of 20 and 21 oocytes), rather than results from independent experiments, and the study did not include a positive control.]

(ii) Prokaryotes and other species

See Table 4.9 (PFOA) and Table 4.10 (PFOS).

Prokaryotes

PFOA has not been found to be mutagenic in various *Escherichia coli* and *Salmonella typhimurium* tester strains (Griffith and Long, 1980; US EPA, 1995a, 1996f; Oda et al., 2007; Fernández Freire et al., 2008; Buhrke et al., 2013; Butenhoff et al., 2014). PFOS has given negative results for mutagenicity in various *E. coli* and *S. typhimurium* tester strains (Simmon and Marx, 1978; US EPA, 1979, 1999a; Oda et al., 2007; NTP, 2019).

[The Working Group noted that PFOA and PFOS might not readily enter cells in the absence of appropriate transporters, which could be the case for the prokaryotic systems and for *Saccharomyces cerevisiae*.]

Lower eukaryotes

Exposure of *Paramecium caudatum* (unicellular freshwater protozoa) to PFOA was associated with increased levels of DNA strand breaks (Kawamoto et al., 2010). PFOA exposure did not alter the frequency of mitotic recombination in *S. cerevisiae* (Butenhoff et al., 2014).

Exposure to PFOS did not affect levels of DNA strand breaks in *P. caudatum* (Kawamoto et al., 2010) and did not induce mitotic recombination in *S. cerevisiae* (Simmon and Marx, 1978; US EPA, 1979).

Other species

Studies on PFOA have shown increased levels of DNA strand breaks in earthworms (*Eisenia fetida*) (Zheng et al., 2016; Wang et al., 2021a), green mussels (*Perna viridis*) (Liu et al., 2014a, b), planarians (*Dugesia japonica*) (Zhang et al., 2020a), and daphnia (*Daphnia carinata*) (Logeshwaran et al., 2021), whereas exposure to PFOA in the common carp (*Cyprinus carpio*) was not associated with changes in levels of DNA strand breaks (Kim et al., 2010). Increased micronuclei frequency was observed in haemolymph cells of PFOA-exposed *Perna viridis* (Liu et al., 2014b).

DNA strand breaks, measured using the comet assay, were increased after exposure to PFOS in *Cyprinus carpio* (Kim et al., 2010), zebrafish (*Dario rerio*) (Du et al., 2016), *Eisenia fetida* (Xu et al., 2013a; Zheng et al., 2016), *Perna viridis* (Liu et al., 2014a, b), *Dugesia japonica* (Shao et al., 2019), *Daphnia carinata* (Logeshwaran et al., 2021), and *Allium cepa* (Sivaram et al., 2021). One study showed unaltered levels of DNA strand breaks in brain and liver cells from

Table 4.9 End-points relevant to genotoxicity in non-mammalian systems exposed to PFOA

End-point (assay)	Species (cell type)	Result[a]	Concentration (LEC or HIC)	Comments	Reference
Prokaryotes					
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538)	–	5 µmol/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	Buhrke et al. (2013)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* WP2 *uvrA*	–	1000 µg/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	Butenhoff et al. (2014)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA102, and TA104)	–	500 µM	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	Fernández Freire et al. (2008)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538)	–	500 µg/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	Griffith and Long (1980)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537)	–	5 mg/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	US EPA (1995a)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537)	–	5 mg/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	US EPA (1996f)
Mutations	*Salmonella typhimurium* TA1535/ pSK1002 (*umu* test)	–	1000 µM	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	Oda et al. (2007)
Lower eukaryotes					
Mitotic recombination	*Saccharomyces cerevisiae* D4	–	500 µM	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	Butenhoff et al. (2014)
DNA strand breaks (comet assay)	*Paramecium caudatum*	(+)	100 µM	Positive control groups included (2-aminoanthracene and MNNG)	Kawamoto et al. (2010)
Other species					
DNA strand breaks (comet assay)	*Eisenia fetida* (in the coelomocytes cells)	+	10 mg/kg soil	No positive control group included	Wang et al. (2021a)
DNA strand breaks (comet assay)	*Eisenia fetida* (in the coelomocytes cells)	+	600 mg/kg soil	No positive control included; there was uncertainty about whether the statistical analysis was based on all comets, independent experiments, or individual specimens	Zheng et al. (2016)
DNA strand breaks (comet assay)	*Perna viridis* (haemolymph)	+	1000 µg/mL	No positive control included	Liu et al. (2014a)

Table 4.9 (continued)

End-point (assay)	Species (cell type)	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks (comet assay)	*Perna viridis* (haemolymph)	+	1000 µg/mL	No positive control included	Liu et al. (2014b)
DNA strand breaks (comet assay)	*Dugesia japonica*	(+)	15 µg/mL	No positive control included; there was uncertainty about whether the statistical analysis was based on all comets, independent experiments, or individual specimens	Zhang et al. (2020a)
DNA strand breaks (comet assay)	*Daphnia carinata*	+	10 µg/mL	Positive control included (H_2O_2)	Logeshwaran et al. (2021)
DNA strand breaks (comet assay)	*Cyprinus carpio* (blood cells)	–	50 µg/mL	No positive control included	Kim et al. (2010)
Micronuclei	*Perna viridis* (haemolymph)	+	1000 µg/mL	No positive control included	Liu et al. (2014b)

H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; LEC, lowest effective concentration; MNNG, *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine; PFOA, perfluorooctanoic acid.
[a] +, positive; –, negative; (+), positive in a study of limited quality.

Table 4.10 End-points relevant to genotoxicity in non-mammalian systems exposed to PFOS

End-point (assay)	Species (cell type)	Result[a]	Concentration (LEC or HIC)	Comments	Reference
Prokaryotes					
Mutations	*Salmonella typhimurium* (TA98 and TA100) and *Escherichia coli* WP2 *uvrA*/pKM101	–	10 mg/plate	Negative with and without metabolic activation; genotoxic effect by exposure to positive controls	NTP (2019)
Mutations	*Salmonella typhimurium* TA1535/pSK1002 (*umu* test)	–	1000 μM	Negative with and without metabolic activation; genotoxic effect by exposure to positive controls	Oda et al. (2007)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538)	–	5 mg/plate	Negative with and without metabolic activation; genotoxic effect by exposure to positive controls; report contains results from two compounds (T-2247 and T-2248) that were not identified by CAS No.[b]	Simmon and Marx (1978)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538)	–	2 mg/plate	Negative with and without metabolic activation; genotoxic effect by exposure to positive controls; results from one independent experiment, except TA100 ($n = 2$)	US EPA (1979)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* WP2 *uvrA*	–	5 mg/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	US EPA (1999a)
Lower eukaryotes					
Mitotic recombination	*Saccharomyces cerevisiae* D3	–	5% (w/v or v/v)	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls; report contains results from two compounds (T-2247 and T-2248) that are not identified by CAS No.[b]	Simmon and Marx (1978)
Mitotic recombination	*Saccharomyces cerevisiae* D4	–	0.5 mg/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls; result from one experiment	US EPA (1979)
DNA strand breaks (comet assay)	*Paramecium caudatum*	–	100 μM	Positive control groups included (2-aminoanthracene and MNNG)	Kawamoto et al. (2010)
Other species					
DNA strand breaks (comet assay)	*Cyprinus carpio* (blood cells)	+	5 μg/mL	No positive control included	Kim et al. (2010)
DNA strand breaks (comet assay)	*Danio rerio* (blood cells)	+	0.4 μg/mL	No chemical positive control included (exposure to ZnO nanoparticles was associated with increased number of DNA strand breaks)	Du et al. (2016)

Table 4.10 (continued)

End-point (assay)	Species (cell type)	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks (comet assay)	*Eisenia fetida* (in the coelomocytes cells)	(+)	0.25 μg/cm²	No positive control included; there was uncertainty about whether the statistical analysis was based on all comets, independent experiments, or individual specimens	Xu et al. (2013a)
DNA strand breaks (comet assay)	*Eisenia fetida* (in the coelomocytes cells)	(+)	470 mg/kg soil	No positive control included; there was uncertainty about whether the statistical analysis was based on all comets, independent experiments, or individual specimens	Zheng et al. (2016)
DNA strand breaks (comet assay)	*Perna viridis* (haemolymph)	+	10 μg/mL	No positive control included	Liu et al. (2014a)
DNA strand breaks (comet assay)	*Perna viridis* (haemolymph)	+	10 μg/mL	No positive control included	Liu et al. (2014b)
DNA strand breaks (comet assay)	*Dugesia japonica*	+	5 μg/mL	No positive control included	Shao et al. (2019)
DNA strand breaks (comet assay)	*Daphnia carinata*	+	1 μg/mL	Positive control included (H_2O_2)	Logeshwaran et al. (2021)
DNA strand breaks (comet assay)	*Allium cepa*	+	25 μg/mL	Positive control included (benzo[*a*]pyrene)	Sivaram et al. (2021)
DNA strand breaks (DNA precipitation assay)	*Larus michahellis* (brain and liver cells of embryos)	–	200 ng/g egg weight	No positive control included	Parolini et al. (2016)
Mutations	λ transgenic medaka (liver)	+	6.7 ng/mL	Mutation spectrum encompassed mainly +1 frameshift mutations	Chen et al. (2016)
Chromosomal aberrations	*Allium cepa*	+	25 μg/mL	Positive control included (benzo[*a*]pyrene)	Sivaram et al. (2021)
Micronuclei	*Danio rerio* (blood cells)	+	0.8 μg/mL	No chemical positive control included (exposure to ZnO nanoparticles was associated with increased number of DNA strand breaks)	Du et al. (2016)
Micronuclei	*Perna viridis* (haemolymph)	+	100 μg/mL	No positive control included	Liu et al. (2014b)

CAS No., Chemical Abstracts Service Registry Number; H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; LEC, lowest effective concentration; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; PFOS, perfluorooctanesulfonic acid; w/v, weight per volume; w/w, weight per weight; ZnO, zinc oxide.

[a] +, positive; –, negative; (+), positive in a study of limited quality.

[b] The compounds have been identified (OECD, 2002) as a 50% (by weight) solution of the diethanolammonium salt of perfluorooctanesulfonate in water (T-2247 CoC) and 22.5% of a reaction product of ethyl and methyl methacrylates and 22.5% of the pyridinium chloride salt of an *N*-methylperfluorooctanesulfonamidoethanol-based glutaryl amide (T-2248 CoC).

yellow-legged gull (*Larus michahellis*) embryos after exposure to PFOS (Parolini et al., 2016).

Increases in the frequencies of micronuclei and chromosome aberrations versus the control group were observed in haemolymph cells of PFOS-exposed *Perna viridis* (Liu et al., 2014b), in *Dario rerio* (Du et al., 2016), and in cells of *Allium cepa* (Sivaram et al., 2021). A higher frequency of +1 frameshift mutations was observed in the liver cells of λ transgenic medaka fish (Chen et al., 2016).

Synopsis

[The Working Group noted that there was a paucity of data in exposed humans regarding genotoxicity, especially investigating associations between PFOA exposure and DNA damage end-points such as mutations and chromosome aberrations. One study in exposed humans showed a positive association between the serum level of PFOA and DNA strand breaks in the leukocytes of adolescents in Menen, Belgium. This positive association was not statistically significant when corrected for multiple comparisons. Two studies on PFOS have shown inconsistent indices of DNA fragmentation in semen samples from exposed humans.

For both PFOA and PFOS, in vitro studies in human primary cells have shown a lack of genotoxicity. Studies in experimental systems in human cell lines and in non-human mammalian systems in vivo and in vitro have shown mixed results for various types of end-points, such as DNA strand breaks (measured with the comet assay or by counting γH2AX foci), micronuclei, and chromosome aberrations. PFOA and PFOS did not exert mutagenic effects in prokaryotes.]

4.2.3 Alters DNA repair or causes genomic instability

(a) Humans

No studies in exposed humans or in human cells in vitro were available to the Working Group.

(b) Experimental systems

Estefanía González-Alvarez et al. (2022) reported that oral treatment with PFOA at the dose of 2.5 mg/kg body weight (bw) for 15 days altered the ovarian contents of proteins that are involved in DNA damage sensing and repair in lean (4 increases and 12 decreases in protein contents) and obese (12 increases and 6 decreases in protein contents) female mice.

In vitro exposure of rhesus monkey trophoblast stem cells to PFOA at 100 nM for 4 weeks produced only subtle effects on gene expression related to DNA damage checkpoint signalling (Midic et al., 2018).

PFOS has been shown to reduce the activity of polymerase α from calf thymus: half-maximal inhibitory concentration (IC_{50}), 24.5 μM, and of the recombinant rat polymerase β, IC_{50} = 46.4 μM (Nakamura et al., 2007).

Synopsis

[The Working Group noted that there was a paucity of data on whether PFOA and PFOS altered DNA repair and caused genomic instability.]

4.2.4 Induces epigenetic alterations

See Tables 4.11 to 4.14.

DNA (CpG, 5′-C-phosphate-G-3′-dinucleotide) methylation, global DNA methylation, altered expression of microRNAs (miRNAs), and histone modifications are all forms of epigenetic change that have been associated with carcinogenesis (Sharma et al., 2010). The following sections detail studies that have investigated the

Table 4.11 End-points relevant to epigenetic alterations in humans exposed to PFOA or PFOS

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Epigenome-wide DNA methylation	Cord blood and peripheral leukocytes	Ohio, USA Prospective birth cohort with cross-sectional analysis	266 mother–child pairs (median: PFOS, 14 ng/mL; PFOA, 5.5 ng/mL) at birth	435 CpGs (PFAS); 2 CpGs PFOS + 12 CpGs PFOA + and –	Cell type composition, child age, child sex, annual household income, maternal race/ethnicity, and maternal smoking during pregnancy	Cohort replication included; comparison of methylation over time highlighting the persistence of epigenetic modifications	Liu et al. (2022a)
Epigenome-wide DNA methylation	Cord blood leukocytes	Taiwan, China Prospective birth cohort	Sapporo cohort of the Hokkaido (Japan) study (190 mother–child pairs from the general population; discovery cohort) (median: PFOS, 5.2 ng/mL; PFOA, 1.4 ng/mL) Taiwan, China Maternal and Infant Cohort Study (37 mother–child pairs from the general population; replication cohort (PFOS, 12.2 ng/mL; PFOA, 1.8 ng/mL)	4 CpGs for PFOS –; 3 CpGs for PFOA + and –	Maternal age, parity, maternal educational level, maternal blood sampling period, maternal pre-pregnancy BMI, maternal smoking during pregnancy, gestational age, infant sex, and cord blood cell estimates	Strengths of this study included the use of a replication cohort	Miura et al. (2018)
Epigenome-wide DNA methylation	Dried blood spots	New York, USA Cross-sectional study	597 neonates (median: PFOS, 1.74 ng/mL; PFOA, 1.12 ng/mL)	2 sex-specific associations for CpGs PFOS + and –; 1 CpG PFOA –	Sample plate and estimated cell count; infant sex, plurality, and epigenetically-derived ancestry (4 principal components); maternal age, race/ethnicity, education level, marital status, pre-pregnancy BMI, smoking during pregnancy, and history of pregnancy loss		Robinson et al. (2021)

Table 4.11 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Epigenome-wide DNA methylation	Cord blood leukocytes	Colorado, USA Cross-sectional study	583 mother–child pairs (median: PFOS, 2.4 ng/mL; PFOA 1.1 ng/mL)	1 DMR – for PFOA	Infant sex, gestational age (days), maternal age (years), education level (completed high school), smoking during pregnancy, race/ethnicity, BMI, previous pregnancies, cell type		Starling et al. (2020)
Epigenome-wide DNA methylation	Blood leukocytes	Shiyan Renmin Hospital Hubei Province, China Cross-sectional study	98 male and female patients (median: PFOS, 2.29 ng/mL; PFOA, 0.85 ng/mL)	87 CpGs and 11 DMRs for PFOS 63 CpGs for PFOA	Age, BMI, sex		Cheng et al. (2022)
Epigenome-wide DNA methylation	Placenta	USA Cross-sectional study	260 pregnant women (median in maternal plasma: PFOS, 4.74 ng/mL; PFOA, 2.2 ng/mL)	PFOS: 3 CpG sites (2 sites + and 1 site –) in placenta	Self-reported maternal race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, Asian), age (in years), offspring sex (male/female), pre-pregnancy BMI (kg/m^2), total plasma lipid concentration (ng/mL, except PFAS), log-transformed plasma cotinine concentration (ng/mL); methylation sample plate (*n* = 5); the first three methylation PCs and the first 10 genotype PCs were used to account for population structure	Included gene expression	Ouidir et al. (2020)
Epigenome-wide DNA methylation	Peripheral blood leukocytes	Ohio, USA Cross-sectional study	44 total (7.5 ng/mL); PFOA high (*n* = 22; 15 ng/mL) and low (*n* = 22; 2.4 ng/mL); median values	1 CpG –	Cell type, child sex, and income	Small sample size; nothing passed FDR	Kingsley et al. (2017)

Table 4.11 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Epigenome-wide DNA methylation	Blood leukocytes	Six European cohorts (France, Spain, Norway, Greece, UK, Lithuania) Cross-sectional study	1173 (PFOA, 1.51 µg/L; PFOS, 2.14 µg/L)	PFOS: 12 CpGs majority −; PFOA +/−	Cell type		Cadiou et al. (2020)
Epigenome-wide DNA methylation	Blood leukocytes	Dordrecht, Netherlands Cross-sectional study	34 (PFOS: 40 ng/g) median	PFOS: 29 CpGs, 38 DMRs	Age and leukocyte counts	Small sample size; did not differentiate between dioxins, PCBs, and PFOS; men only	van den Dungen et al. (2017a)
Epigenome-wide DNA methylation/ Epigenetic age	Whole blood leukocytes	Ronneby, Sweden Cross-sectional	63 participants (PFOS: controls, *n* = 32: 2.8 ng/mL, high exposure group, *n* = 31: 295 ng/mL) (PFOA: controls, *n* = 32: 1.4 ng/mL, high exposure group, *n* = 31: 19 ng/mL); medians	12 DMPs, PFAS +	Neutrophil fraction	Study did not differentiate between PFOS and PFOA; study design was unclear (case–control?)	Xu et al. (2022)
Global (Alu elements, LINE-1) DNA methylation	Cord blood leukocytes	Taipei, Taiwan, China Cross-sectional	363 participants (PFOS, 6.07 ng/mL; PFOA, 2.05 ng/mL)	Alu methylation: PFOS −; PFOA +/−; LINE-1 methylation +/−	Parental education level, maternal BMI, maternal age, delivery method (vaginal delivery or caesarean section), parity, infant sex, gestational age, and cotinine level		Liu et al. (2018a)
Global DNA methylation of LINE-1	Peripheral blood leukocytes	Ohio, USA Cross-sectional	685 participants (PFOS, 14.1 ng/mL; PFOA, 57.9 ng/mL)	LINE-1 methylation PFOS +; no association with PFOA	Age, sex, BMI, smoking status (ever/never), and current alcohol consumer (yes/no)		Watkins et al. (2014)
Global DNA methylation of LINE-1 and Alu methylation	Umbilical cord blood	Maryland, USA Cross-sectional	30 participants (PFOS, 5.8 ng/mL; PFOA, 1.8 ng/mL)	PFOS +/−; PFOA was marginally associated ($P = 0.06$) with a decrease in global DNA methylation	Maternal age or gestational age	Small sample size	Guerrero-Preston et al. (2010)

Table 4.11 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Global DNA methylation	Sperm	Greenland, Denmark; Poland; Ukraine Cross-sectional	262 participants (PFOS, 27.2 ng/mL; PFOA, 4.0 ng/mL)	DNA global methylation PFOS +/–; PFOA +/–	Age and smoking status	No results were significant when the entire sample was considered, but when broken down by sub-study, there were significant associations	Leter et al. (2014)
Global DNA methylation	Blood leukocytes	Taiwan, China Cross-sectional	1425 participants (mean PFOS, 4.95 ng/mL)	5mdC/dG +	Model 1: adjusted for age, sex, smoking, alcohol consumption, BMI, and household income; Model 2: adjusted for Model 1 parameters plus HTN, DM, or hyperlipidaemia	The overall epigenetic sampling was performed in a smaller sample	Lin et al. (2022)
Targeted DNA methylation	Cord blood leukocytes	Sapporo, Japan Cohort	177 mother–child pairs (PFOS, 5.2 ng/mL; PFOA, 1.3 ng/mL)	IGF2 methylation; PFOA –	Maternal age, maternal education, infant sex, maternal smoking during pregnancy, and blood sampling period		Kobayashi et al. (2017)
miRNA expression	Blood leukocytes	Ronneby, Sweden Cross-sectional	53 pregnant women (median: PFOS: low-exposure group, 3 ng/mL; high-exposure group, 230 ng/mL) (median: PFOA: low-exposure group: 2 ng/mL; high-exposure group, 8 ng/mL)	PFOS: ↓ miR-101-3p, ↓ miR-144-3p, ↓ miR-19a-3p	None	No covariates included; only women tested	Xu et al. (2020b)
Targeted DNA methylation	Cord blood leukocytes	Taiwan, China Cross-sectional	486 participants (PFOS, 6.09 ng/mL; PFOA, 2.04 ng/mL)	*Mest* promoter methylation PFOS –	Maternal age, infant sex, parental educational level, cotinine level in cord blood, maternal alcohol consumption, pre-pregnancy BMI, parity, type of delivery, and gestational age	Sex-specific findings (women higher than men)	Ku et al. (2022)

Table 4.11 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Targeted DNA methylation (*IGF2*, *NR3C1*), LINE-1 DNA methylation	Placenta	Hebei Province, China Cross-sectional	180 participants; median: PFOS, 1.21 ng/g; PFOA, 1.33 ng/g	LINE-1 methylation: PFOS –; PFOA –	Age, pre-pregnancy BMI, gestational weeks, GDM, parity, newborn sex, mean intakes of carbohydrate, protein, and fat during pregnancy, and physical activity		Wang et al. (2023a)
Epigenome-wide DNA methylation, targeted DNA methylation, epigenetic age	Blood leukocytes	Arizona, California, and Massachusetts, USA Firefighters Cross-sectional	197 firefighters (*n*-PFOS, 4.02 ng/mL; *sm*-PFOS, 2.06 ng/mL; PFOA, 1.79 ng/mL)	Epigenetic age: positive association with 2 epigenetic clocks for *sm*-PFOS; positive association with 6 epigenetic clocks for *n*-PFOA Targeted DNA methylation: *sb*-PFOA positively associated with CpG sites in *PPARG* and *CD36*; *n*-PFOA inversely associated with methylation at one CpG site in *ACOT2* Epigenome-wide DNA methylation: positively associated with a CpG site within *CAPN12*; *n*-PFOS was inversely associated with methylation near the transcription start site of *RAD1*	Age, sex, race/ethnicity, cell type estimates and PCs representing technical variation from the Infinium MethylationEPIC array, and ethnicity in site-specific analysis and EWAS		Goodrich et al. (2021)

Table 4.11 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Targeted miRNA expression	Blood leukocytes	Fluorochemical plant, Jiangsu Province, China Cross-sectional	55 workers: (PFOS, 33 ng/mL; PFOA, 1272 ng/mL), 132 nearby residents: PFOS, 30.92 ng/mL; PFOA, 249 ng/mL)	PFOA positively associated with the expression of miR-26b and miR-199-3p	Age, BMI, smoking, alcohol consumption status		Wang et al. (2012a)
Targeted miRNA expression	Blood leukocytes	China Cross-sectional	80 participants with MetS and 64 controls (PFOS, 3.3 ng/mL; PFOA, 2.1 ng/mL)	*n*-PFOA negatively associated with miR-140-5p	Age	All men	Yang et al. (2020)

BMI, body mass index; CpG, cytosine–guanosine dinucleotide; dG, 2′-deoxyguanosine; DM, diabetes mellitus; DMP, differentially methylated position; DMR, differentially methylated region; EWAS, epigenome-wide association analysis study; FDR, false discovery rate; GDM, gestational diabetes mellitus; HTN, hypertension; LINE-1, long interspersed nuclear element-1; 5mdC, 5-methyl deoxycytosine; MetS, metabolic syndrome; miRNA, microRNA; *n*-, linear isomer; PC, principal component; PCB, polychlorinated biphenyl; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; *sb*-, sum of branched isomers; *sm*-PFOS, sum of perfluoromethylheptane sulfonate isomers; UK, United Kingdom; USA, United States of America.

[a] +, increased methylation; –, decreased methylation, +/–, increase or decrease in methylation; ↑, increased gene expression; ↓, decreased gene expression; ↑↓ increased and decreased gene expression.

association between PFOA or PFOS exposure and these epigenetic modifications.

(a) Humans

(i) Exposed humans

Exposure to PFOA

Several epigenome-wide association analysis studies (EWASs) have examined the relation between maternal exposure to PFOA during pregnancy and DNA methylation in neonatal cord blood.

Noteworthy among these studies was that by Liu et al. (2022a), which focused on a prospective birth cohort of mother–child pairs from the Health Outcomes and Measures of the Environment (HOME) Study (2003–2006; Cincinnati, Ohio, USA). Pregnant women were enrolled in the study at around week 16 of gestation, and children were followed-up at age 4 weeks and at age 1, 2, 3, 4, 5, 8, and 12 years. Overall, 291 participants with data on PFOA exposure and DNA methylation were considered in the study (266 at baseline and 160 at follow-up). For this study, the median PFOA concentration in maternal serum was 5.5 ng/mL and the 25th and 75th percentile values were 3.9 and 7.9 ng/mL, respectively. After adjustment for potential confounders and multiple comparisons, the authors identified that the maternal concentration of PFOA was associated with differential methylation of 12 CpGs measured in cord blood. The CpGs displayed both hyper- and hypomethylation. Notably, several of these DNA methylation changes persisted up to age 12 years. The associations were consistent at birth and at age 12 years, having the same direction and comparable effect sizes (Liu et al., 2022a). In addition, several CpGs were annotated to genes that have been linked to cancer of the breast, prostate, pancreas, and/or brain, such as *MAGI1, KRT18, SRPRB, TNR,* and *SERPINA5* (Liu et al., 2022a). [The Working Group noted that a strength of this study was the comparison of differential DNA methylation in cord blood at birth and blood collected during adolescence in the same participants, highlighting the stability of the findings. This study also benefited from the inclusion of a replication cohort. The Working Group also noted that this study analysed the association between maternal exposure to other PFAS and CpG methylation and found that there were specific changes associated with PFOA that persisted over time, further supporting the chemical specificity of the finding. As a potential limitation, it is possible that exposure to PFAS during the postnatal period could have influenced CpG methylation in later life. However, the probability of the chance identification of common CpG methylation sites between the early (e.g. at birth) and adolescent time points is low. Finally, the Working Group noted that a potential limitation of this study was that serum PFAS were not measured during the same trimester of pregnancy in all participants. However, this would lead to non-differential exposure misclassification and thus bias towards the null.]

Miura et al. (2018) also performed an EWAS in 190 mother–child pairs from the prospective Sapporo cohort of the Hokkaido Study (discovery cohort) and from 37 mother–child pairs from the Taiwan Maternal and Infant Cohort Study (replication cohort), examining the relation between PFOA in maternal serum and cord blood DNA methylation. The median PFOA concentration was measured to be 1.4 ng/mL and exposure was associated with both hypo- and hypermethylation of CpGs (Miura et al., 2018). [The Working Group noted that a strength of this study was the inclusion of a replication cohort. As above, the Working Group noted a potential limitation of this study was that serum PFAS concentrations were not measured during the same trimester of pregnancy in all participants. As noted, this would lead to non-differential exposure misclassification that would bias towards the null.]

Robinson et al. (2021) examined associations between PFOA levels and DNA methylation in a

cohort study in 597 neonates in New York, USA, in which both were assessed in dried blood spots from newborns. The median PFOA concentration measured was 1.12 ng/mL. Overall, log-transformed values of PFOA were not related to site-specific DNA methylation. When comparing the participants in the top decile of PFOA concentration with the other participants, exposure was associated with decreased DNA methylation of one CpG methylation site annotated to genes *SCRT2* and *SRXN1* (Robinson et al., 2021).

Starling et al. (2020) performed an analysis of data from a prospective cohort study of mother–infant pairs in Colorado, USA (n = 583). The median PFOA concentration was 1.12 ng/mL, and the range was 0.1–15.4 ng/mL. The maternal serum level of PFOA was associated with decreased DNA methylation of a CpG site annotated to *TJAP* in the cord blood of infants (Starling et al., 2020). Additionally, PFOA was associated with altered methylation of 15 differentially methylated regions (DMRs).

In the study by Cheng et al. (2022), plasma PFOA was assessed in 98 patients and the median concentration was 0.85 ng/mL. There were 63 CpG sites and eight DMRs associated with the measured plasma PFOA levels. Among the identified CpGs were those that were annotated to the genes *AFF3*, *CREB5*, *NRG2*, and *USF2*, and one of the DMRs was annotated to *IRF6* (Cheng et al., 2022).

[The Working Group noted that, taken together, the EWAS studies relating maternal PFOA to cord blood-based DNA methylation identified statistically significant associations. Although the specific genes that were identified across studies were not the same, in numerous cases the CpG sites were located within cancer-associated genes.]

In addition to the EWAS described above, several studies have also investigated the relations between PFOA and gene-specific/targeted CpG methylation. For example, Kobayashi et al. (2017) examined prenatal exposure to PFOA in a cohort in Japan (177 participants). The mean level was 1.3 ng/mL and the concentrations ranged from below the detection limit to 5.3 ng/mL. Exposure to PFOA was associated with lower methylation of the imprinted gene *IGF2* in cord blood (Kobayashi et al., 2017).

Similarly, Goodrich et al. (2021) investigated the PFOA-associated DNA methylation in blood leukocytes in firefighters from three states in the USA, namely Arizona, California, and Massachusetts. The mean level of linear *n*-PFOA was 1.79 ng/mL, and the 25th and 75th percentile values were 1.40 and 2.20 ng/mL. [The Working Group noted that there were lower concentrations and detection frequencies for branched *sb*-PFOA in this cohort, with a detection frequency of 31%.] The results indicated that exposure to *n*-PFOA was associated with lower methylation at one CpG site annotated to *ACOT2*. The results also indicated that the sum of the branched isomers of perfluorooctanoate (*sb*-PFOA) was associated with greater methylation at two CpG sites annotated to *PPARG* and *CD36*. When assessed via an epigenome-wide approach, *sb*-PFOA was associated with greater methylation at a CpG site that was annotated to *CAPN12* (Goodrich et al., 2021).

The relation between PFOA and epigenetic ageing (i.e. a measure of biological ageing) has been assessed. Indicators of epigenetic age, referred to as epigenetic clocks, have been developed with the use of CpG methylation data (Li et al., 2022b). Epigenetic age is a predictor of age and/or mortality. Recent research in diverse cancer types has highlighted the crucial role of epigenetic ageing in the initiation of tumours and its potential utility in predicting cancer risk (Yu et al., 2020). Goodrich et al. (2021) highlighted a positive association between exposure to *n*-PFOA and epigenetic ageing, assessed using various epigenetic clocks. However, there were no associations identified between *sb*-PFOA and epigenetic ageing.

The relationship between exposure to PFOA and the expression levels of miRNAs has been assessed in several studies.

Wang et al. (2012a) performed a cross-sectional analysis of a cohort of 55 workers in a fluorochemical plant and 132 nearby residents (controls) in a suburban area of Changshu City, Jiangsu Province, China. The geometric mean levels of PFOA were 1272.31 ng/mL in the workers and 249.93 ng/mL in the residents. To explore the effect of PFOA on circulating miRNAs, serum samples from 10 workers and 10 residents were used for miRNA microarray analysis. The high serum level of PFOA (high PFOA group) was positively associated with the increased expression of miR-26b and miR-199–3p in blood leukocytes (Wang et al., 2012a).

Yang et al. (2020) performed a cross-sectional analysis in a cohort of male participants with (n = 80) and without (n = 64) metabolic syndrome from China in whom the mean level of PFOA was 2.1 ng/mL. The serum concentration of *n*-PFOA was found to be negatively associated (β = −0.772; 95% CI, −0.244 to −0.300; $P < 0.01$; $q < 0.05$) with the expression of miR-140-5p in blood leukocytes (Yang et al., 2020).

Two studies assessed PFOA in relation to the global methylation of long interspersed nuclear element 1 (LINE-1) and/or small dimeric elements ALU methylation. Specifically, Guerrero-Preston et al. (2010) performed a cross-sectional analysis of a cohort of newborns in Maryland, USA (30 participants). The mean PFOA concentration, measured in umbilical cord blood just after birth, was 1.8 ng/mL. PFOA was marginally associated (P = 0.06) with a low level of global DNA methylation in the umbilical cord blood (Guerrero-Preston et al., 2010).

Similarly, Watkins et al. (2014) examined the association between PFOA and LINE-1 in a subset of adults enrolled in the C8 Health Project in Ohio, USA (685 participants). The mean serum concentration of PFOA was 57.9 ng/mL. No association was observed between PFOA and LINE-1 (Watkins et al., 2014) (see Table 4.11).

Exposure to PFOS

EWAS have been used to investigate the relationship between exposure to PFOS during pregnancy and CpG methylation in neonatal cord blood. A prospective birth cohort study collected data from 266 mother–child pairs from the general population in Ohio, USA. The median maternal serum PFOS concentration was measured as 14 ng/mL and the 25th and 75th percentile values were 9.9 and 17.8 ng/mL. After adjusting for potential confounders and multiple comparisons, the study found that prenatal exposure to PFOS was associated with persistent hypermethylation of two CpGs, in both cord blood and peripheral blood later in life (at age 12 years) (Liu et al., 2022a). One of these CpGs was annotated to *HPSE2*, which has been linked to breast cancer (Zhang et al., 2021a).

[The Working Group noted two strengths of this study: (i) the comparison of DNA methylation at different time points in the same participants; and (ii) the inclusion of a replication cohort.]

Miura et al. investigated the relation between maternal serum PFOS and cord blood DNA methylation in mother–child pairs recruited from the Sapporo cohort of the Hokkaido Study (discovery cohort) and mother–child pairs from the Taiwan Maternal and Infant Cohort Study (replication cohort). A total of 190 mother–child pairs, with exposure and methylation data, were analysed. The median PFOS concentration was 5.2 ng/mL, and the exposure was associated with the hypomethylation of four CpGs, as well as one DMR (Miura et al., 2018). [The Working Group considered the inclusion of a replication cohort to be a strength of this study. However, the Working Group noted a potential limitation of this study, which was that the serum PFOS concentrations were not measured during the same trimester of pregnancy in all participants. This might lead to

non-differential exposure misclassification and thus bias towards the null.]

In a separate study, Cheng et al. (2022) examined the relation between plasma PFOS and DNA methylation in leukocytes sampled from both male and female patients (n = 98) from the Shiyan Renmin Hospital of Hubei Province in China. The patients were being treated for benign diseases or for cosmetic needs. The median plasma concentration was 2.29 ng/mL. A total of 87 CpG sites and 11 DMRs displayed associations with plasma PFOS concentrations (Cheng et al., 2022).

Ouidir et al. (2020) assessed the association between PFOS exposure (plasma median concentration, 4.74 ng/mL) in pregnant women (n = 260) enrolled in the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies of a singleton cohort and epigenome-wide DNA methylation in the placenta. The authors identified three CpG sites with both hyper- and hypomethylation (Ouidir et al., 2020).

Robinson et al. (2021) investigated the association between PFOS exposure and DNA methylation in the Upstate KIDS cohort study, New York, USA, comprising 597 newborns, for whom the median PFOS concentration, measured in dried blood spots, was 1.74 ng/mL (25th to 75th quartile, 1.11 to 2.54 ng/mL). The results showed that PFOS was associated with lower DNA methylation, measured in dried blood spots, at one CpG site in boys, and higher DNA methylation at a different site in girls. In addition, the associations were observed only at the highest concentrations of PFOS, above the 90th percentile (Robinson et al., 2021).

Other studies have examined the relation between exposure to PFOS and epigenetic ageing. As reported above, DNA methylation can be used to estimate epigenetic age (Li et al., 2022b). Epigenetic ageing has been associated with tumour initiation and cancer risk prediction (Yu et al., 2020).

Goodrich et al. (2021) conducted a cross-sectional analysis of 197 firefighters from Arizona, California, and Massachusetts, USA. They observed that the mean serum concentration of n-PFOS was 4.02 ng/mL and the 25th and 75th percentile values were 3.00 and 5.80 ng/mL, respectively. The total concentration of perfluoromethylheptane sulfonate isomers (*sm*-PFOS) was 2.06 ng/mL and the 25th and 75th percentile values were 1.40 and 3.10 ng/mL, respectively. The results showed that for n-PFOS there was no association with epigenetic ageing, whereas for *sm*-PFOS there were associations with epigenetic ageing, specifically for two of the clocks (Goodrich et al., 2021).

Besides the EWAS approach, several studies have investigated the relationship between PFOS and gene-specific or targeted DNA methylation. For example, Ku et al. (2022) analysed 486 mother–infant pairs from the Taiwan Birth Panel Study cohort, China. The mean PFOS concentration was 6.09 ng/mL and the highest measured concentration was 67.92 ng/mL. The researchers identified that, in the multivariable model after adjustments, prenatal exposure to PFOS was associated with decreased methylation in the promoter region of *MEST* in the cord blood of infants (Ku et al., 2022). [The Working Group noted that *MEST* is an imprinted gene that encodes a protein belonging to the α/β hydrolase superfamily and has been found to be linked to adipocyte differentiation (Kamei et al., 2007).]

The associations of exposure to PFOS with global methylation, LINE-1, and/or ALU methylation have also been assessed. Wang et al. (2023a) performed a cross-sectional analysis of 180 pregnant women enrolled in a cohort study from Tangshan City, northern China, to examine the relation between PFOS and DNA methylation in the placenta. The median PFOS concentration in the placenta was 1.39 ng/g and ranged from 0.19 to 3.70 ng/g. The level of PFOS in the placenta was inversely associated with the overall methylation of LINE-1 (Wang et al., 2023a).

Similarly, Liu et al. (2018a) analysed 363 mother–infant pairs from the Taiwan Birth Panel birth cohort study in Taiwan, China. Maternal and cord blood samples were collected at birth. The mean PFOS concentration in the cord blood was 6.07 ± 1.93 ng/mL (geometric mean ± standard deviation, SD). Prenatal PFOS was associated with decreases in cord blood-derived ALU methylation (Liu et al., 2018a).

Guerrero-Preston et al. (2010) conducted a cross-sectional analysis of cohort of newborns (n = 30) in Maryland, USA. The mean PFOS concentration in the cord blood was 5.8 ng/mL. No association between PFOS and global DNA methylation was observed.

Another form of epigenetic modification is the altered expression of miRNAs. Several studies have examined the relation between PFOS and miRNA expression. For example, Xu et al. (2020b) performed a cross-sectional analysis of 53 women from the Ronneby area, Sweden, in whom the PFOS levels were up to 315 ng/mL because of contamination of drinking-water. The results highlighted that PFOS is associated with decreases in the expression of three miRNAs (miR-101-3p, miR-144-3p, and miR-19a-3p) in blood leukocytes (Xu et al., 2020b). These miRNAs are predicted to target genes annotated to cancer or endocrine dysfunction, such as *DNMT3A, EGFR, HMGCR, NR1H3, PPARA*, *PTGS2*, and *TGFA*.

[The Working Group noted several factors as strengths of the studies, including: (1) an examination of the persistence of the epigenetic mark over time; (2) the inclusion of gene expression measures; and (3) the use of replication cohorts. The Working Group noted that numerous studies deployed designs where chemical exposure was analysed in relation to epigenetic end-points in a cross-sectional manner. A potential limitation of this design is that a chemical measurement and/or epigenetic modification assessed at a single time point may not capture variation over time. In support of the above, it has been reported that PFAS levels can vary over the course of gestation: blood levels are higher in the first trimester of pregnancy than in later trimesters. Most of the association analyses presented here examined the relationship between PFAS exposure assessed later in pregnancy and the epigenetic end-point. This time frame of exposure assessment would thus be expected to lead to bias towards the null. The Working Group also noted that most studies assessed the epigenetic marks in blood leukocytes, and the relevance of the epigenetic marks to tumorigenesis has not been completely established. Despite the limitations of measurements in leukocytes, the Working Group noted features of the studies that have relevance to cancer, including PFOA and PFOS-associated DNA methylation of cancer-associated genes, altered expression of miRNAs that are known to be involved in carcinogenesis, and cancer-associated features, such as global hypomethylation.]

Other studies with inconclusive results or limitations in the quality of design were considered less informative. These studies investigated the associations of PFOA (Kingsley et al., 2017) or PFOS (van den Dungen et al., 2017a; Cadiou et al., 2020; Xu et al., 2022) with genome-wide methylation, global DNA methylation, or target DNA methylation (Leter et al., 2014; Lin et al., 2022).

(ii) Human cells in vitro

See Table 4.12.

The effects of in vitro exposure to PFOA and PFOS on DNA methylation have been assessed in a variety of human cells, including breast (MCF-7, MCF-10A), brain (SH-SY5Y), liver (HepG2), lung (A549), and placenta (HTR-8/SVneo) cell lines (Tian et al., 2012; Bastos Sales et al., 2013; Guo et al., 2017; van den Dungen et al., 2017b; Jabeen et al., 2020; Zhao et al., 2022).

Table 4.12 End-points relevant to epigenetic alterations in human cells in vitro exposed to PFOA or PFOS

End-point	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
DNA methylation	Brain, SH-SY5Y cells	PFOA –	PFOA: 0.4 or 4 µg/L in medium for 4 d		Zhao et al. (2022)
DNMT activity (DNMT1 and DNMT3A); global DNA methylation; *DNMT* expression	Breast, MCF7; liver, HepG2 cells	PFOA: global methylation –, ↓ *DNMT1* expression, ↑ *DNMT3A* in MCF-7	PFOA: 0, 20, 100, 200, or 400 µM for 48 h		Liu and Irudayaraj (2020)
Global DNA methylation	Brain, neuroblastoma cell line (SK-N-AS)	PFOS +/–; PFOA +/–	PFOS: 10 µM; PFOA 10 µM		Bastos Sales et al. (2013)
Global DNA methylation	Breast, MCF-10A cells	PFOS + and PFOA +	PFOS (10 µM) and PFOA (100 µM) for 72 h		Pierozan et al. (2020)
Global DNA methylation	Liver, L02 cells	PFOA +/–	PFOA: 5, 10, 25, 50, or 100 mg/L		Tian et al. (2012)
Global DNA methylation	Liver, HepG2	PFOA –	PFOA: 0–400 µM for 24 h		Wen et al. (2020)
Global methylation	Breast, MCF-10A	PFOS + and PFOA +	PFOS: 10 µM; PFOA: 100 µM		Pierozan et al. (2020)
mRNA expression of DNMTs and BDNF, miRNA-16, miRNA-22, and miRNA-30a-5p	Brain, SK-N-SH cells	↑ miRNA-16, ↑ miRNA-22, and ↑ microRNA-30a-5p, ↓ *DNMT1* mRNA, ↓ DNMT1 protein, ↑ *DNMT3A* mRNA, ↑ DNMT3A protein, ↑ *DNMT3B* mRNA, ↑ DNMT3B protein, ↓ BDNF protein, ↓ *BDNF* mRNA	PFOS: (0–150 µM)		Guo et al. (2017)
Targeted DNA methylation: DMRs, DMPs, 84 adipogenic genes	Human mesenchymal stem cells from bone marrow	DNA methylation –, 2 DMRs (*AXIN1*, *DKK1*) –, 45 DMPs (majority –)	PFOS: 0–30 µM, from day –1 to day 10; measurements on day 10	Treatment during cell differentiation	van den Dungen et al. (2017b)
Targeted gene expression (DNA methylation machinery (DNMTs, TETs)	Lung, A549 cells	Expression: PFOS: ↓ *TET1* mRNA, ↑ *TET2* mRNA, ↑ *TET3* mRNA, ↑↓ *DNMT1* mRNA, ↓ *DNMT3B* mRNA, ↓ *DNMT3A* mRNA, ↑↓ *CCNE1* mRNA, ↑↓ *CCNA2* mRNA, ↓ *CCNB1* mRNA PFOA: ↓ *TET1* mRNA, ↑ *TET3* mRNA, ↓ *DNMT1* mRNA, ↓*DNMT3B* mRNA, ↑ *DNMT3A* mRNA, ↑ *CCNE1* mRNA, ↓ *CCNA2* mRNA, ↑↓ *CCNB1* mRNA	PFOS: 0–400 µM; PFOA: 0–400 µM	DNA methylation was not assessed	Jabeen et al. (2020)
Targeted miRNA expression (miR-155 expression)	Liver, HepG2 cells	↑ miR-155	PFOS: 0–50 µM for 24 h		Wan et al. (2016)

Table 4.12 (continued)

End-point	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
Targeted miRNA expression and gene expression (MEG3)	HTR-8/SVneo cells	PFOS: ↓ miR-770; ↓ *MEG3*, ↑ *PTX3*	PFOS: 0–10 µM		Li et al. (2022b)
Targeted miRNA expression: miR-19a and miR-19b expression; targeted DNA methylation (H19 methylation)	HTR-8/SVneo cells	↓ miR-19a; ↓ miR-19b; ↑ *H19* expression	PFOS: 0, 0.1, 1, or 10 µM for 24 or 48 h		Li et al. (2020b)
Targeted miRNA expression (miRNA-22)	Brain, SH-SY5Y cells	↓ miR-16 expression; ↑ miR-22 expression, ↓ *BDNF*, ↓ *CREB* (100 µM), ↑ *TrkB*	PFOS: 0–100 µM for 48 h		Li et al. (2015)
Targeted gene expression (DNMTs, SIRTs), global DNA methylation, targeted miRNA expression (miR-29b)	HTR-8/SVneo cells	PFOS: ↓ *DNMT1*, ↓ *DNMT3A*, ↓ *DNMT3B*, ↓ *SIRT1*, ↓ *SIRT3*; global DNA methylation; expression: ↑ miR-29b	1, 10, and 50 µM		Sonkar et al. (2019)

BDNF, brain-derived neurotrophic factor; d, day(s); DMP, differentially methylated position; DMR, differentially methylated region; DNMT, DNA methyltransferase; miRNA, microRNA; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; SIRT, sirtuin; TET (enzymes), ten eleven translocation, alias for tet methylcytosine dioxygenases.

[a] +, increased methylation; –, decreased methylation, +/–, increase or decrease in methylation; ↑, increased gene expression; ↓, decreased gene expression; ↑↓, increased and decreased gene expression.

Exposure to PFOA

Pierozan et al. (2020) examined the effects of PFOA at a concentration of 100 μM for 72 hours and found increased global DNA methylation in breast MCF-10A cells. In another study in which PFOA was tested at a range of 0 to 400 μM, exposure was associated with a dose-dependent decrease in global DNA methylation in HepG2 cells (Wen et al., 2020).

Liu and Irudayaraj (2020) exposed breast MCF7 and liver HepG2 cells to PFOA at concentrations ranging from 0–400 μM. PFOA was associated with reduced global DNA methylation and altered expression of *DNMT1* in both cell types. *DNMT3A* displayed increased expression in MCF7 cells. An inconclusive trend in the expression level of *DNMT3B* was observed in both cell types (Liu and Irudayaraj, 2020).

Exposure to PFOS

Pierozan et al. (2020) examined the effects of PFOS exposure on MCF-10A cells using a concentration of 10 μM. PFOS was associated with increases in global DNA methylation in breast MCF-10A cells (Pierozan et al., 2020).

In relation to miRNAs, Wan et al. studied PFOS at concentrations ranging from 0–50 μM. They found that PFOS was associated with increased expression of miR-155 in HepG2 cells (Wan et al., 2016).

Li et al. (2020b) examined the effects of PFOS in HTR-8/SVneo cells at concentrations ranging from 0–10 μM. They found that PFOS altered the expression of several miRNAs in HTR-8 cells, including the reduction of the expression of miR-19a and miR-19b (Li et al., 2020b). PFOS was subsequently shown to reduce the expression of miR-770 in HTR-8 cells (Li et al., 2022c). The effects of PFOS were tested in SH-SY5Y at concentrations ranging from 0–100 μM; PFOS caused decreased expression of miR-16 and increased expression of miR-22 (Li et al., 2015).

PFOS treatment for 24 or 48 hours, even at 10 μM, decreased gene and protein expression of the DNA methyltransferases, and significantly increased reactive oxygen species (ROS) production in the first-trimester human HTR-8/SVneo trophoblast cell line. In addition, PFOS reduced global DNA methylation and increased protein lysine acetylation (Sonkar et al., 2019).

(b) Experimental systems

(i) Non-human mammalian systems in vivo

See Table 4.13.

Exposure to PFOA

In relation to histone modifications, Li et al. (2019a) exposed female pregnant Kunming mice to PFOA doses of 1, 2.5, 5, 10, or 20 mg/kg bw per day from pregnancy day 0, i.e. gestational day (GD)1, to GD17, and found that PFOA was associated in a dose-dependent manner with decreased histone acetylation in the liver: the histone acetyltransferase activity of the female offspring was reduced significantly up to the dose of 5 mg/kg, and the histone deacetylase activity was increased significantly up to the highest dose of 10 mg/kg. The expression of both acetyl-histone H3 and acetyl-histone H4 proteins was reduced significantly (Li et al., 2019a).

Rashid et al. (2020a) exposed CD-1 mice (age 30 days) to PFOA at 1, 5, 10, or 20 mg/kg per day for 10 days and found that PFOA exposure was associated with 879 DMRs and increased *DNMT1* expression in the kidney (Rashid et al., 2020a).

Ahmad et al. (2021) exposed CD-1 mice to PFOA at 5 or 20 mg/kg per day for 10 days and observed decreases in the methylation of the gene encoding transmembrane serine protease *Tmprss2* (a prognostic marker for lung adenocarcinoma) in lung tissue where also PFOA accumulated (Ahmad et al., 2021). The gene expression of *Dnmts* and *Tets* was also decreased (Ahmad et al., 2021).

Table 4.13 End-points relevant to epigenetic alterations in non-human mammals in vivo exposed to PFOA or PFOS

End-point	Species, strain	Tissue	Result[a]	Concentrations or dosing regimen	Route, duration	Comments	Reference
Global DNA methylation level, targeted gene expression (histone demethylases *Kdm1a* and *Kdm4c*)	Mouse, CD-1	Kidney	Global DNA methylation level –, gene expression of histone demethylases Kdm1a and Kdm4c ↑	PFOS, 5, 10, 20 mg/kg per day	Oral, 14 d		Wen et al. (2022)
Global DNA methylation, LINE-1 methylation, *GSTP* promoter region methylation	Rat, Sprague-Dawley	Liver of offspring, on PND21	Global DNA methylation –, LINE-1 methylation –, *GSTP* promoter region methylation changes +	PFOS, 0.1, 0.6, or 2.0 mg/kg bw per day	Oral gavage to dams from GD2 to GD21	Prenatal treatment	Wan et al. (2010)
Histone acetylation	Mouse, Kunming	Liver of female offspring on PND21	PFOA: ↓ histone acetylation, ↓ HAT activity, ↑ HDAC activity	PFOA, 1, 2.5, 5, or 10 mg/kg/bw (0.2 mL per day) to dams	Gavage, solution in deionized water, dams, GD1 to GD17	Prenatal treatment; liver specimens collected from female offspring killed on PND21	Li et al. (2019a)
Histone acetylation	Rat, Wistar	Testis	PFOS: H3K9me2 +, H3K9ac +, H3K18ac +, H3K9me3 –	PFOS, 0.015 and 0.15 mg/kg per day	Oral gavage, 60 d		Alam et al. (2021)
Histone acetylation	Mouse, ICR	Ovary	PFOS: histone H3K14 acetylation of *StAR* promoter –	PFOS, 0.1 mg/kg per day	Drinking-water, 4 mo		Feng et al. (2015)
Targeted DNA methylation, targeted gene expression	Mouse, CD-1	Kidney	879 differentially methylated regions; ↑ *Dnmt1* expression, ↓ *Dnmt3a* expression, ↑ *Dnmt3b* expression, ↓ *Tet1* expression, *Tet2* expression +/–, ↑ *Tet3* expression; global DNA methylation +/–; ↑ Hdac1, Hdac3, Hdac4, Hdac 2–10 +/–; ↓ *RASAL1* mRNA expression, ↑ *Acta2* mRNA expression, *Lrnf2* and *Dlg2* mRNA expression +/–, ↑ *Tgfb* mRNA expression	PFOA, 1, 5, 10, or 20 mg/kg per day	Oral, 10 d		Rashid et al. (2020a)

Table 4.13 (continued)

End-point	Species, strain	Tissue	Result[a]	Concentrations or dosing regimen	Route, duration	Comments	Reference
Targeted gene and miRNA expression (angiogenesis-related mRNA, miRNA, and lncRNA)	Pregnant mouse, CD-1	Placenta of dams	↓ lncRNA Xist expression	PFOS, 0.5, 2.5, 12.5 mg/kg bw per day	Oral gavage, GD1 to GD17	Analysis performed on GD18	Chen et al. (2018)
Targeted gene expression (*Dlk1–Dio3* imprinted cluster)	Mice, Kunming	Testes of offspring	↓ *Dlk1–Dio3* on PND21	PFOA, 1, 2.5, or 5 mg/kg per day	Gavage during gestation, GD1 to GD17	Prenatal treatment	Song et al. (2018)
Targeted gene expression (Dnmts and Tets); targeted CpG methylation	Mouse, CD-1	Lung tissue	PFOA: ↓ Dnmts and ↓ Tets expression: – CpG *Tmprss2*	PFOA, 5 or 20 mg/kg per day	Oral gavage, 10 d		Ahmad et al. (2021)
Targeted gene expression (epigenetic machinery)	Mouse, CD-1	Small intestine, colon	Small intestine: mRNA: ↓ *Dnmt1*, ↓ *Dnmt3a* (↑↓), ↓ *Dnmt3b*; ↑ *Tet1*, ↑ *Tet2*, ↓ *Tet3*, ↓ *Cldn2*, ↓ *Cldn8*, ↓ *Cldn12*, ↑ *Cld4*, *Cldn3* (↑ and ↓), ↑ *Cldn15*, *Cldn7* (↑↓), *Tjp1* (↑↓), *Tjp2* (↓↑), *Ocln* +/– Colon: mRNA: ↓ *Dnmt3b*, ↓*Dnmt3a*, *Dnmt1* +/–; ↓ *Tet1*, *Tet2* +/–, *Tet3* +/–, ↑ *Cldn2*, ↑ *Cldn3*, ↑ *Cldn8*, ↓ *Cldn7*, *Cldn4* +/–, *Cldn12* +/–, *Cldn15* +/–, ↓ *Tjp1*, ↓ *Ocln*, *Tjp2* +/–	PFOA, 1, 5, 10, or 20 mg/kg per day	Oral gavage, 10 d		Rashid et al. (2020b)
Targeted gene expression (H19)	Pregnant mouse, CD-1	Placenta of dams	↑ *H19* expression, H19 methylation –	PFOS, 0, 0.5, 2.5, or 12.5 mg/kg per day	Oral, GD1 to GD17	Analysis performed on GD18	Li et al. (2020b)
Targeted miRNA and gene expression (miR-770 expression; *Meg3* expression)	Pregnant mouse, CD-1	Placentas of dams	PFOS: ↓ miR-770 expression, ↓ *Meg3* expression, *Meg3* methylation +, ↑ *Ptx3* expression	PFOS, 0.5, 2.5, or 12.5 mg/kg per day	Gavage, GD0 to GD17	Analysis performed on GD18	Li et al. (2022c)
Targeted miRNA expression	Rat, Wistar	Brain	↓ miR-466b, ↓ miR-672, ↓ miR-297	PFOS, 0–3.2 mg/kg per day	Feed, GD1 to PND7		Wang et al. (2012b)

Table 4.13 (continued)

End-point	Species, strain	Tissue	Result[a]	Concentrations or dosing regimen	Route, duration	Comments	Reference
Targeted miRNA expression	Chicken, Plymouth Rock	Heart	PFOA: ↑ miR-490-5p	PFOA, 0–2 mg/kg per day	Cell injection, ED0 to ED21		Guo et al. (2022a)
Targeted miRNA expression	Mouse, BALB/c	Testes	9 ↓miRNAs, 8 ↑ miRNAs, including miR-133b-3p	PFOA, 5 mg/kg per day	Oral gavage, 28 d		Lu et al. (2017)
Targeted miRNA expression	Mice, BALB/c	Serum	miR-28-5p, miR-32-5p, miR-122-5p, miR-192-5p, and miR-26b-5p (all ↑)	PFOA, 1.25 or 5 mg/kg per day	Oral gavage, 28 d		Yan et al. (2014)
Targeted MiRNA expression	Mice, ICR	Sertoli cells and Leydig cells	↑ miR-9-3p, ↑↓ miR-1954, ↑↓ miR-710	PFOS, (0.5–10 mg/kg per day)	Oral gavage, 4 wk		Huang et al. (2022a)
Targeted miRNA expression (387)	Rat, Wistar	Liver	Significantly altered miRNAs included ↑ miR-19b, miR-21*, miR-17-3p, miR-125a-3p, miR-16, miR-26a, miR-1, miR-200c, and miR-451. PND1: 35 miRNAs ↑, 11 ↓ miRNAs; PND7: 8 ↑ miRNAs, 1 ↓ miRNA; 4 miRNAs on both PND1 and PND7	PFOS, 3.2 mg/kg per day	Feed (dam), GD1 to PND7 Pups, until PND7		Wang et al. (2015b)
Targeted miRNA expression (miR-34a)	Mice, BALB/c	Liver	miR-34a ↑	PFOA, 5 mg/kg per day	Gavage, 28 d		Cui et al. (2019)
Targeted gene expression, histone modifications	Rat, Wistar	Testes	PFOA: *Lhr* ↑, *Star*↑, *Hsd3b* ↓, *Hsd17b* ↓, *Arom* ↓, *Cyp11a1* +/–, *Cyp17a1* +/–; histone modification: ↓ H3K9me1, H3K9me2, H3K9me3, H3K9ac, H3K18me1, H3K18ac, H3K23me1, and H3K23ac	PFOA, 0.015 or 0.15 mg/kg per day	Gavage, 60 d		Han et al. (2022)

bw, body weight; d, day(s); CpG, cytosine–guanosine dinucleotide; DMP, differentially methylated position; ED, embryonic day; GD, gestational day; HAT, histone acetyltransferase; HDAC, histone deacetylase; HIC, highest ineffective concentration; lncRNA, long non-coding RNA; LEC, lowest effective concentration; LINE-1, long interspersed nuclear element-1; mo, month(s); miRNA, microRNA; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PND, postnatal day; wk, week(s).

[a] +, increased methylation; –, decreased methylation; +/–, increase or decrease in methylation; ↑, increased gene expression; ↓, decreased gene expression; ↑↓, increased and decreased gene expression.

Targeted gene expression analyses have shown that PFOA exposure was associated with an increase in *DNMT* expression in the mouse kidney (Rashid et al., 2020a). PFOA was associated with decreases in the expression of *Dnmt1*, *Dnmt3a*, and *Dnmt3b* in the intestines of CD-1 mice exposed to PFOA at 1, 5, 10, or 20 mg/kg per day (Rashid et al., 2020b). Targeted gene expression analysis has shown that *Dlk1 and Dio3* have decreased expression in the testes of Kunming offspring mice exposed prenatally to PFOA at doses of 1, 2.5 or 5 mg/kg during gestation (Song et al., 2018).

In relation to miRNAs, in male BALB/c mice exposed to PFOA at 0.08, 0.31, 1.25, 5 or 20 mg/kg per day for 28 days, increases in the expression of miR-28-5p, miR-32-5p, miR-122-5p, miR-192-5p, and miR-26b-5p were identified in mouse serum at the doses of 1.25 and 5 mg/kg (Yan et al., 2014).

Developmental exposure of fertile hatchling chicken eggs (incubated to hatch) at PFOA doses of 0.5, 1, or 2 mg/kg per egg weight was also associated with increased expression of miR-490-5p in heart tissues compared with the vehicle control group (Guo et al., 2022a).

Exposure to PFOS

Wen et al. (2022) exposed CD-1 mice to PFOS at doses of 5, 10, or 20 mg/kg per day for 14 days and found decreases in global DNA methylation levels in the kidney.

Wan et al. (2010) exposed Sprague-Dawley rats to PFOS at doses of 0.1, 0.6, or 2.0 mg/kg per day from GD2 to GD21. PFOS was found to accumulate in the kidney in a dose-dependent manner and was associated with increased expression of the kidney injury markers *Acta2* and *Bcl2l1*. In addition, PFOS was found to be associated with decreased global DNA methylation and decreased LINE-1 methylation in the livers of the offspring (Wan et al., 2010). These authors also found that PFOS was associated with increased methylation the *Gstp* promoter region in the livers (Wan et al., 2010). [The Working Group noted the relevance of this finding, because it represents DNA methylation in a region of the *Gstp* gene, which is a member of the glutathione *S*-transferase (GST) gene family, involved in carcinogenesis.]

In relation to targeted gene expression, Wen et al. (2022) exposed CD-1 mice to PFOS at doses ranging from 5–20 mg/kg per day for 14 days. PFOS was associated with increased expression of the histone demethylases *Kdm1a* and *Kdm4c* in the kidney (Wen et al., 2022).

In relation to miRNAs, Wang et al. (2012b) observed decreased expression of miR-466b, miR-672, and miR-297 in the brains of neonatal albino Wistar rats on postnatal day (PND) 1 and PND7 that were born from mothers fed with PFOS at a dose of 3.2 mg/kg per day from GD1 to PND7, compared with neonatal brain tissue derived from mothers treated with vehicle (Wang et al., 2012b).

Li et al. (2022c) observed an inverse association between PFOS, at the highest dose, and miR-770 expression in the placenta sampled on GD18 from CD-1 pregnant mice treated with PFOS at doses ranging from 0.5 to 12.5 mg/kg per day by gavage from GD0 to GD17. Similarly, the expression of *MEG3*, a cancer suppressor gene, was significantly decreased in the placenta, and there was hypermethylation in a CpG site in its promoter region (Li et al., 2022c).

Wang et al. (2015b) fed pregnant albino Wistar rats with chow containing PFOS at a dose of 3.2 mg/kg per day from PND1 to PND7 and reported that 35 miRNAs were highly expressed on PND1, eight miRNAs were highly expressed on PND 7, and four miRNAs (miR-125a-3p, miR-23a*, miR-25*, and miR-494) were significantly expressed on both PND1 and PND7.

Related to histone modifications, PFOS was found to be associated with increased H3K9me2, H3K9ac, and H3K18ac, and decreased H3K9me3, in the testes of male Wistar rats treated with PFOS at a dose of 0.015 or 0.15 mg/kg per day for 60 days (Alam et al., 2021).

PFOS was also associated with decreases in histone acetylation of the StAR promoter in the ovaries of ICR mice exposed to PFOS at a dose of 0.1 mg/kg per day for 4 months (Feng et al., 2015).

PFOS was associated with decreases, observed on GD18, in the expression of lncRNA Xist in the placentas of CD-1 mouse dams exposed to PFOS at doses of 0.5, 2.5, or 12.5 mg/kg from GD1 to GD17 (Chen et al., 2018) (see Table 4.13).

(ii) Non-human mammalian systems in vitro

See Table 4.14.

In vitro testing has been carried out in cells that represent the mouse brain (mHypoE-N46), mouse liver, embryonic stem cells, and macrophages; as well as in rat mitochondria and kidneys. In addition, zebrafish embryos and bovine tissues have been assessed.

Exposure to PFOA

Kim et al. (2021) examined the effects of PFOA exposure at concentrations of 0.25–250 μmol/L on the embryonic hypothalamic cell line N46 (mHypoE-N46). PFOA was found to increase global DNA methylation.

Mouse fibroblast preadipocytes (3T3-L1) exposed to PFOA at concentrations of 0.01–100 μg/mL showed decreases in global DNA methylation compared with control (Ma et al., 2018).

No apparent DNA methylation was observed in a study by Starkov and Wallace performed in mitochondria isolated from the livers of Sprague-Dawley rats and exposed to 100 μM PFOA (Starkov and Wallace, 2002), or in the kidneys of Balb/c mice treated with PFOS at 0.1 or 1 mg/kg intraperitoneally every other day for 3 months, and rat renal tubular epithelial NRK-52E cells treated with PFOS (0–500 nM) for 6, 12 or 24 hours (Chou et al., 2017). However, Chou et al. (2017) observed increases in the upregulation of Sirt1, and in the deacetylation of peroxisome proliferator-activated receptor gamma (PPARγ) that mediated epithelial–mesenchymal transition-associated renal fibrosis.

Exposure to PFOS

PFOS exposure was associated with increased expression of miR-9-3p in Sertoli cells and Leydig cells of male ICR mice treated orally with PFOS at doses ranging between 0.5 and 10 mg/kg bw per day for 4 weeks. PFOS exposure was not associated with changes in miR-1954 or miR-710 in Sertoli cells (Huang et al., 2022a).

Blanc et al. (2019) exposed zebrafish embryos and liver (ZF-L) cells to PFOS at a concentration equivalent to its EC_{10} (93 μM) for 96 hours and showed an increase in global DNA methylation of 13% versus control. Hallberg et al. exposed bovine oocytes complexes to PFOS at concentrations of 2 ng/g or 53 ng/g for 22 hours during their maturation and found both increases and decreases in target DNA methylation on day 8 after fertilization (Hallberg et al., 2021). The authors identified that the most altered pathways were those involved in cell death and survival, with the p53 pathway the most altered (see Table 4.15).

Synopsis

[The Working Group noted that numerous studies investigating epigenetic alterations in humans exposed to PFOA and PFOS were in cohorts with background exposures representative of the general population. Many of these studies were prospective birth cohort studies that evaluated the relation between maternal/in utero exposure and epigenetic alterations in the neonate. These studies are of great importance, because they investigate the potential for developmental reprogramming that may influence cancer susceptibility. Numerous studies in exposed humans have identified associations between exposure to PFOA or PFOS and altered DNA methylation in cancer-associated genes. For PFOA and PFOS, the specific gene targets identified in these studies differed. The Working

Table 4.14 End-points relevant to epigenetic alterations in non-human mammalian systems in vitro exposed to PFOA or PFOS

End-point	Species	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
Global DNA methylation	Mouse	Brain, embryonic hypothalamic cell line N46 (mHypoE-N46)	Global DNA methylation +	PFOA, ranging study of 0–250 µmol/L for 24 h or 48 h; cells were then exposed to PFOA at EC_{50} = 27.5 µmol/L, for 24 h		Kim et al. (2021)
Global DNA methylation; DNA methyltransferase gene expression	Mouse	Fibroblasts, preadipocytes (3T3-L1)	Global DNA methylation –; DNA methyltransferase genes +	PFOA, 0.01–100 µg/mL for 4–8 days		Ma et al. (2018)
Permeability of mitochondrial membranes	Rat	Liver, mitochondria	+	PFOA, 100 µM; PFOS: 10 µM; concomitant measurements	No apparent DNA methylation or miRNA	Starkov and Wallace (2002)
Targeted DNA methylation	Cow	Egg, bovine cumulus oocyte	PFOS +/–; gene-dependent	PFOS, 2 or 53 ng/g for 22 h		Hallberg et al. (2021)
Targeted gene expression	Rat	Kidney, renal tubular epithelial cells (NRK-52E)	+ expression of EMT and renal injury biomarkers (e.g. *N*-cadherin, vimentin, *Snai1*, *Kim1*, and *Lcn2*); – expression of *Tjp1*; + expression of *Sirt1*	PFOA, 0–500 nM for 24 h	No apparent DNA methylation or miRNA	Chou et al. (2017)
Targeted gene expression (Sirt1–7)	Mouse	Macrophages (RAW 264.7 cells)	SIRT1–7 mRNA: PFOS +/–; PFOA +/–	PFOS or PFOA, 0, 0.5, 5, or 50 µM for 24 h		Park et al. (2019)
Targeted gene expression, histone modifications; global DNA methylation	Zebrafish	Embryo, liver, ZFL cells	Global DNA methylation: PFOS +	PFOS at EC_{10} = 93 µM, for 48 h		Blanc et al. (2019)
Targeted miRNA expression (miR-145 and miR-490-3p) and mRNA and protein expression	Mouse	Embryonic stem cells	PFOS: miR-145 expression +, miR-490-3p expression +, *Sox2* mRNA –, Sox2 protein –, *Nanog* mRNA –, Nanog protein –, *Oct4* mRNA +/–, Oct4 protein +/–, *Chrm2* expression +	PFOS, 0.2, 2, 20, or 200 µM for 24 h		Xu et al. (2013b)

Table 4.14 (continued)

End-point	Species	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
Targeted miRNA expression (miR-134, miR-145, miR-490-3p)	Mouse	Embryonic stem cells, D3	miR-134 ↓, miR-145 ↓, miR-490-3p ↓	PFOS, 0.2, 2, 20, or 200 µM; medium changed on days 0, 2, and 4; measurement on day 6		Xu et al. (2015)

EMT, epithelial–mesenchymal transition; h, hour(s); mRNA, messenger RNA; miRNA, microRNA; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.

[a] +, increased methylation; −, decreased methylation; +/−, increase or decrease in methylation; ↑, increased gene expression; ↓, decreased gene expression; ↑↓, increased and decreased gene expression.

Table 4.15 End-points relevant to oxidative stress in humans exposed to PFOA or PFOS

End-point Assay	Biosample type	Location, setting Study design	Exposure level No. of participants	Response[a]	Covariates adjusted for	Comments	Reference
MDA, by TBARS 8-OHdG, by HPLC-MS/MS	Urine	Seoul, Republic of Korea Placebo-controlled crossover trial	PFOS, 10.04 ng/mL; PFOA, 4.61 ng/mL; 141 participants, age > 60 years	↑ MDA and 8-OHdG in a dose-dependent response with PFOS, but not PFOA	Age, sex, BMI, cotinine level, PM_{10}, outdoor temperature, dew point, treatment arm, and treatment	Vitamin C did not significantly reduce MDA or 8-OHdG in the PFOA or PFOS group	Kim et al. (2016b)
8-oxodG, 15-F2t-isoP, by ELISA	Urine, plasma	Ceske Budejovice, Prague, and Ostrava, Czechia Cross-sectional study	PFOA means: 1.05 (Ceske), 0.96 (Prague), 0.98 (Ostrava) ng/mL; PFOS means: 3.49 (Ceske), 3.23 (Prague), 3.35 (Ostrava) ng/mL in plasma 126 healthy non-smoking policemen; mean age, 38–40 years	↑ 8-OHdG and ↓ 15-F2t-isoP. Statistically significant ($P < 0.05$) association only between PFOS and 8-OHdG	Sampling period and locality, non-smoking policemen	Plasma PFOS and PFOA concentrations did not differ between the three different areas	Ambroz et al. (2022)
8-OHdG 8-Nitrosoguanine by HPLC-ESI-MS/MS	Urine	Taipei, Taiwan, China Cross-sectional study	*n*-PFOA, 3.77 ng/mL; branched PFOA, 0.08 ng/mL; *n*-PFOS, 12.92 ng/mL; branched PFOS, 0.44 ng/mL 597 participants (519 men and 78 women, aged 22–63 years; mean, 45.8 years)	↑ in a dose-dependent manner across the four quartiles of linear PFOS, but not PFOA Positive association with linear PFOS using 3 models	Model 1 was adjusted for age and sex; Model 2 was adjusted for the Model 1 parameters plus smoking status, alcohol intake, education level, BMI, hypertension and diabetes mellitus; Model 3 was adjusted for the Model 2 parameters plus LDL-C and urinary creatinine as covariates (multiple linear regression)	Recruited participants were controls and patients with acute coronary heart disease from another study; therefore, few women were enrolled in this study Both branched and linear PFOS and PFOA were measured in serum	Lin et al. (2020b)

Table 4.15 (continued)

End-point Assay	Biosample type	Location, setting Study design	Exposure level No. of participants	Response[a]	Covariates adjusted for	Comments	Reference
8-OHdG, by LC-MS/MS	Urine	Taiwan, China Cross-sectional study	Geometric mean and 95% CI, 3.21 (3.00–3.46) ng/mL for PFOA, 6.44 (6.05–6.89) ng/mL for PFOS 848 participants (331 men and 517 women, aged 12–30 years	No association with PFOA or PFOS using Models 1 and 2; Model 1 was adjusted for age and sex; Model 2 was adjusted for age, sex, and other risk factors, such as smoking status, BMI, systolic blood pressure, LDL-C, HDL-C, and hs-CRP	Age, sex, systolic blood pressure, LDL-C, HDL-C, insulin resistance, serum hs-CRP, history of medication, income, smoking, alcohol consumption, BMI, hypertension, diabetes mellitus	Sufficient sample size; serum PFC and microplastics were analysed	Lin et al. (2016)
8-OHdG, by ELISA	Urine	Flanders, Belgium	PFOA, 2.55 µg/L; 596 adolescents (324 males and 282 females, aged 14–15 years)	Serum PFOA concentration (*n* = 197) weakly associated (not statistically significant) with 8-OHdG (*n* = 195), and associated with increased DNA damage (alkaline comet assay, *n* = 598)	Sex, age, BMI, smoking habits, alcohol consumption, education level, season of sample collection	Study limitation: use of ELISA method; the presence of contaminants, including lead, chromium, cadmium, arsenic, methyl mercury, and PAH complicated the interpretation	Franken et al. (2017)
Bilirubin (an antioxidant) Albumin (extracellular antioxidant) NHANES 2007–2008 Laboratory Data Overview (National Center for Health Statistics, 2023)	Serum	USA (NHANES 2005–2012) Cross-sectional study	Serum 95th percentile of PFOA, 8.90 (max. 104.0) ng/mL; PFOS, 49.40 (max. 281.0) ng/mL 6652 participants (3246 men, 3406 women, aged 49.48 ± 18.07 years)	Positive associations with PFOA and PFOS; dose-dependent	Age, sex, education, ethnicity, income level, cotinine, and BMI	Large sample size. However, the classical and most informative oxidative end-points were not measured. These two end-points are not considered to be specific to oxidative stress	Omoike et al. (2021)

Table 4.15 (continued)

End-point Assay	Biosample type	Location, setting Study design	Exposure level No. of participants	Response[a]	Covariates adjusted for	Comments	Reference
α-klotho antioxidant, by ELISA	Serum	USA (NHANES 2007–2016) Cross-sectional study	3981 participants (1940 men, 2041 women, aged 40–79 years)	Positive associations of PFOA and PFOS with α-klotho in participants with GF-3B/4 stage, without albuminuria. Inverse associations between PFOS and α-klotho, with healthy kidney	Age, sex, ethnicity, obesity status, hypertension, diabetes, smoking, anaemia, alcohol consumption, glomerular filtration stage, and albuminuria	Large sample size. However, the classical oxidative biomarkers were not measured	Jain and Ducatman (2022)
Metabolome biomarkers of oxidative/ nitrosative stress: hydroxybutyric acid, pyroglutamic acid, oxoglutaric acid, D-glucurono-6,3-lactone, deoxyarabinohexonic acid, tetrahydrobiopterin, α-carboxyethyl hydroxychromanol, and arachidonic acid, by LC/orbitrap-MS	Serum	China	Median concentration: PFOA, 7.56 nM; PFOS, 12.78 nM 181 male participants, aged 22–48 years	PFOA and PFOS were associated with direct or indirect biomarkers of oxidative/nitrosative stress	Age, BMI, smoking, alcohol consumption	Small sample of men in a single region with incomplete demographic data; a metabolomic approach, involving the measurement of biomarkers directly or indirectly involved in the oxidative/nitrosative pathways using a state-of-the-art instrument. The metabolism biomarkers were not specific for oxidative/nitrosative stress	Wang et al. (2017)

Table 4.15 (continued)

End-point Assay	Biosample type	Location, setting Study design	Exposure level No. of participants	Response[a]	Covariates adjusted for	Comments	Reference
ROS, by OxiSelect ROS/RNS assay kit	Umbilical cord plasma	Shanghai, China Prospective study	PFOA: girls, 0.70–29.97 ng/mL; boys, < LOD to 25.99 ng/mL PFOS: girls, 0.39–18.68 ng/mL; boys,0.62–65.61 ng/mL Newborns (299 boys, 282 girls)	Positive association with PFOS in female newborns; however, in male newborns, there were no relations with PFOA or PFOS	Maternal and paternal age, maternal education, maternal pre-pregnancy BMI, mode of delivery, gestational age at birth, infant sex, infant birth weight, and antepartum obstetric risk	It was impossible to distinguish the separate effects of PFOA and PFOS	Liu et al. (2018b)
8-isoprostane-PGF2α, PGF2α, 2,3-dinor-8-iso-PGF2α, and 2,3-dinor-5,6-dihydro-8-iso-PGF2α, by LC-MS/MS	Urine	Illinois, USA Prospective birth cohorts	Geometric means: PFOA, 0.75 ng/mL; PFOS, 2.03 ng/mL 428 pregnant (15-week) mothers, aged 18–40 years	Association with PFOS but not PFOA	Sociodemographic backgrounds and geographical locations; clinical characteristic and trimesters	Relatively small sample size	Taibl et al. (2022)
HO-1, by ELISA kit	Plasma	Shanxi, China Case–control study	PFOS median, 1.79 ng/mL; PFOA median, 0.79 ng/mL 144 spontaneous preterm births, and 375 full-term deliveries as controls	No association with HO-1 observed	Demographic characteristics	A nested case–control study, minimizing selection and recall bias; however, subgroups were small	Liu et al. (2020a)

BMI, body mass index; h, hour(s); CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionization; GF-3B/4 stage, glomerular function stage of kidney disease; HDL-C, high-density lipoprotein-cholesterol; HO-1, haem oxygenase 1; HPLC, high-performance liquid chromatography; hs-CRP, high-sensitivity C-reactive protein; LC, liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LDL-C, low-density lipoprotein-cholesterol; LOD, limit of detection; LTL, leukocyte telomere length; MDA, malonaldehyde; NHANES, National Health and Nutrition Examination Survey; *n*-, linear isomer; 8-NO2Gua, 8-nitrosoguanine; 8-OHdG, 8-hydroxydeoxyguanosine; 8-oxodG, 8-oxo-2′-deoxyguanosine; PAH, polycyclic aromatic hydrocarbon; PFC, perfluorochemicals; PFOA, perfluorooctanoic acid; PFDA, perfluorodecanoic acid; PFNA, perfluorononanoic acid; PFOS, perfluorooctanesulfonic acid; PG, prostaglandin; PM_{10}, particulate matter of < 10 µm in diameter; RNS, reactive nitrogen species; ROS, reactive oxygen species; SD, standard deviation; TBARS, thiobarbituric acid-reactive substance; USA, United States of America; yr, year(s).

[a] ↓, decrease; ↑, increase.

Group analysed the strengths and limitations of individual studies and considered the relevance of the KC-associated end-points across systems. The Working Group noted that the significant findings in humans are unlikely to be attributable to chance. The Working Group noted that the data in experimental systems corroborate the evidence observed in exposed humans. Specifically, in vivo and in vitro studies using rodents and human- and rodent-derived cells suggest that PFOA and PFOS alter DNA methylation, LINE methylation, histone modifications, and miRNA expression.]

4.2.5 Induces oxidative stress

(a) Humans

See Table 4.15.

(i) Exposed humans

Eleven studies on PFOA and PFOS (or both) relevant to exposed humans were identified in which oxidative stress-related end-points were measured.

Kim et al. (2016b) conducted a double-blind, randomized, placebo-controlled crossover trial to study the effect of vitamin C on PFOA and PFOS-induced insulin resistance in 141 healthy elderly participants aged > 60 years from the Korean Elderly Environmental Panel (KEEP) study in Seoul, Republic of Korea. Serum PFOA and PFOS concentrations were 4.61 ± 1.86 ng/mL (11.1 ± 4.5 µM) and 10.04 ± 4.12 ng/mL (20.1 ± 8.2 µM), respectively. PFOS, but not PFOA, was positively associated with increased urinary end-points of oxidative stress malondialdehyde (MDA, $P = 0.02$) and 8-hydroxy-2′-deoxyguanosine (8-OHdG, $P = 0.001$) levels. Vitamin C treatment (1000 mg/day for 4 weeks) did not significantly alter MDA or 8-OHdG levels induced by PFOA or PFOS. The measurement of both end-points included the use of radiolabelled internal standards. MDA was measured using the thiobarbituric acid-reactive substance (TBARS) assay. The urinary 8-OHdG concentration was measured using HPLC-tandem mass spectrometry (MS/MS). [The Working Group noted that the TBARS method was not specific for malonaldehyde but, nevertheless, the MDA results complemented those for 8-OHdG.]

In a recent cross-sectional study (Ambroz et al., 2022), 126 healthy, non-smoking adult policemen from three areas of Czechia were sampled for their plasma PFOA, PFOS, and 15-F_{2t}-isoprostane (IsoP), and urinary 8-oxo-2′-deoxyguanosine (8-oxodG). 15-F_{2t}-IsoP, and urinary 8-oxodG were measured using enzyme-linked immunosorbent assay (ELISA) kits. The PFOA and PFOS concentrations correlated with elevated urinary 8-oxodG levels and reduced plasma 15-F_{2t}-IsoP, but only the association between PFOS and 8-OHdG was statistically significant.

A cross-sectional study (Lin et al., 2020b) was conducted in 597 adult (519 men and 78 women) from a middle-aged cohort (mean age, 45.8 years) from the National Taiwan University Hospital, China, to assess the associations of serum isomers of PFOA and PFOS (branched and linear) with urinary 8-OHdG and 8-nitroguanine (8-NO_2Gua). The levels of urinary 8-OHdG and 8-NO_2Gua were measured using HPLC-electrospray ionization (ESI)-MS/MS with satisfactory accuracy. Branched PFOA and PFOS were 2.1% and 3.2% of the total PFOA and PFOS concentrations, respectively. The geometric means of urinary 8-OHdG and of 8-NO_2Gua significantly increased across the four quartiles (< 8.39 and > 22.3 ng/mL for *n*-PFOS; from 6.82 to 8.65 µg/mL for 8-OHdG, P for trend = 0.016; and from 0.78 to 1.21 µg/mL for 8-NO_2Gua; P for trend, 0.041) in multiple linear regression analysis, after controlling for potential confounders; however, this was not true for PFOA. The results indicate that *n*-PFOS in serum was significantly associated with urine oxidative/nitrative stress end-points in a relatively large cohort in Taiwan.

Lin et al. (2016) investigated the relation between serum PFOA and PFOS and urinary 8-OHdG in 848 participants aged 12–30 years (331 men and 517 women) in a cross-sectional study in Taiwan, China. Urinary 8-OHdG concentrations were measured using liquid chromatography (LC)-MS/MS, with the inclusion of a suitable internal standard ($^{15}N_5$8-OHdG). The geometric means of serum PFOA and PFOS were 3.21 ng/mL (95% CI, 3.00–3.46 ng/mL) and 6.44 ng/mL (95% CI, 6.05–6.89 ng/mL) (i.e. 7.8, 7.2–8.4 μM and 12.9, 12.1–13.8 μM), respectively. There were no associations of serum PFOA and PFOS concentrations with 8-OHdG levels in the urine. [The Working Group noted that the cohort was younger, and the exposure level was lower than those in the most 8-oxodG studies in humans exposed to PFOS.]

In a cross-sectional study of a cohort of approximately 600 adolescents (both males and females aged 14–15 years) from the Flanders region of Belgium, urinary 8-OHdG as a measure of DNA damage was assessed by ELISA in 596 adolescents (see also Section 4.2.4) (Franken et al., 2017). In addition, damage to DNA was assessed with alkaline comet assay, and specifically, oxidative damage to DNA was also assessed with the Fpg-modified comet assay in a subpopulation of the cohort. Increased serum PFOA levels were associated with a 9% increase in DNA damage, as measured by the alkaline comet assay (95% CI, 1.5–17.0%; $n = 196$); however, in 195 participants the PFOA level was only weakly and not significantly associated with increased 8-OHdG.

In another cross-sectional study, Omoike et al. (2021) analysed data from the National Health and Nutrition Examination Survey (NHANES) cohort (2005–2012; $n = 6652$, 3246 men and 3406 women; age, 49.40 ± 18.07 years) that included the measurement of serum antioxidants bilirubin and albumin (extracellular antioxidants) as indicators of oxidative stress. The 95th percentile values for PFOA and PFOS were 8.90 (maximum, 104) ng/mL (i.e. 21.5, max. 251.2 μM) and 42.70 (max. 281) ng/mL (i.e. 85.4, max, 561.9 μM), respectively. PFOA and PFOS were found to be positively associated with bilirubin and albumin levels. [The Working Group noted that the actual serum levels of bilirubin and albumin were not reported in this study. Also, although the study included a substantial number of participants, the most informative oxidative stress-related end-points were not assessed; those measured were not specific for oxidative stress.]

Another NHANES cross-sectional study (Jain and Ducatman, 2022) analysed data for 3981 US adults (aged 40–79 years), 3461 with and 530 without albuminuria. The study authors hypothesized that PFAS may adversely affect the antioxidant response of the normal kidney. Serum PFAS, including PFOA and PFOS, and α-klotho, an anti-ageing protein that plays a key role in the production of antioxidant enzymes in the kidney, were measured. A positive association between PFOA or PFOS and α-klotho was observed in participants without albuminuria and kidney function in glomerular function (GF) stage 3B/4 ($15 \leq \text{eGFR} < 45$ mL/min/1.73 m^2). In stage GF-1 ($\text{eGFR} \geq 90$ mL/min/1.73 m^2), an inverse association between PFOS and α-klotho was observed in individuals without albuminuria (Jain and Ducatman, 2022).

Wang et al. (2017) studied serum PFAS, including PFOA and PFOS, and serum metabolome markers related to oxidative/nitrosative stress in 181 Chinese men (aged 22–48 years; mean ± SD, 33.2 ± 6.4 years). Metabolism end-points included hydroxybutyric acid, pyroglutamic acid, oxoglutaric acid, D-glucurono-6,3-lactone, deoxyarabinohexonic acid, tetrahydrobiopterin, α-carboxyethyl hydroxychromanol, and arachidonic acid, and were measured using LC-Orbitrap-MS. The metabolism markers directly or indirectly correlated with lipid oxidation. Associations between PFOA or PFOS and oxidative/nitrosative stress-related end-points were observed. The authors suggested that low environmental levels of PFAS, including PFOA

and PFOS, may increase the early risk of metabolic diseases, including diabetes and cardiovascular diseases. [The Working Group noted that this study included a small male cohort from a single region with incomplete demographics, which complicates the interpretation of the observations. It was noted that the metabolic markers measured in the study might not all be specific for oxidative/nitrosative stress. However, they are involved and/or result from an oxidative stress process.]

Liu et al. (2018b) studied umbilical cord plasma PFAS, including PFOA and PFOS, and ROS and fetal leukocyte telomere length (LTL) in 299 newborn boys and 282 newborn girls from a prospective cohort in Shanghai, China. The ROS/reactive nitrogen species (RNS) ratios in the cord plasma were measured using a commercial kit. Shorter LTL and high levels of ROS were observed in female newborns, and these were associated with PFOS. No association with PFOA or PFOS was observed in male newborns. [The Working Group noted that in this study the umbilical cord plasma PFOA level was higher than that for PFOS, in contrast to what is normally observed in adults. It was impossible to distinguish the effect of individual PFOA and PFOS exposure, because they are strongly positively correlated, as acknowledged by the authors.]

Taibl et al. (2022) tested the hypothesis that excess ROS might be a contributor to preterm birth (at less than 15 weeks of gestation) in 428 pregnant mothers (aged 18–40 years), not pregnant with multiple fetuses, from the Illinois Kids Development Study (IKIDS) and Chemicals in Our Bodies (CIOB) prospective birth cohorts between 2014 and 2019 in the USA. Serum PFAS levels (second trimester) and urinary levels of end-points of oxidative stress (second and third trimesters), including prostaglandin-F2α (PGF2α), 8-isoprostane-prostaglandin F2α (8-iso-PGF2α), 2,3-dinor-8-iso-PGF2α, and 2,3-dinor-5,6-dihydro-8-iso-PGF2α were measured using LC-MS/MS. The 95th percentiles and geometric means ± SD of PFOA and PFOS were 2.17 (0.75 ± 2.14) ng/mL (5.2, 1.7 ± 5.2 μM) and 7.01 (2.03 ± 2.47) ng/mL (14, 4.1 ± 4.9 μM), respectively. PFOS was found to have a modest positive association with increases in the oxidative end-points measured.

Liu et al. (2020a) conducted a nested case–control study in Shanxi, China, that included 144 women who underwent spontaneous preterm birth and 375 who underwent full-term delivery as controls. Among the 17 PFAS measured in the maternal plasma, the median values of PFOA and PFOS were 0.79 ng/mL (1.9 μM) and 1.79 ng/mL (3.6 μM), respectively. No significant differences in haem oxygenase 1 (HO-1) in the maternal plasma were observed between the spontaneous preterm birth group and the controls, with no association between HO-1 level and PFOA or PFOS. [The Working Group noted that the nested case–control study was designed to minimize selection and recall bias. However, the small sample size was considered a limitation.]

Of the eleven human studies evaluated, six were in Asian countries including China (including Taiwan) and the Republic of Korea; three studies were in the USA; one in Belgium; and one in Czechia. Among the studies, eight demonstrated a positive association between PFOS exposure and oxidative stress, and four showed that PFOA might cause oxidative stress in humans. Five studies included the measurement of the 8-OHdG or 8-oxodG form of the oxidative product, a relevant end-point of oxidative DNA damage. Three of the five studies reported a positive association for PFOS, and only one a positive association for PFOA, with 8-OHdG.

Among the studies that reported a positive association for PFOS, only two measured 8-oxodG with a highly specific analytical method. The evidence for PFOA is weak compared with that for PFOS. It was also noted that the serum/plasma PFOS concentrations were generally higher than the PFOA concentrations, except

in the umbilical cord plasma, as reported by Liu et al. (2018b).

[The Working Group noted that four prospective studies reported associations of the urinary excretion of 8-oxodG with the risks of specific cancers, although they did not evaluate any PFAS with this effect. Two of the studies showed associations with lung cancer (Loft et al., 2006, 2012) and the other two studies showed associations with breast cancer (Loft et al., 2013; Broedbaek et al., 2015). It was noted that the study by Broedback et al. was in patients with type 2 diabetes. In these studies few cancer cases were included; however, the studies were considered relevant because the authors reported on effects on this particular oxidative damage-related end-point.]

[The Working Group also noted there was a recent systematic review article (Chen et al., 2023a) that analysed the associations of exposure to persistent organic pollutants (POPs) in humans, including PFAS (PFOA and PFOS were included), with oxidative stress end-points. The review included typical oxidative stress end-points, such as 8-OHdG, ROS, MDA, reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), and some clinical diagnostic markers (gamma-glutamyl transferase, GGT), uric acid, and bilirubin). The meta-analysis of Chen et al. (2023a) acknowledged that the association of PFOA or PFOS with oxidative stress in exposed humans would require further studies.]

(ii) Human cells in vitro

See Table 4.16.

Human primary cells

There have been fewer studies using human primary cells in vitro compared with immortalized cells to investigate oxidative stress induced by PFOA or PFAS. Alterations of oxidative stress end-points were observed in cells treated with non-cytotoxic concentrations ranging from submicromolar to under 100 µM in most studies. Most studies set $P < 0.05$ for significant differences.

Han et al. (2020) exposed human epidermal keratinocytes and human dermal fibroblasts within a human full-thickness skin model (EpiDermFT (EFT-400), MatTek Corporation, Ashland, MA, USA) to PFOA at 250 µM or 2500 µM for 6 days. MDA lipid peroxidation marker levels, measured using TBARS, were significantly increased at both concentrations in the treated cells, but no significant changes in 8-OHdG were observed, as measured using an ELISA kit (Han et al., 2020).

For experiments using human primary cells, Orbach et al. (2018) used primary liver cells to assemble a multicellular organotypic culture model in 96-well plates (µOCMs) and collagen sandwich (CS) culture. Commercially available primary hepatocytes, derived from two adult males, were exposed to ½ or 1 median lethal concentration (LC_{50}) (250 or 500 µM) of PFOA for 24 hours. GSH was measured to evaluate PFOA-induced oxidative stress, and PFOA was found to reduce GSH to < 10% of control (Orbach et al., 2018). [The Working Group noted that GSH was measured using the commercial HTS GSH-Glo assay kit and that GSH reduction because of binding or oxidation did not occur because cytochrome P450 (CYP) enzymes do not oxidize PFOA. It was also noted that the doses of ½ LC_{50} and 1 LC_{50} were considered high.]

In a study from Pan et al. (2018), human erythrocytes were isolated from blood samples collected from six healthy, non-smoking adults from China. Cells were exposed to three PFAS compounds, including PFOA, at 0, 5, 10, 50, or 100 µM for 3 hours, then MDA, GSH, glutathione peroxidase (GPx), SOD, and CAT were measured using the TBARS, 2,3-naphthalenedicarboxaldehyde, 5,5-dithiobis (2-nitrobenzoic acid), SOD assay kit, and ammonium molybdate methods, respectively. PFOA at 100 µM induced a significant increase in MDA level, GSH levels were reduced by 10 and 100 µM, and the CAT and

Table 4.16 End-points relevant to oxidative stress in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
Primary cells						
8-oxodG, MDA	ELISA for 8-oxodG, TBARS for MDA	EpiDermFT skin model (human epidermal keratinocytes and human dermal fibroblasts cells)	No change in 8-oxodG ↑ MDA	PFOA: 250 or 2500 µM, 6 days of treatment every other day	No positive control; levels of 8-oxodG in unexposed controls (0.3 ng/µg DNA, corresponding to 1740 lesions/10^6 dG); significant increase in MDA	Han et al. (2020)
GSH	HTS GSH-Glo glutathione assay	Primary human hepatic cells	↓ GSH at 250 µM	PFOA: 250 or 500 µM (1/2 LC_{50} and LC_{50}), 24 h	PFOA reduced GSH to < 10%; CYP enzymes did not oxidize PFOA, GSH depletion due to binding or oxidation did not occur; doses of ½ LC_{50} and 1 LC_{50} were considered to be high	Orbach et al. (2018)
MDA, GSH, GPx, SOD, CAT	TBA, 2, 3-NDA, DTNB, SOD by assay kits with ammonium molybdate	Erythrocytes	↑ MDA, ↓ GSH, ↓ GPx, ↓ CAT, no change in SOD	PFOA: 10 or 100 µM, 3 h for GSH; 5, 10, 50, or 100 µM for MDA, GPx, CAT, SOD	MDA: (100 µM) significant increase; GSH (10 and 100 µM), GPx (100 µM), and CAT (100 µM): significant decrease; SOD: no change. The results suggest PFOA induces oxidative stress in erythrocytes	Pan et al. (2018)
ROS	Muse Oxidative Stress Kit, followed by flow cytometry	Embryonic stem cell system – primary spermatocytes, secondary spermatocytes, and spermatids	No increase in ROS	PFOA: 11, 25, or 100 µM; PFOS: 24, 48, or 126 µM on days 1–10 of the differentiation process	No significant effect on ROS generation PFOS (all concentrations) and PFOA (11 and 25 µM) exposure resulted in significantly lower ROS levels	Steves et al. (2018)
ROS	ROS kit	Sperm	↑ ROS	PFOA 0.25, 2.5, or 25 µg/mL (0.6, 6, or 60 µM) for 0.5 or 4 h	60 µM significantly induced ROS production after 4 h	Yuan et al. (2020)
ROS, MDA, GSH, GSSG	DCFH-DA, TBARS, *o*-phthalaldehyde fluorescence detection	Lymphocytes	↑ ROS, ↑ MDA, ↓ GSH, ↑ GSSG	PFOS, 75, 150, or 300 µM for 2, 4, 6, 8, 10, or 12 h	20 healthy adults: aged 18–30 yr, sex not reported ↑ ROS in a time- and dose–dependent manner ↑ MDA after 6 h at 150 and 300 µM; ↓ GSH and ↑ GSSG after 4, 8, 10, and 12 h, but not after 6 h Biomarker data supportive of oxidative stress	Zarei et al. (2018)

Table 4.16 (continued)

End-points	Assay	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
ROS	Electron spin resonance (ESR) spectroscopy, DHE-fluorescence–confocal microscopy, DHE–fluorescence microplate reader	Microvascular endothelial (HMVEC) cells	↑ ROS	PFOS, 10, 20, 50, or 100 µM for 1 h; 2 µM for 1, 2, 3, or 5 h (time study)	↑ ROS at 50 or 100 µM by DHE method; ↑ ROS at 2 µM at all time points (1–5 h)	Qian et al. (2010)
ROS, GPx, HO-1	DCFH-DA, western blotting	Proximal tubular epithelial (HK-2) cells	↑ ROS, ↓ GPx4, ↓ HO-1	PFOS, 200 µM for 12 h	Significant ↑ ROS, ↓ GPx4, and ↓ HO-1 provided evidence of oxidative stress Only a single concentration and time point	Wang et al. (2022b)
ROS	DCFH-DA	Corneal epithelial (HCEpiC) cells	↑ ROS	PFOA 100, 200, or 400 ppm (mg/kg) for 6 h	The solubility of the PFOA in the culture medium and hence its bioavailability to cells was unknown	Tien et al. (2020)
ROS	DCF-DA	Insulin-producing EndoC-βH1 cells	No change in ROS	PFOA 1 nM or 1 µM for 24 h	No change in ROS	Dos Santos et al. (2022)
ROS	DCFH-DA	Placental trophoblast (HTR-8/SVneo) cells	↑ ROS	PFOA 0, 100, 200, 400, 600, 800, or 1000 µM for 24 h	Increased ROS in a dose–response manner	Du et al. (2022)
ROS	DCFH-DA	Placental trophoblast (HTR-8/SVneo) cells	↑ ROS	PFOS, 1, 10, or 50 µM for 24 h or 48 h	Significant ↑ ROS at 10 and 50 µM in a time- and dose-dependent manner	Sonkar et al. (2019)
ROS	DCFH-DA	Umbilical vein endothelial (HUVEC) cells	↑ ROS	PFOS, 100 mg/L (200 µM) for 1, 5, 12, 24, or 48 h	ROS increased in a time-dependent manner	Liao et al. (2012)
ROS	DCFH-DA	Umbilical vein endothelial (HUVEC) cells	↑ ROS	PFOS, 100 mg/L (200 µM) for 24 h or 40 h	PFOS increased ROS production after 24 or 40 h of exposure. In cells co-treated with anti-oxidant *Flos lonicerae* extract or chlorogenic acid for 40 h, ROS production was reduced to levels comparable to those of cells exposed to PFOS for 24 h. ROS levels were not reduced by co-treatment of cells exposed to PFOS for 24 h only	Liao et al. (2013)

Table 4.16 (continued)

End-points	Assay	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
ROS	ROS sensor (CellROX)	Mesenchymal stem (hMSC) cells	↑ ROS	PFOS, 0.1 or 10 µM at 12, 24, 36 and 48 h; 7 and 14 days	↑ ROS at 10 µM at all time points, ↑ ROS at 7 days at 0.1 µM	Gao et al. (2020)
Cell lines						
Oxidatively damaged DNA	Fpg-modified comet assay	HepG2 cells	No change	PFOA or PFOS, 100 or 400 µM	Positive control (Ro19-8022 + light) increased DNA damage	Eriksen et al. (2010)
8-oxodG	Immunostaining	HaCaT cells	↑ ROS	PFOA, 50 µM for 24 h + 8 days recovery	No positive control; increased 8-oxodG at 8 days recovery	Peropadre et al. (2018)
8-oxodG	HPLC-MS/MS	TK6 cells	↑ ROS	PFOA, 125 or 250 µg/mL for 2 h	No positive control	Yahia et al. (2016)
ROS, 8-OHdG	DCFH-DA for ROS, immunocytochemical detection for 8-OHdG	HepG2 cells	↑ ROS, ↑ 8-OHdG	PFOA, 100, 200, or 400 µM for 3 h	ROS production increased in a dose–response manner; no positive control for ROS; 8-OHdG increased in a dose–response manner with H_2O_2 used as a positive control	Yao and Zhong (2005)
ROS (H_2O_2 and superoxide anions)	Flow cytometry: DCFH-DA for H_2O_2; DHE for superoxide anion	HepG2 cells	↑ ROS	PFOA, 200 or 400 µM for 3 h	Minimum 3 replicates per treatment; ROS measured after 1.5, 3, 5, and 24 h and was found to peak at 3 h	Panaretakis et al. (2001)
ROS	DCFH-DA by flow cytometry; GSH, SOD, CAT by ELISA	Hep2G cells	↑ ROS, ↑ GSH, ↑ CAT, no change in SOD	PFOA, 10, 25, or 50 µM for 24 h	PFOA at 10, 25, or 50 µM significantly increased ROS (≤ 5.3-fold at 50 µM); GSH (1.7-fold), and CAT (1.4-fold) only at 10 µM; no change in SOD	Abudayyak et al. (2021b)
ROS	DCFH-DA	HepG2 cells	↑ ROS, ↓ GSH, ↓ GPx, ↑ SOD, ↑ CAT, GST	PFOA and PFOS 50, 100, 150, or 200 µM for 5, 10, or 15 h (ROS) and 48 h (GSH, GPx, SOD, CAT)	Significant increase in ROS at ≥ 100 µM after 5, 10, or 15 h; GSH and GPx increased at ≥ 100 µM; SOD and CAT increased at ≥ 150 µM; GST increased at 200 µM	Hu and Hu (2009)
ROS	DCFH-DA	HepG2 cells	↑ ROS	PFOA or PFOS at 0.4, 4, 40, 200, 400, 1000, or 2000 µM; every 15 min for 3 h	Significant increases at all time points, but no dose-dependency	Eriksen et al. (2010)
ROS, TAC	Carboxy-DCFH-DA, antioxidant assay kit	HepG2 cells	↑ ROS, ↓ TAC	PFOA or PFOS: ROS: 0.2, 2, or 20 µM for 24 h; TAC: 0.02, 0.2, 2, 20, or 200 µM for 24 h	PFOA or PFOS significantly induced ROS; PFOA significantly reduced TAC; PFOS reduced TAC, but not significantly	Wielsøe et al. (2015)

Table 4.16 (continued)

End-points	Assay	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
ROS	DCFH-DA	HepG2 cells	↑ ROS	PFOA or PFOS: 0, 5, 25, 50, 100, 200, 400, or 800 µM for 3 or 24 h	PFOA or PFOS induced ROS in a dose-dependent manner	Amstutz et al. (2022)
ROS, NO	DCF, and Greiss reagent	HepG2 cells	↑ ROS, ↑ NO	PFOA: 100 or 200 µM for 24 h	PFOA increased ROS and NO levels and *NOS2A* mRNA expression	Yarahalli Jayaram et al. (2020)
ROS	CellRox green reagent	HepaRG and HepG2 cells	↑ ROS	PFOA: 10, 100, or 1000 nM for 72 h	Generated PFOA-resistant cells; conducted acute (1–3 days) and chronic (30–60 days) exposure experiments for the steatosis and fibrosis study	Qi et al. (2023)
ROS, NOx	DCFH-DA or fluorescent MAK145 for ROS; 2, 3-diaminonaphthalene (fluorometric assay kit for NOx)	HepG2 cells and keratinocyte (HaCaT) cell line	↑ ROS, ↑ NOx in both cells	PFOA: 10 µM for 24 h	The DCFH-DA assay yielded higher intensity compared with the MAK14 assay	Magnifico et al. (2022)
Nrf2–ARE	Luciferase assay	ARE reporter HepG2 cell line	↑ Nrf2–ARE	PFOA or PFOS: 1, 2, 3, 4, 5, or 6 µM for 24 h	PFOA and PFOS induced Nrf2–ARE activation; the effective concentration of induction ratio 1.5 ($EC_{IR1.5}$) was 1.38 µM for PFOA and 1.17 µM for PFOS; supportive of oxidative stress being involved	Ojo et al. (2022b)
ROS	DCFH-DA	HepG2 cells	↑ ROS	PFOS: 10, 20, 30, 40, or 50 µM for 24 h; PFOA: 50 µM for 1, 3, 6, 12, or 24 h	Significant ↑ ROS at PFOS concentrations ≥ 30 µM after 24 h of treatment; when treated with 50 µM, ↑ ROS gradually, peaking after 12 h, then lower after 24 h	Wan et al. (2016)
MDA, ROS, superoxide anion, SOD, CAT, GSH	TBARS for MDA, assay kits for other biomarkers	HepG2 cells	↑ ROS, no significant changes in other biomarkers	PFOS: 50, 100, or 200 µM for 24 or 72 h	Significant ↑ ROS with PFOA ≥ 100 µM after 24 h, but not 72 h; no significant changes in the other biomarkers measured; cells pretreated with NAC showed a reduction in PFOA-induced ROS production	Yan et al. (2015a)
ROS	DCFH-DA	HepG2 cells	No change in ROS	PFOA: 5, 10, 50, 100, 200, or 400 µM; PFOS: 5, 10, 50, 100, 200, or 300 µM; 1 or 24 h	No significant increase at any time point Avoided additional stress by not trypsinizing cells before adding DCFH-DA	Florentin et al. (2011)

Table 4.16 (continued)

End-points	Assay	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
ROS, GSH	DCFH-DA for ROS, GSH-Glo assay kit	HepG2 cells	No change in ROS, ↓ GSH	PFOA: 0.2, 2, or 20 µM for 24 h PFOS: 0.2, 2, or 20 µM for 24 h	No increase in ROS but a decrease in GSH as the PFOA or PFOS concentration increased; 20 µM PFOA or PFOS induced a significant decrease in GSH; oxidative stress implicated, but unclear; the avoidance of additional physical stress by not trypsinizing cells before adding DCFH-DA may have been a factor	Ojo et al. (2021)
ROS, GSH/GSSG ratio, MDA	DCFH-DA for ROS, HPLC/fluorescence detector for GSH and GSSG, assay kit for MDA	HepG2 cells	No change in ROS, MDA, GSH/GSSG	PFOA or PFOS at 100 µM for 3 h	No change in ROS, MDA, or GSH/GSSG; only a single level of exposure; no detailed data were provided	Shan et al. (2013)
ROS, MDA, GSH, SOD, CAT	ROS by DCFH-DA/flow cytometry; MDA, GSH, SOD, CAT by ELISA	Pancreatic epithelioid carcinoma (PANC-1) cell line	No changes in ROS, ↑ GSH, ↑ CAT, ↑ SOD, ↑ MDA	PFOA at 10, 50, or 100 µM for 24 h	No increase in ROS levels; GSH levels increased only at 10 µM; MDA and SOD increased to similar levels regardless of the dose; the involvement of oxidative stress was unclear; CAT increased at all doses in a non-dose-dependent manner	Abudayyak et al. (2021a)
ROS	ROS-Glo H_2O_2 assay; DCFH-DA assay	Differentiated neuroblastoma (SH-SY5Y) cells	No changes in ROS	PFOA, 1, 10, 100, 150, 200, or 250 µM for 4, 24, 48, or 72 h	DCFDA detected ROS increases at 100, 200, and 250 µM after 4 h and at 250 µM after 24 h, but no change after 48 h at any concentration	Souders et al. (2021)
ROS, MDA, GSH, SOD	DCFH-DA followed by fluorescence photography, TBARS, GSH assay kit, SOD assay kit	Lung carcinoma (A459) cells	↑ ROS, ↑ MDA, ↑ SOD, ↓ GSH	PFOS, 25, 50, 100, or 200 µM for 24 h	PFOS at 50, 100, or 200 µM significantly increased ROS, MDA, and SOD, and reduced GSH; ROS increase was measured using fluorescence photography, but not quantified	Mao et al. (2013)
mtROS	Immunofluorescence microscopy	Ovarian granulosa-like tumour (KGN) cells	↑ mtROS	PFOA, 250, 500, or 750 µM for 24 h	Significant increase in mtROS at all doses	Zhang et al. (2023a)

Table 4.16 (continued)

End-points	Assay	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
ROS	DCFH-DA	Embryonic kidney (HEK293) cells	↑ ROS	PFOS, 10–60 µM for 24 h	Significant increase in ROS production; CBD (2–80 µM) partially restored PFOS-induced ROS; however, the concentrations of PFOS and CBD that had their effects were unclear	Du et al. (2023)

ARE, antioxidant responsive element; CAT, catalase; CBD, cannabidiol; CYP, cytochrome P450; DCFH-DA, 2′,7′-dichlorodihydrofluorescein diacetate; DCF, dichlorofluorescein; dG, 2′-deoxyguanosine; DHE, dihydroethidium; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); ELISA, enzyme-linked immunosorbent assay; Fpg, formamidopyrimidine DNA glycosylase; GPx, glutathione peroxidase; GSH, glutathione; GSSG, oxidized glutathione; GST, glutathione *S*-transferase; h, hour(s); H_2O_2, hydrogen peroxide; HK-2, human kidney proximal tubular epithelial cells; HMVEC, human microvascular endothelial cells; HO-1, haem oxygenase 1; HPLC, high-performance liquid chromatography; HUVEC, human umbilical vein endothelial cells; LC_{50}, median lethal concentration; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOD, limit of detection; MDA, malondialdehyde; min, minute(s); NAC, *N*-acetylcysteine; 2,3-NDA, 2,3-naphthalenedicarboxaldehyde; NOx, nitrogen oxides; NRf2, NF-E2-related factor 2; 8-OHdG, 8-hydroxydeoxyguanosine; 8-oxodG, 8-oxo-2′-deoxyguanosine; PANC-1, human pancreatic cells; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; ROS, reactive oxygen species; mtROS, mitochondrial reactive oxygen species; SD, standard deviation; SOD, superoxide dismutase; TAC, total antioxidant content; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substances; yr, year(s).

[a] ↑, increase; ↓, decrease.

GPx levels were reduced by 100 μM. No change was observed for SOD (Pan et al., 2018). [The Working Group noted that the authors concluded that PFOA induces oxidative stress in human erythrocytes.]

Steves et al. (2018) used a human stem cell-based model of spermatogenesis to assess the effects of PFOA and PFOS individually or in mixtures. Cells were treated with PFOS (24, 48, or 126 μM) or PFOA (11, 25, or 100 μM), starting on day 1, for the entire 10-day cell differentiation process. ROS production was measured using the Muse Oxidative Stress Kit, followed by flow cytometry. No significant changes in ROS production in the cells exposed to PFOA at 100 μM were observed, whereas PFOS at 126 μM significantly reduced ROS compared with the control group (0.25% dimethyl sulfoxide only). Both PFOA and PFOS significantly reduced ROS production at the two lowest concentrations. The authors concluded that ROS production induced by PFOA or PFOS is unlikely to influence the viability of spermatogenic cells in vitro.

In another study, Yuan et al. (2020) treated human sperm with PFOA at 0, 0.25, 2.5, or 25 μg/mL (0, 0.6, 6, or 60 μM) for 30 minutes or 4 hours. ROS was measured using a ROS assay kit. The highest exposure concentration (60 μM) after 4 hours induced a significant increase in ROS generation. Sperm have high levels of polyunsaturated fatty acids and low antioxidant enzyme levels. Hence, they are especially vulnerable to oxidative stress, and high levels of ROS may disrupt sperm function.

Human lymphocytes for a PFOS exposure study were isolated from blood samples of 20 healthy adults aged 18–30 years (sex not reported) (Zarei et al., 2018). The cells were treated with PFOS at 75, 150, or 300 μM for 2, 4, 6, 8, 10, or 12 hours. ROS production was measured using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), MDA using TBARS, and GSH and oxidized glutathione (GSSG) using the *o*-phthalaldehyde fluorescence method. PFOS significantly ($P < 0.05$) induced ROS production in a time– and dose–responsive manner: the levels of ROS (measured by DCFH-DA) increased at concentrations of 150 and 300 μM PFOS at 2 hours and at all concentrations and later time points (4–12 hours), in comparison with the control. MDA levels were also increased after 6 hours of treatment with 150 or 300 μM PFOS. A reduction in GSH and increase in GSSG were observed at 4, 8, 10, and 12, but not at 6 hours, at all concentrations. Butylated hydroxytoluene (50 μM), an antioxidant, was found to inhibit PFOS-induced oxidative stress.

In another study (Qian et al., 2010), human microvascular endothelial cells (HMVEC) were exposed to PFOS at 10, 20, 50, or 100 μM for 1 hour, or 2 μM for 1, 2, 3, or 5 hours. Dihydroethidium-fluorescence-confocal microscopy and dihydroethidium-fluorescence microplate reader methods were used to measure ROS. PFOS increased ROS production at all concentrations (10–100 μM for 1 hour) and time points (2 μM for 1–5 hours). The low concentrations of PFOS applied to HMVEC cells matched occupational and environmental levels.

Wang et al. (2022a) treated human L02 liver cells with 0, 8, 64, or 256 μM PFOA for 24 hours. PFOA significantly increased ROS production at 64 and 256 μM. The authors concluded that PFOA may induce endoplasmic reticulum stress (ERS) and oxidative stress. [The Working Group noted that the L02 cells used in the study might be contaminated with HeLa cells (SIB, 2024). The HeLa cell line is derived from cervical cancer cells. Therefore, contamination of L02 cells with HeLa cells would complicate the interpretation of the results of this study.]

In another study (Wang et al., 2022b), human proximal tubular epithelial cells (HK-2) were exposed to PFOS at 200 μM for 12 hours. PFOS significantly increased the level of ROS production and reduced expression of the antioxidants GPx4 and HO-1. In this study, the authors proposed that PFOS may cause ferroptosis because of an

intracellular increase in iron, which would lead to an increase in ROS production and a reduction in GSH concentration.

Tien et al. (2020) identified consistently high concentrations of PFOA in the particulate matter ($PM_{2.5-1,}$) fraction of indoor dust to which people can be exposed and assessed the effects of PFOA on human corneal epithelial (HCEpiC), endothelial (HCEC), and retinal pigment epithelial (RPE) cells. HCEpiC cells were treated with PFOA at concentrations of 100, 200, or 400 ppm for 6 hours. PFOA at 200 and 400 ppm increased ROS production in HCEpiC cells, and a significant increase was observed for 400 ppm exposure. The authors concluded that PFOA in dust might induce ROS production in the retina and that this may have a risk implication for age-related macular degeneration (Tien et al., 2020). [The Working Group noted that the response could have been because of the dust particles as well as the PFOA in the dust.]

Elumalai et al. (2023) reported that PFOS-induced oxidative stress contributed to apoptosis in human pancreatic 1.1b4 β cells. Human 1.1b4 cells were treated with 100 μM PFOS for 48 or 36 hours. PFOS was found to increase mitochondrial ROS generation with significantly decreased GSH/GSSG ratios through NOX2-gp91Phox activation and the inhibition of cyclic adenosine monophosphate-protein kinase A (cAMP-PKA).

However, in another study, Dos Santos et al. (2022) treated the human insulin-producing EndoC-β-cell line (a human pancreatic β-cell model) with relatively low concentrations of PFOA (1 nM or 1 μM). No significant changes in ROS production, as measured as 2′,7′-dichlorofluorescein diacetate (DCF-DA) fluorescence, were observed.

Du et al. (2022) treated human placental trophoblast cells immortalized with SV40T antigen (HTR-8/SVneo cells) with PFOA at 0, 100, 200, 400, 600, 800, or 1000 μM for 24 hours. PFOA was found to increase ROS production in a dose-dependent manner. The study also showed that PFOA induced ERS, which triggered both the proliferation and apoptosis of trophoblasts via ROS generation or activation of the unfolded protein response (UPR) signalling pathway.

Sonkar et al. (2019) also studied PFOS-induced ROS production in HTR-8/SVneo cells. The cells were exposed to PFOS (1, 10, or 50 μM) for 24 or 48 hours. ROS production in the cells was significantly induced at concentrations ≥ 10 μM at both 24 and 48 hours.

Liao et al. (2012) exposed human umbilical vein endothelial cells (HUVECs) to PFOS (100 mg/L; 200 μM), and ROS production was measured after 1, 5, 12, 24, and 40 hours using the DCFH-DA fluorescence assay. A significant increase in ROS production was observed in a time-dependent manner.

In a follow-up study, Liao et al. (2013) reported that PFOS (at 100 mg/L, i.e. 200 μM) significantly increased ROS production, as measured by DCFH-DA, in HUVECs. The study showed that PFOS treatment alone significantly increased ROS production. Co-treatment with PFOS and either *Flos lonicerae* extract containing chlorogenic acid (CGA) or CGA for 40 hours reduced ROS production to levels comparable to those in cells exposed to PFOS only for 24 hours. However, the increased ROS levels of cells exposed to PFOS for just 24 hours were not affected by co-treatment with either *Flos lonicerae* or CGA.

Gao et al. (2020) studied the effects of PFOS on human mesenchymal stem cells (hMSCs) by treating the cells with 0.1 or 10 μM PFOS for 12, 24, 36, or 48 hours. Treatment with 10 μM PFOS was found to significantly increase ROS production at all time points. The lower concentration of PFOS only increased ROS production significantly in cells treated for 7 days. The results suggest an accumulation effect of low-dose PFOS in hMSC cells.

[The Working Group noted that primary hepatic cells were the most-used human cells for the study of PFOA and PFOS induction of oxidative stress in vitro, with studies also using

primary cells derived from other organ systems. PFOA and PFOS exposure concentrations typically ranged from low doses (submicromolar) to medium doses (up to 100–200 μM) and high doses (above 500 μM). Except for a few studies reporting inverse associations between the exposure and response, both PFOA and PFOS were shown to have varying potencies to induce ROS production in primary liver cells and cells derived from other organ systems.]

Human cell lines

Among immortalized cell lines, HepG2 cells are the most widely used. This cell line was used for exposure experiments involving exposure to a wide range of concentrations of PFOA or PFOS for different periods of time.

Two studies examining the effect of PFOA and/or PFOS on the induction of oxidative DNA damage found no effects. The first study used the Fpg-modified comet assay, which included a Ro19-8022 + light as a positive control for the generation of 8-oxodG, to examine the effect of PFOA and PFOS treatment on HepG2 DNA. PFOA and PFOS treatment did not generate oxidative damage to DNA (Eriksen et al., 2010). The authors observed an increase in ROS production. Specifically, HepG2 cells were treated with PFOA or PFOS at concentrations ranging from 0.4 μM to 2 mM (0.4, 4, 40, 200, 400, 1000, or 2000 μM), and ROS production was measured every 15 minutes up to 3 hours. PFOA and PFOS induced a moderate, non-significant increase in ROS production in the cells; however, the response was not concentration-dependent (Eriksen et al., 2010). The second study (Han et al., 2020) exposed a human skin equivalent to PFOA for 6 days. A slight but non-significant increase in 8-oxodG, measured by ELISA, was observed at 0.25 mM.

In three other studies, PFOA exposure was shown to increase the levels of 8-oxodG in human hepatoma cells (HepG2), in lymphoblastoid cells (TK6), and in epidermal keratinocytes (HaCaT) [although the Working Group considered the reliability of the measurements to be questionable] (Yao and Zhong, 2005; Yahia et al., 2016; Peropadre et al., 2018). The first study reported relatively high background levels of 8-oxodG, measured by HPLC-MS/MS (i.e. 8.3 lesions/10^6 dG) (Yahia et al., 2016), which is almost one order of magnitude higher than the generally accepted background level of 8-oxodG in mammalian cells (1 lesion/10^6 dG) and exceeds the level at which methodological artefacts become a serious concern (5 lesions/10^6 dG) (ESCODD, 2002). [The Working Group considered that the results from three studies with antibody-based detection of 8-oxodG in cells were inconclusive because of a lack of positive controls to verify the specificity of the assay (Yao and Zhong, 2005; Peropadre et al., 2018). The detection of 8-oxodG by an antibody-based system was considered questionable, because of non-specific binding to other biomolecules in cells or biological matrices (Chao et al., 2021).]

Studies demonstrating the induction of ROS production are summarized below.

PFOA (200 or 400 μM) was found to significantly increase ROS production in HepG2 cells after a 3-hour exposure (Panaretakis et al., 2001). ROS were measured using flow cytometry as hydrogen peroxide (H_2O_2) and the superoxide anion O_2^- after reaction with DCFH-DA and dihydroethidine, respectively.

HepG2 cells were exposed to PFOA at concentrations of 10, 25, or 50 μM for 24 hours (Abudayyak et al., 2021b). In this study, ROS production was increased by all concentrations (≤ 5.3-fold at 50 μM), GSH was increased (1.7-fold at 10 μM), and CAT was increased (1.4-fold at 10 μM), but changes in SOD activity were not observed at any concentration. Fluorescein isothiocyanate-labelled DCFH-DA was used to measure ROS, whereas ELISA kits were used for the measurement of other end-points.

Hu and Hu (2009) measured the following end-points of oxidative stress – ROS, GSH, GPx, SOD, and CAT – in HepG2 cells exposed to PFOA or PFOS (50, 100, 150, or 200 µM) for 5, 10, or 15 hours. PFOS significantly increased ROS production in a time- and dose-dependent manner (at 100, 150, and 200 µM). PFOA induced ROS production in a similar manner, although no data were shown. At 48 hours, significant changes in antioxidant activity were observed: both PFOA and PFOS significantly reduced GSH and GPx at 100, 150, and 200 µM and increased SOD and CAT activities at the two highest concentrations, 150 and 200 µM. PFOA and PFOS induced an increase in GST only at 200 µM.

Wielsøe et al. (2015) studied the effects of exposure to seven different PFAS, including PFOA and PFOS, on oxidative stress end-points, such as ROS production and the total antioxidant capacity (TAC) in HepG2 cells. The cells were exposed to concentrations of 0.02, 0.2, 2, 20, or 200 µM for 24 hours. ROS production was significantly induced by 0.2, 2, and 20 µM of PFOA and PFOS. TAC was reduced at all the tested concentrations of PFOA and PFOS; however, the reduction only reached significance with PFOA exposure.

Amstutz et al. (2022) exposed HepG2 cells to 5, 25, 50, 100, 200, 400, or 800 µM of PFOA, PFOS, or several other PFAS for 3 or 24 hours. PFOA and PFOS induced concentration-dependent increases in ROS generation. Yarahalli Jayaram et al. (2020) exposed HepG2 cells to PFOA at 100 or 250 µM for 24 hours. PFOA exposure at 250 µM significantly increased ROS production and NO levels, measured using dichlorofluorescein (DCF) and Greiss reagent, respectively. The mRNA expression of *NOS2A* also increased upon exposure to PFOA, providing further evidence for oxidative effects of PFOA.

Qi et al. (2023) studied the pro-steatotic and fibrotic effects of PFOA in HepG2 and HepaRG cells. Cells were exposed to PFOA at 10, 100, or 1000 nM for 72 hours, which led to increased ROS production. Additionally, PFOA was found to increase steatosis and fibrosis in both cell lines, as indicated by the upregulation of specific genes involved in UPR signalling and non-alcoholic fatty liver disease (NAFLD).

HepG2 cells and HaCaT cells were treated with 10 µM PFOA for 24 hours. Afterwards, ROS levels were measured using two different fluorescent probes, MAK145 (red fluorescence) and DCFH-DA, with the DCFH-DA probe being more sensitive than the MAK145 probe (Magnifico et al., 2022). In addition, levels of nitrogen oxides (NO_x) were measured using the fluorescent probe 2,3-diaminonaphthalene from a nitrate/nitrite fluorometric assay kit. Fluorescence intensity, proportional to the total NO production, was measured using a fluorometer. The results showed that PFOA significantly increased ROS production and nitrosative stress in both cell lines.

One study by Ojo et al. (2022b) investigated the effects of several PFAS, including PFOA and PFOS, on the Nrf2–antioxidant responsive element (ARE) pathway, individually or in combination, using ARE reporter–HepG2 cells. The induction of Nrf2–ARE is indicative of an event of cellular oxidative stress. The cells were exposed to PFOA or PFOS at 1, 2, 3, 4, or 5 µM for 24 hours. The results showed a significant induction of oxidative stress. The 1.5-fold induction ratios (EC_{IR} = 1.5) for PFOA and PFOS were determined to be 1.38 µM and 1.17 µM, respectively.

Wan et al. (2016) treated HepG2 cells with PFOS at concentrations of 0, 10, 20, 30, 40, or 50 µM for 24 hours or 50 µM for 1, 3, 6, 12, or 24 hours. ROS levels increased significantly at PFOS concentrations ≥ 30 µM. When treated with 50 µM, ROS levels gradually increased over time, peaking at 12 hours, and then decreased to 24 hours. GSH levels significantly decreased at PFOS concentrations ≥ 20 µM and at all time points after 6 hours during the time-course experiment.

Yan et al. (2015a) exposed HepG2 cells to PFOA at concentrations of 0, 50, 100, or 200 μM for 24 or 72 hours. ROS production was significantly increased in cells treated with PFOA concentrations ≥ 100 μM at 24 hours but was decreased at 72 hours. ROS production was decreased in cells pre-treated with *N*-acetylcysteine (NAC).

Several studies have reported no effects of PFOA or PFOS exposure on the production of ROS in HepG2 cells. For example, Florentin et al. (2011) assessed the induction of ROS production after exposure to PFOA or PFOS in HepG2 cells. The cells were exposed to PFOA at 0, 5, 10, 50, 100, 200, or 400 μM, or 0, 5, 10, 50, 100, 200, or 300 μM PFOS for 1 or 24 hours. No increase in oxidative stress was observed. No effect on ROS production was also reported by Ojo et al. (2021) after they exposed HepG2 cells to 0.2, 2, or 20 μM PFOA or PFOS for 24 hours. It was noted that the doses used in this study were lower and more relevant to environmental exposure levels.

In another study, Shan et al. (2013) exposed HepG2 cells to a single concentration (100 μM) of PFOA or PFOS for 3 hours. No significant differences in ROS production, GSH, GSSG, or MDA were observed between the PFAS test groups and the negative control. [The Working Group noticed that the study focused on the combined effects of PFOA or PFOS with pentachlorophenol. No tabulated or graphical data were provided for PFOA and PFOS alone, only descriptive text. Nevertheless, this was another study available in the literature that used relatively low concentrations of PFOA and PFOS and with no effects on the cell oxidative status.]

The concentrations of PFAS and the treatment conditions of the cells before the addition of the ROS-detection probe were considered of relevance (Ojo et al., 2021). It was noted that the cell trypsinization stage included in the ROS measurement method could have induced additional stress in the cells, as noted by Florentin et al. (2011). Exposure concentrations may also affect ROS production. It was reported that low concentrations may protect against ROS generation, and high concentrations may induce ROS production (Steves et al., 2018).

Yao and Zhong (2005) reported that 8-OHdG content, measured using immunocytochemical staining, was significantly increased in a dose-dependent manner when HepG2 cells were exposed to PFOA (100, 200, or 400 μM) for 3 hours. Hydrogen peroxide was used as a positive control. ROS, measured using DCFH-DA, was also increased in a dose-dependent manner. No positive control was included in the ROS assay. As mentioned above, Eriksen et al. (2010) also did not observe a significant increase in ROS.

Fewer studies have examined the effects of PFOA and PFOS on the induction of oxidative stress in human cells other than HepG2. Abudayyak et al. (2021a) exposed the human pancreatic epithelioid carcinoma cell line PANC-1 to PFOA (0, 10, 50, or 100 μM) for 24 hours. ROS levels were not increased by any tested concentration. However, MDA, SOD, and CAT were significantly increased upon PFOA treatment, although not in a dose-dependent manner. It was noted that GSH was increased in cells treated with 10 μM PFOA, but not higher concentrations.

Souders et al. (2021) exposed differentiated human SH-SY5Y neuroblastoma cells to PFOA for a metabolic profiling study. Cells were treated with 1, 10, 100, 150, 200, or 250 μM PFOA, and ROS production was measured at 4, 24, and 48 hours using the ROS-Glo H_2O_2 and DCFH-DA assays. The ROS-Glo assay did not detect changes in ROS production, except a reduction after 4 hours at 250 μM, whereas the DCFH-DA assay detected a significant increase in ROS levels at 4 hours for 100, 200, and 250 μM and at 24 hours for 250 μM, but no increase at 48 hours.

In another study (Mao et al., 2013), human lung carcinoma A459 cells were treated with PFOS (25, 50, 100, or 200 μM) for 24 hours. ROS were measured using DCFH-DA, MDA

using TBARS, and the GSH and SOD levels were measured using assay kits. PFOS significantly increased ROS, MDA, and SOD, and reduced GSH at all concentrations ≥ 50 μM. ROS was measured qualitatively using fluorescence photography, and an increase in fluorescence levels was observed with increasing PFOS concentrations. PFOS-induced ROS generation was also inhibited by pre-treatment with the thiol antioxidant NAC.

More recently, Zhang et al. (2023a) exposed human ovarian granulosa-like tumour cells (KGN) to PFOA at concentrations ranging from 250 to 750 μM for 24 hours. Mitochondrial ROS (mt-ROS), determined by the superoxide indicator fluorescence ratio of red MitoSOX to MitoTracker Green was significantly increased in a dose-dependent manner across all tested concentrations.

Du et al. (2023) observed PFOS-induced ROS production (DCFH-DA method) in human embryonic kidney cells (HEK293) exposed for 24 hours to a medium containing PFOS at 40 μM and/or cannabidiol (CBD) at 20 μM. Co-exposure with CBD reduced the level of PFOS-induced ROS production.

(b) Experimental systems

(i) Non-human mammalian systems in vivo

See Table 4.17.

Some studies have shown mixed results regarding 8-oxodG formation in the kidneys, liver, and testes of mice and rats after oral exposure to PFOA (Takagi et al., 1991; Abdellatif et al., 2003–2004; Zou et al., 2015; Ma et al., 2023). [The Working Group noted that some of the studies did not include positive controls. In addition, there was uncertainty about the reliability of the results, because of high background levels of 8-oxodG in rat tissues (Takagi et al., 1991; Abdellatif et al., 2003–2004) and the measurement of 8-oxodG by a non-specific antibody-based method in the mouse tissues (Zou et al., 2015; Ma et al., 2023).]

Abdellatif et al. (2003–2004) studied the effects of dietary PFOA exposure on peroxisomal enzymes and 8-OHdG in male Wistar rats (minimum 15 rats per group), following a biphasic initiation procedure involving dosing with diethylnitrosamine (DEN) or a triphasic initiation involving dosing with DEN, 2-acetylaminofluorene, and carbon tetrachloride (CCl_4) (Abdellatif et al., 2003–2004). For the rats that underwent biphasic initiation, basal rodent diet or diet containing 0.005% or 0.02% PFOA (daily dose in mg/kg per day was not estimated) was ingested for 14 or 25 weeks. Those that underwent triphasic initiation ingested a diet without PFOA or a diet containing 0.015% PFOA for 25 weeks. CAT, measured spectroscopically, was significantly increased in the rat livers after 14 or 25 weeks compared with the basal diet group. To study the effect of PFOA on 8-OHdG induction, male Wistar rats underwent the initiation treatment and were fed either a basal diet or a basal diet containing 0.02% PFOA for 5 or 9 weeks. No significant increase in 8-OHdG was observed in the liver after 5 or 9 weeks compared with the basal diet group. [The Working Group noted that the study authors suggested that PFOA was a liver cancer promoter that may not require significant DNA damage. It was also noted that the study did not include a positive control group and that the background 8-OHdG was relatively high in the livers from the basal diet group.]

Ma et al. (2023) investigated the potential of rutin to ameliorate the oxidative effects of PFOA in mice (10 mice per group). Male ICR mice were orally dosed with PFOA (20 mg/kg bw) daily for 28 days in the presence or absence of rutin. The levels of 8-OHdG, GPx, SOD, and MDA were measured. 8-OHdG was significantly increased in the serum and testes; and MDA, GPx, and SOD were significantly increased in the testes of

Table 4.17 End-points relevant to oxidative stress in in non-human mammalian systems in vivo exposed to PFOA or PFOS

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
8-OHdG, CAT, by HPLC-ECD, spectroscopic method	Rat, Wistar, male	Liver	No change in 8-OHdG, significant increase in CAT	PFOA 0.02% in feed for 8-OHdG, 0.005% or 0.02% with DEN biphasic initiation, or 0.015% PFOA after DEN, 2-acetylaminofluorene and CCl_4 triphasic initiation	Oral, 5 or 9 wk for 8-OHdG, 14 or 25 wk for CAT	No positive control; tissues from unexposed rats have high background levels of 8-oxodG (i.e. 0.126 ng/µg DNA, corresponding to 730 lesions/10^6 dG)[a]	Abdellatif et al. (2003–2004)
8-OHdG, MDA, GPx, SOD 8-OHdG by ELISA kit; MDA, SOD, and GPx by microplate reader	Mouse, ICR, male	Serum and testicular tissue	Serum and testis: ↑ 8-OHdG Testis: ↑ MDA, ↑ GPx, ↑ SOD	PFOA (20 mg/kg per day)	Daily oral gavage for 28 days	Rutin ameliorates PFOA-induced oxidative stress; no positive control group; results on 8-oxodG reported as pg/mg protein	Ma et al. (2023)
8-OHdG, by HPLC-ECD	Rat, Fischer 344, male	Kidney, liver	↑ 8-OHdG (liver) ≥ 3 days No changes (kidney)	PFOA, 100 mg/kg	Peritoneum single injection, dosing at 1, 3, 5, or 8 days post-injection	No positive control group; tissues from unexposed rats had high background levels of 8-oxodG (> 30 lesions/10^6 dG)	Takagi et al. (1991)
8-OHdG, by HPLC-ECD	Rat, Fischer-344, male	Kidney, liver	↑ 8-OHdG (liver) No changes (kidney)	PFOA, 0.02% in feed	Oral, 2 wk, feed	No positive control group; tissues from unexposed rats had high background levels of 8-oxodG (i.e. 17 lesions/10^6 dG)	Takagi et al. (1991)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
8-OHdG, MDA, H_2O_2, SOD, CAT by ELISA for 8-OHdG, commercial kits for other biomarkers	Mouse, Kunming, male	Liver	↑ 8-OHdG, ↑ H_2O_2, ↑ MDA, ↓ SOD, ↓ CAT	PFOA, 10 mg/kg per day	Oral, daily for 14 days	No positive control group; results for 8-oxodG were reported as pg/mg protein; commercial kits may not be specific for the particular end-points Quercetin, a flavonoid, given at 75 mg/kg per day for 14 days limited the oxidative effects of PFOA	Zou et al. (2015)
8-OHdG, by HPLC (UV and electrochemical detectors)	Mouse, wildtype mice (129S4/SvlmJ) and Pparα-null mice (129S4/SvJae-Pparαtm1Gonz/J), male	Liver	↑ 8-OHdG (only in Pparα-null mice)	PFOA, 12.5, 25, or 50 µmol/kg per day	Oral, daily for 4 wk	No change in 8-OHdG in the wildtype mice; the ablation of Pparα exacerbated PFOA-induced oxidative stress	Minata et al. (2010)
8-OHdG, MDA, 8-OHdG by ELISA, MDA by HPLC	Rat, Sprague-Dawley, male	Urine	↑ 8-OHdG, ↑ MDA	PFOA, 10, 33, or 100 mg/kg per day	Oral gavage on days 4, 5, and 6	Urine 8-OHdG and MDA tested from day 1 to day 10; both biomarkers increased from day 3 or 4, peaked on day 5–7, then returned to baseline by day 8 or 10	Rigden et al. (2015)
ROS, MDA, GSSG, CAT, SOD, and GSH, by DCFH-DA for ROS, assay kits for other biomarkers	Rat, Sprague-Dawley, male	Liver	↑ 8ROS, ↑ MDA, ↑ GSSG, ↓ SOD, ↓ CAT, ↓ GSH, ↓ GSH/GSSH	PFOS, 1 or 10 mg/kg per day	Oral, daily for 28 days	Significant dose-dependent changes in biomarkers; the observed induction of oxidative stress was also supported by increases in serum NO and liver *Nos2* mRNA expression	Han et al. (2018a)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, GSH, SOD, and CAT, by TBARS assay, GSH ELISA, SOD kit, CAT kit	Mouse, Balb/c, male	Liver	↓ MDA, ↑ GSH, ↓ SOD, ↑ CAT	PFOA, 0, 0.08, 0.31, 1.25, 5, or 20 mg/kg per day	Gavage, daily for 28 days	Multiple groups and daily dosing; PFOA disturbed the antioxidant defence system in the liver, but did not significantly induce oxidative stress	Yan et al. (2015a)
8-iso-pg-PGF2α; *Sod1*, *Sod2*, *Gpx2*, *Cat*, *Nqo1* expression, by LC/MS for 8-iso-pg-PGF2α, qRT-PCR for the other biomarkers	Mouse, C57BL/6, male	Liver, pancreas	Pancreas: ↑ all biomarkers, except for *Cat* Liver: ↑ 8-iso-pg-PGF2α, ↑ *Sod1*, ↑ *Sod2*, ↑ *Cat*, and ↑ *Nqo1* dosed with 2.5 or 5 mg/kg; no change in *Gpx2* expression	PFOA, 0, 0.5, 2.5, or 5 mg/kg per day	Gavage, daily for 7 days	LC-MS is a robust method for 8-iso-pg-PGF2α measurement; qRT-PCR is considered to be reliable	Kamendulis et al. (2014)
MDA, SOD, CAT, TrxR, by commercial kits	Mouse (conditional *Kras*^{G12D} mouse model [*LSL-Kras*^{G12D} and Pdx-1 Cre mice])	Pancreas	↑ MDA, ↑ SOD, ↑ CAT, ↑ TrxR	PFOA, 5 ppm in drinking-water	Drinking-water, 4 mo or 7 mo exposure; mice were of age 6 or 9 mo at the end of the dosing period	MDA protein level increased only at age 6 mo; SOD enzyme activity increased at both 6 and 9 mo, whereas CAT and TrxR enzyme activities were increased only at age 9 mo	Kamendulis et al. (2022)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
GSH, CAT, GPx, GR, GST, by spectrometry	Rat, Wistar, male and female	Liver	↑ CAT, ↑ GPx, No change in GSH, ↑ GR, ↓ GST (male), GST – no effect (female), ↑ peroxisomal β-oxidation, ↑ microsomal NADP-dependent lipid peroxidation (male, age 26 weeks)	PFOA, 0.01% of diet	Oral, 2 or 26 wk	Increases in peroxisomal β-oxidation in male and female rats after 2 or 26 wk of PFOA exposure, plus an increase in microsomal NADP-dependent lipid peroxidation, and changes in other biomarkers supported the induction of oxidative stress in male and female rats by PFOA	Kawashima et al. (1994)
Oxidized lipid products (15-F_{2t}-IsoP, 5-F_{2t}-IsoP, 8-F_{3t}-IsoP, 4(RS)-4-F_{4t}-NeuroP, 17(*RS*)-F_{2t}-dihomo-Iso-P, Iso-F, NeuroF, 17(*RS*)-SC-Δ^{15}-11-dihomo-IsoF, 7β-hydroxycholesterol, 7-ketocholesterol, 27-hydroxycholesterol, 9(*S*)-HETE, 11(*S*)-HETE, 9(*S*)-HETE, 20-HETE, 5(S)-HETE, 8(S)-HETE, 12(*S*)-HETE, 15(*S*)-HETE), SOD, CAT, by LC-MS/MS, and assay kits	Mouse, CD-1, pregnant and adult females and fetuses	Liver, brain, kidney	No changes in SOD or CAT, significant increases in various oxidized lipid products in fetal liver, brain, and kidney	PFOS, 3 mg/kg per day	Oral, pregnant mice from GD1 to GD17, adult female mice daily for 14 days	Comprehensive evaluation of oxidized lipid products derived from the lipid peroxidation of polyunsaturated fatty acid; both non-enzymatic and enzymatic oxidation (CYP and LOX) products were measured. Changes in oxidative stress biomarkers were more significant in the fetuses	Lee et al. (2015)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, H_2O_2, SOD, CAT, by TBARS, assay kits	Mouse, Kunming, male	Testis	↑ MDA, ↓ SOD, ↓ CAT	PFOA, 0, 2.5, 5, or 10 mg/kg per day	Oral, daily for 14 days	Oxidative stress was further supported by a reduction in Nrf2 expression	Liu et al. (2015a)
MDA, H2O2, SOD, CAT, by assay kits	Mouse, Kunming, male	Liver	↑ MDA, ↑ H_2O_2, ↓ SOD, ↓ CAT	PFOA, 10 mg/kg per day	Oral, daily for 14 days	Oxidative stress was further supported by a reduction in Nrf2 expression	Liu et al. (2015b)
MDA, RONs, NO, GPx, GST, SOD, CAT, GSH, By TBARS, DCFH-DA, Griess reaction, 1-chloro-2,4-dinitrobenzene, autoxidation of epinephrine, H_2O_2, *o*-phthaldialdehyde	Rat, Wistar, male	Liver, kidney	↑ RONs, ↑ NO, ↓ GPx, ↓ GST, ↓ SOD, ↓ CAT, ↓ GSH, ↑ MDA	PFOA, 5 mg/kg per day	Oral, daily for 28 days	Evidence of oxidative stress is further supported by increases in xanthine oxidase and myeloperoxidase. NAC alleviated the level of oxidative stress	Owumi et al. (2021a)
MDA, RONs, NO, GPx, GST, SOD, CAT, GSH, by TBARS, DCFH-DA, Griess reaction, 1-chloro-2,4-dinitrobenzene, autoxidation of epinephrine, H_2O_2, *o*-phthaldialdehyde	Rat, Wistar, male	Testis, epididymis	↑ MDA, ↑ RONs (testes only), ↑ NO, ↓ GPx (epididymis only), ↓ GST (testes only), ↓ SOD, ↓ CAT, ↓ GSH	PFOA, 5 mg/kg per day	Oral, daily for 28 days	Evidence of oxidative stress is further supported by increases in xanthine oxidase and myeloperoxidase; NAC alleviated the level of oxidative stress	Owumi et al. (2021b)
MDA, H_2O_2, by assay kits	Mouse, Kunming, male	Liver	↑ MDA, ↑ H_2O_2	PFOA, 0, 2.5, 5, or 10 mg/kg per day	Oral, daily for 14 days	Significant dose-dependent increases in biomarkers	Yang et al. (2014)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, GSH, GSSG, by assay kits	Mouse, C57BL/6, male	Liver	↑ MDA, ↓ GSH/GSSG	PFOS, 0, 0.003%, 0.006%, or 0.012% of diet	Oral, daily for 14 days 0.003% for 6 wk	Increase in MDA and decrease in GSH/GSSG ratio mice consuming either a normal diet or a marginal methionine/choline-deficient diet containing PFOS; oxidative stress biomarker data were only available for the 0.003% PFOS groups; choline reduced oxidative stress	Zhang et al. (2016a)
ROS, by CM-DCF-DA	Mouse, ICR, female	Oocyte	↑ ROS	PFOA, 0, 1, or 5 mg/kg per day	Oral, daily for 28 days	ROS significantly increased in a dose-dependent manner; increase in γH_2AX, a DNA damage marker provided supportive evidence for oxidative stress	Zhang et al. (2022a)
MDA, SOD, GPx, ROS, by TBARS assay for MDA, nitroblue tetrazolium-illumination method for SOD, benzoic acid method for GPx, DCFH-DA for ROS	Mouse, Balb/c, male	Epididymis, sperm cells	↑ MDA, No change in SOD, ↓ GPx, ↓ SOD/MDA	PFOA, 0, 1.25, 5, or 20 mg/kg per day	Gavage, daily for 28 days Sperm cells were exposed to PFOA at 0, 100 or 400 µM for 1, 2, 4, or 8 h	No available method for ROS measurement in epididymis; therefore, sperm cells were isolated from mice for in-vitro PFOA exposure	Lu et al. (2016b)
MDA, SOD, GPx, by TBARS for MDA, xanthine oxidase assay for SOD, DTNB assay for GPx	Mouse, Kunming, pregnant female	Liver	↑ MDA, ↓ SOD, ↓ GPx	PFOA, 0, 1, 5, 10, 20, or 40 mg/kg per day	Gavage, daily from GD1 to GD7, killed on GD9	Dose-dependent effects observed for all biomarkers tested in the liver; uterine cell apoptosis was observed	Zhang et al. (2021b)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, H_2O_2, SOD, CAT, by commercial kits	Mouse, Kunming, pregnant female	Ovary	↑ MDA, ↑ H_2O_2, ↓ SOD, ↓ CAT	PFOA, 0, 2.5, 5, or 10 mg/kg per day	Gavage, daily from GD1 to GD7 or GD13	Significant increases in MDA and H_2O_2, and decreases in SOD and CAT on GD13 (dose-dependent); significant increase in MDA on GD7 (not dose-dependent); mixed and non-significant results for H_2O_2, SOD, and CAT on GD7	Chen et al. (2017)
mtROS, by immunofluorescent microscopy (MitoSOX Red/MitoTracker Green)	Mice, unspecified strain, female	Granulosa cells/ oocytes	↑ mtROS	PFOA, 4 mg/kg per day for 30 days	Oral, in drinking-water		Zhang et al. (2023a)
MDA, SOD, GPx, by ELISA kits	Rat, Sprague-Dawley, male	Testis	↑ MDA, ↓ SOD, ↓ GPx	PFOA, 0.01 g/kg per day	Oral gavage daily for 30 days	Both low and high doses of lipoic acid protected rats against reproductive damage by reducing oxidative stress biomarkers induced by PFOA	Zhang et al. (2023c)
MDA, SOD, GPx, by commercial kits	Rats, strain not specified (albino), male	Jejunum	↑ MDA, ↓ SOD, ↓ GPx,	Potassium salt of PFOS, 5 mg/kg per day	Oral gavage daily for 28 days	Co-exposure with lemongrass essential oil (100 mg/kg per day) restored the levels of the biomarkers in rats dosed with 5 mg/kg per day	Shalaby et al. (2023)
MDA, SOD, GPx, NOX4, by commercial kits and immunofluorescence staining	Mouse, C57BL/6, male	Serum (MDA, SOD, GPx); kidney (NOX4)	↑ MDA, ↓ SOD ↓ GPx	PFOS, 5 mg/kg per day	Oral gavage, 28 days	Cannabidiol partially restored the levels of markers	Du et al. (2023)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, GPx, ROS, by assay kits (A003-1, A005-1) for MDA and GPx, DCFH-DA for ROS (in vitro)	Rat, Sprague-Dawley, male	Kidney	↑ MDA, ↓ GPx	PFOS, 1 or 10 mg/kg	Intraperitoneal injections every other day for 15 days	↑ MDA and ROS (significant at either dose), ↓ G-Px (significant only at 10 mg/kg); approx. 12% apoptosis at 10 mg/kg dose	Tang et al. (2022)
HO-1, SOD, Nrf2, by gene expression (mRNA) assays	Mouse, C57BL/6, male	White adipose cells	↑ Nrf2 expression, HO-1 and SOD: non-significant increases	PFOS, 100 µg/kg per day	Oral gavage for 36 days	Gene expression of oxidative stress biomarkers was measured	Xu et al. (2016)
MDA, H_2O_2, SOD, GPx, by TBARS, hydrogen peroxide kit, ELISA kits	Mouse, DBA/1J, male	Paw tissue	↑ MDA, ↓ GPx, ↓ SOD, H_2O_2 non-significant increase	PFOS, 10 mg/kg	Rheumatoid arthritis was induced on days 1 and 21; daily oral gavage of PFOS on days 21–35	Paw tissue was extracted for analyses; PFOS induced oxidative stress in normal mice; the effect was enhanced in mice with rheumatoid arthritis	D'Amico et al. (2022)
MDA, CAT, GSH, by commercial kits	Mouse, C57BL/6, male	Ileum and colon	↑ MDA (colon and ileum), no changes in CAT, ↑ GSH versus PFBS	PFOS, 500 µg/L in drinking-water	PFOS in drinking-water for 28 days	TNF-α and IL-1β expression was also significantly increased in ileum but not in colon	Chen et al. (2023b)
MDA, H_2O_2, SOD, by assay kits	Mouse, Kunming, male	Liver	↑ MDA, ↑ H_2O_2, ↓ SOD	PFOS, 10 mg/kg per day	Daily oral gavage for 21 days	Grape seed proanthocyanidin extract was shown to be protective against PFOS-induced oxidative stress	Huang et al. (2020)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, SOD, CAT, GPx, by assay kits	Mouse, C57BL/6J male	Liver	↑ MDA, ↓ SOD, ↓ CAT, ↓ GPx	PFOA, 5 mg/kg per day	Daily oral gavage for 6 wk, followed by 0.5 mL saline, with or without gastrodin, daily for 8 wk	Gastrodin, isolated from the root of *Gastrodia elata* Blume, partially protected against the oxidative stress caused by PFOS	Ma et al. (2021)
MDA, GSH, SOD, MPO, by assay kits	Rat, Sprague-Dawley, pregnant female	Lung	↑ MDA, ↑ MPO ↓ SOD, ↓ GSH	PFOS, 0, 0.1, or 2 mg/kg per day	Daily from GD0 to GD21	2 male and 2 female pups were randomly selected for the oxidative biomarker assays; significant oxidative stress was observed in the pups' lungs	Chen et al. (2012)
Peroxidation (MDA), by TBARS	Mouse, C57BL/6, male	Liver	No change in MDA	PFOA, 0, 0.1, 1, or 5 mg/kg	Oral, via drinking-water containing 0.55, 5.5, or 28 mg/L PFOA	No significant increase in liver MDA in PFOA-treated mice; liver MDA significantly increased in CCl_4-treated mice (positive control)	Crebelli et al. (2019)
mtROS, by DCFH-DA	Mouse, NMRI, pregnant female	Liver, brain, heart of fetus and placenta	↑ ROS in liver, brain, and heart of fetus but not placenta	PFOA, 0, 1, 10, or 20 mg/kg	Intraperitoneal injections, GD5–GD9	Significant increase in mtROS production with PFOA at 10 and 20 mg/kg (brain), and 20 mg/kg (liver, heart)	Salimi et al. (2019)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, SOD, T-AOC, by TBARS, xanthine oxidase, Fe^{3+} reduction methods	Mouse, Kunming, male and female pups	Liver, brain	No change in MDA, ↓ SOD, ↓ T-AOC	PFOA, 50 mg/kg	0 or 50 mg/kg by subcutaneous injection on PND7, 14, 21, 28, and 35	No changes in MDA levels in liver or brain at any time point; ↓ SOD in male pup brain on PND7 and PND21 and in female pup liver on PND14; ↓ T-AOC in male pup brain on PND21, in female pup liver on PND21, and in male pup liver on PND7 and PND14; ↑ T-AOC in male pup liver on PND21	Liu et al. (2009)
GSH, GR, by biochemical assay kits	Mouse, Balb/c, male	Liver	↑ GSH, ↑ GR	PFOS, effect at 5 mg/kg per day only	0.2, 1, or 5 mg/kg per day; oral gavage, 28 days		Wang et al. (2022c)
MDA, GSH, GPx, CAT, Cu-Zn-SOD, by TBARS for MDA, DTNB for GSH, assay kits for other biomarkers	Mouse, Balb/c, male	Liver, brain	Liver (no change in MDA, ↑ GSH, ↑ CAT, ↓ Cu-Zn-SOD, ↓ GPx). Brain (↑ MDA only for 30 mg/kg, ↓ GPx, no changes in other biomarkers)	PFOA, 15 or 30 mg/kg per day	Oral, 10 days One group received PFOA 30 mg/kg for another 10 days	Depletion of the antioxidant system suggested potential oxidative stress in the liver and brain	Endirlik et al. (2022)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
Hepatic peroxisomal β-oxidation and CAT, by spectrophotometry at 340 nm for β-oxidation and 240 nm for CAT activity	Rat, Fischer 344, male	Liver	↑ β-oxidation ↓ CAT	PFOA, 150 mg/kg	150 mg/kg bw PFOA in corn oil given to rats of age 4, 10, 20, 50, and 100 wk	Rats were killed at pre-set time points between days 0 and 28 after exposure for oxidative stress determination; β-oxidation significantly increased in all age groups; CAT significantly reduced only in the rats aged 100 wk, probably due to senescence	Badr and Birnbaum (2004)
β-oxidation, by [1–^{14}C]palmitoyl-CoA as the substrate	Rat, Crl:CD BR, male	Liver	↑ β-oxidation	PFOA (ammonium salt): 0.2, 2, 20, or 40 mg/kg per day	0.2, 2, 20, or 40 mg/kg per day PFOA, daily oral gavage for 14 days	No other oxidative stress biomarkers were measured	Liu et al. (1996)
MDA, H_2O_2, GSH, SOD, by assay kits	Mouse, strain not specified, male	Liver	↑ MDA, ↑ H_2O_2, ↓ SOD, ↓ GSH	PFOS, 10 mg/kg per day	10 mg/kg per day PFOS, oral gavage daily for 3 wk	The study also showed the protective effects of naringin against PFOS-induced oxidative stress	Lv et al. (2018)
mtROS, by fluorescent microscopy	Mouse, strain not specified, female	Cumulus–oocyte complexes	↑ mtROS at 250, 500, and 750 μM	PFOA, 50–2000 μM	PFOA for 24 h	Significant dose-dependent increases in mtROS	Zhang et al. (2023b)

bw, body weight; CAT, catalase; CCl_4, carbon tetrachloride; CoA, coenzyme A; CYP, cytochrome P450; DCF, dichlorofluorescein; DCFH-DA, 2′,7′-dichlorodihydrofluorescein diacetate; DEN, diethylnitrosamine, dG, 2′-deoxyguanosine; ECD, electrochemical detection; ELISA, enzyme-linked immunosorbent assay; ESR, electron spin resonance; GD, gestational day; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; GST, glutathione *S*-transferase; h, hour(s); γH_2AX, γ-H2A histone family member X; HETE, hydroxyeicosatetraenoic acid; H_2O_2, hydrogen peroxide; HPLC, high-performance liquid chromatography; IsoP, isoprostane; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOX, lipoxygenase; MDA, malondialdehyde; mo, month(s); MPO, myeloperoxidase; mRNA, messenger RNA; mtROS, mitochondrial reactive oxygen species; NAC, *N*-acetylcysteine; NeuroP, neuroprostane; NO, nitrogen oxide; NR, not reported; NRf2, NF-E2-related factor 2; 8-OHdG, 8-hydroxydeoxyguanosine; 8-oxodG, 8-oxo-2′-deoxyguanosine; PFBS, perfluorobutanesulfonate; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PGF, prostaglandin; PND, postnatal day; ppm, parts per million; qRT-PCR, quantitative reverse transcription–polymerase chain reaction ; RONS, reactive oxygen and nitrogen species; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TBARS, thiobarbituric acid-reactive substances; wk, week(s).

[a] +, positive; ↑, increase; ↓, decrease.

the PFOA-treated mice. Rutin was able to reduce the oxidative stress induced by PFOA.

Takagi et al. (1991) measured 8-OHdG in the DNA of liver and kidney from rats dosed with PFOA. In the first experiment, male Fischer 344 rats were exposed to a single i.p. dose of PFOA (100 mg/kg) in corn oil (5 rats per group) and killed 1, 3, 5, or 8 days after dosing. In a second experiment (a feeding trial), rats were fed a powdered diet ad libitum containing 0.02% PFOA for 2 weeks (5 rats per group; the dose as mg/kg per day was not estimated). After necropsy, liver and kidney DNA was isolated. Significant increases in 8-OHdG levels were found in the livers of rats injected with a single dose of PFOA on days 3, 5, and 8. No effect of PFOA on 8-OHdG level was observed in the kidney. In the feeding trial, 8-OHdG was significantly increased in the liver but not in the kidney. [The Working Group noted that relatively high 8-OHdG levels were reported in both liver and kidney DNA of the control group rats. In addition, HPLC with electrochemical detection, as performed in this study, was considered to be a better technique than assay kits for 8-OHdG measurement.]

Zou et al. (2015) orally dosed male mice ($n = 8$) with PFOA at 10 mg/kg per day for 14 days. Oxidative end-points including ROS, 8-OHdG, H_2O_2, MDA, SOD, and CAT were measured in the liver using commercial kits. The levels of 8-OHdG, H_2O_2, and MDA were significantly increased in PFOA-treated mice compared with control mice, whereas SOD and CAT levels were significantly decreased. When the mice were co-treated with PFOA (10 mg/kg per day) and the flavonoid quercetin (75 mg/kg per day) for 14 days, quercetin was found to be able to reverse the PFOA-induced effects on these end-points.

In a study by Minata et al. (2010), 39 wildtype (129S4/SvlmJ) and 40 PPARα-null (129S4/SvJae-Pparαtm1Gonz/J) male mice were randomly divided into four groups and orally dosed with PFOA (0, 12.5, 25, or 50 μmol/kg per day) for 4 weeks. The level of 8-OHdG in the liver was measured by HPLC using ultraviolet and electrochemical detectors. No changes in 8-OHdG levels were observed in wildtype mice, whereas the 8-OHdG levels were increased in a dose-dependent manner in the PPARα-null mice, with a significant increase in the group at 50 μmol/kg.

Rigden et al. (2015) dosed male Sprague-Dawley rats with PFOA and measured urinary 8-OHdG and MDA. Groups of five rats were dosed by gavage with PFOA (0, 10, 33, or 100 mg/kg bw once per day on days 4, 5, and 6). Urine samples were collected from day 1 to day 10. The two highest doses of PFOA induced significant increases in urinary MDA. The urinary 8-OHdG levels were significantly increased at a PFOA dose of 100 mg/kg. The levels of 8-OHdG started rising from day 3 and peaked from day 4 to day 7, depending on the dose, then decreased to baseline on days 8–10. The MDA levels started to increase from day 4, peaked on day 5, then decreased to baseline on day 8. The rises and peaks in 8-OHdG and MDA levels seemed to occur at about the same time after PFOA dosing.

[The Working Group noted that, altogether, the results support oxidative effects of PFOA, although the commercial kits may not be specific for the particular end-points measured, and the studies did not include a positive control group.]

Groups of six Sprague-Dawley male rats were dosed with PFOS at 0, 1, or 10 mg/kg per day for 28 days (Han et al., 2018a). The oxidative stress end-points measured in the liver included ROS production (measured by DCFH-DA), MDA, GSSG, CAT, SOD, and GSH (measured with assay kits). The MDA and GSSG levels were found to be significantly increased in a dose–response manner, whereas GSH, SOD, CAT, and GSH/GSSG were significantly reduced. An increase in serum NO, coupled with an increase in mRNA expression of inducible nitric oxide synthase (iNOS) in the liver, provided additional evidence for oxidative stress induction.

Yan et al. (2015a) dosed male Balb/c mice (age 6–8 weeks) with PFOA by oral gavage (0, 0.08, 0.31, 1.25, 5, or 20 mg/kg per day) daily for 28 days (the second gavage was used for the administration of additional chemicals, but the mice that yielded the results described here only received water in this second gavage). The MDA, GSH, SOD, and CAT levels were measured in the liver after necropsy. The levels of MDA in the liver were found to be significantly decreased at all doses compared with the control. The liver GSH protein levels decreased, but not significantly, at the three lowest doses but significantly increased when the mice were dosed with 5 or 20 mg/kg. The authors also reported increased CAT (≥ 3 mg/kg) and decreased SOD (≥ 5 mg/kg) enzyme activities in the livers of the mice. However, the dosing regimen was not provided in the manuscript. The study also analysed the mRNA expression of oxidative stress-responsive genes, including *Cat*, *Sod1*, *Sod2*, and *Sesn1*, in the livers of mice after exposure to PFOA for 28 days. *Cat* mRNA expression was unchanged after PFOA exposure. *Sesn1* mRNA expression was significantly increased at the highest dose of 20 mg/kg, *Sod1* mRNA was significantly increased at doses of 1.25 and 5 mg/kg, and *Sod2* mRNA was significantly increased at 5 mg/kg. However, the mRNA expression changes for these three genes were considered moderate. This study suggested that the oxidative stress defence system was affected by repeated doses of PFOA, although oxidative stress, as indicated by the MDA concentration, did not increase in the mouse liver.

Kamendulis et al. (2014) dosed male C57BL/6 mice with PFOA at 0.5, 2.5, or 5 mg/kg for 7 days. The lipid peroxidation product 8-iso-PGF2α (by LC-MS) and the expression of the antioxidation response genes *Sod1*, *Sod2*, *GPx2*, *Cat*, and *Nqo1* (by quantitative reverse transcription-polymerase chain reaction, qRT-PCR) were measured in the liver and pancreas. Cerulein was used as a positive control. The levels of 8-iso-PGF2α were significantly increased in the liver and pancreas upon treatment with PFOA. In the pancreas, all end-points, except for CAT, were significantly and positively correlated with PFOA exposure. In the liver, *Sod1*, *Sod2*, *Cat*, and *Nqo1* expression was significantly increased by PFOA doses of 2.5 and 5 mg/kg. PFOA treatment did not have an effect on *Gpx2* expression in the liver. It was noted that cerulein treatment did not significantly induce any biomarkers, except for 8-iso-PGF2α, compared with the negative control. In another study (Kamendulis et al., 2022), PFOA exposure was thought to promote pancreatic cancer in a conditional *Kras*^{G12D} mouse model (*LSL-Kras*^{G12D} and *Pdx-1 Cre* mice). Drinking-water containing PFOA at 5 ppm was administered to mice (age 2 months) for 4 or 7 months. The mice, which were aged 6 or 9 months at the end of the dosing period, were examined for levels of MDA, and the antioxidant enzymes SOD, CAT, and thioredoxin reductase in the pancreas were measured using commercial kits. The MDA protein levels were significantly increased in mice only at age 9 months, whereas SOD enzyme activity was significantly increased at age 6 and 9 months. The CAT and thioredoxin reductase enzyme activities were only significantly increased in mice aged 6 months.

Male and female Wistar rats were fed a rodent diet containing PFOA at 0.01% w/w for 2 or 26 weeks (Kawashima et al., 1994). Microsomal NADPH-dependent lipid peroxidation, GSH, GPx (with H_2O_2 or cumene hydroperoxide), glutathione reductase (GSR), GST, CAT (H_2O_2 assay), and peroxisomal β-oxidation were measured in the liver. Peroxisomal β-oxidation was significantly increased in both male and female rats after 2 and 26 weeks. CAT levels were also increased compared with the controls at that time. In the livers of male rats, no changes in GSH were observed after 2 or 26 weeks, whereas microsomal NADPH-dependent lipid peroxidation, GPx, and GSR were increased, but were only significantly higher in male rats after 26 weeks of exposure. The GST activities

towards 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene were significantly reduced in male rats after both 2 and 26 weeks of PFOA exposure, whereas in female rats, the GST activities towards 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene were unchanged.

Lee et al. (2015) studied the effects of prenatal PFOS exposure on the fetal development of CD-1 mice. Pregnant mice were orally dosed with PFOS at 3 mg/kg per day from GD1 until GD17. In another group, non-pregnant female adult mice were dosed with PFOS at 3 mg/kg per day for 14 days. Oxidative stress biomarkers were measured in the mothers, fetuses, and non-pregnant mice. Oxidized lipid products derived from the lipid peroxidation of polyunsaturated fatty acids were measured by LC-MS/MS, and SOD and CAT were measured using assay kits. CAT was not measured in the fetuses because of insufficient sample sizes. No significant changes in SOD or CAT levels were observed in the livers or placentas of the pregnant mice. Levels of non-enzymatically oxidized lipid products, including isoprostanes (IsoP) 15-F_{2t}-IsoP, 5-F_{2t}-IsoP, and 8-F_{3t}-IsoP, and neuroprostane (NeuroP) 4(*RS*)-4-F_{4t}-NeuroP, 7β-hydroxycholesterol, and 7-keto-cholesterol, were significantly increased in fetal livers compared with levels in livers of control mice. Effects on enzymatically oxidized lipid products of the lipoxygenase (LOX) and CYP450 pathways were also examined. Upon PFOS treatment, the level of 15(*S*)-hydroxyeicosatetraenoic acid (HETE) decreased in fetal livers, whereas the level of 5(*S*)-HETE increased in maternal livers. As for products of the CYP450 pathway, increased levels of 27-hydroxycholesterol were only observed in fetal livers. In a second experiment, the effects of PFOS exposure on adult female brains and kidneys were compared with those on fetuses. With respect to the non-enzymatic pathway, arachidonic acid, adrenic acid, and neurofuran levels were significantly elevated in PFOS-dosed adult brains compared with non-exposed adults. However, only 8-F_{3t}-IsoP levels were significantly increased in the brains of PFOS-exposed fetuses compared with controls. With respect to the enzymatic pathway, oxidized lipid products were significantly increased in fetal brains, but not in adults. These included 9(*S*)-HETE (CYP-mediated), 5(*S*)-HETE, and 8(*S*)-HETE (LOX-mediated). These results indicate that oxidative stress due to PFOS exposure is more severe in fetuses than in their dams. In the kidneys of PFOS-dosed fetuses, significant increases in all the measured non-enzymatically oxidized lipid products, including 15-F_{2t}-IsoP, 5-F_{2t}-IsoP, 8-F_{3t}-IsoP, 4(RS)-4-F_{4t}-NeuroP, 17(*RS*)-F_{2t}-dihomo-IsoP, isofuran (IsoF), neurofuran, and 17(*RS*)-SC-Δ^{15}-11-dihomo-IsoF, were observed; whereas in PFOS-dosed adult female mice, only 15-F_{2t}-IsoP was increased, and a decrease in levels was observed for 5-F_{2t}-IsoP, 4(RS)-4-F_{4t}-NeuroP, 17(*RS*)-F_{2t}-dihomo-IsoP, and 17(*RS*)-SC-Δ^{15}-11-dihomo-IsoF. The results indicated that oxidative stress induced by PFOS is more severe in fetuses than in their dams or adult female mice.

In a study by Liu et al. (2015a), adult male Kunming mice were dosed with PFOA at 2.5, 5, or 10 mg/kg per day for 14 days. The testicular levels of MDA, measured by TBARS, were significantly increased in mice exposed to PFOA at 5 or 10 mg/kg. Additionally, a significant increase in H_2O_2, measured using the reaction with molybdenum acid, was observed. The induction of oxidative stress was further indicated by the reduction in *Nrf2* gene and protein expression, as well as reduced SOD and CAT activities (assay kits) in all the PFOA-treated mice.

Liu et al. (2015b) exposed male Kunming mice to PFOA orally at 10 mg/kg per day for 14 days. A second group of PFOA-dosed mice was co-treated orally with grape seed proanthocyanidin extract at 150 mg/kg per day. MDA, H_2O_2, SOD, and CAT were measured in liver homogenates using assay kits (Jiancheng Institute of Biotechnology, Nanjing, China). The hepatic levels of MDA and H_2O_2 were significantly

increased in PFOA-treated mice, whereas the SOD and CAT activities were reduced. In addition, *Nrf2* mRNA expression and protein levels were also decreased. The co-treatment with grape seed proanthocyanidin extract was able to restore the oxidative status of the livers of PFOA-dosed mice.

Another study investigated both the hepatic and renal effects of PFOA in male Wistar rats (Owumi et al., 2021a). Groups of 10 rats were dosed orally with PFOA at 0 or 5 mg/kg per day for 28 days. Oxidative biomarkers were measured in the liver and kidney. The levels of reactive oxygen and nitrogen species (RONS) (measured with DCFH-DA), NO (Griess reaction), xanthine oxidase, and myeloperoxidase were significantly increased in both the liver and kidney upon PFOA exposure. The levels of GPx (measured using H_2O_2 and Ellman reagent), GSH (H_2O_2), GST (1-chloro-2,4-dinitrobenzene), SOD (autoxidation of epinephrine), and CAT (H_2O_2) were significantly decreased in the liver and kidneys of PFOA-exposed rats. Co-treatment with NAC was found to alleviate the oxidative stress of the PFOA-dosed rats. The effects of oxidative stress on testicular function in male Wistar rats were reported in another study (Owumi et al., 2021b). Groups of 10 rats were dosed orally with PFOA at 0 or 5 mg/kg per day for 28 days. The testes and epididymides were removed for the same suite of biomarker measurements to that described above (Owumi et al., 2021a). The levels of MDA, NO, xanthine oxidase, and myeloperoxidase were significantly increased in both the testes and epididymides of PFOA-exposed rats, whereas RONS levels were significantly increased only in the testes. The antioxidants SOD, CAT, and GSH were all significantly decreased upon PFOA treatment in both the testes and epididymides, the GPx levels were significantly decreased in the epididymides, and the GST levels were significantly decreased in the testes. Co-treatment with NAC alleviated the oxidative stress observed in the PFOA-dosed rats.

Yang et al. (2014) demonstrated oxidative stress in male Kunming mice dosed with PFOA at 2.5, 5, or 10 mg/kg per day for 14 days. The biomarkers MDA and H_2O_2 were measured using assay kits from the Jiancheng Institute of Biotechnology, Nanjing, and were found to be significantly increased in liver tissue after 2.5, 5, and 10 mg/kg, and 5 and 10 mg/kg doses, respectively.

Zhang et al. (2016a) fed male C57BL/6 mice a normal diet or a marginal methionine/choline-deficient (mMCD) diet, both containing 0.003%, 0.006%, or 0.012% PFOS, for 23 days or 21 days (second replicate). A potential modulatory mechanism affecting hepatic steatosis and oxidative stress was investigated. Both PFOS-containing diets caused weight loss in the mice, and this weight loss was more severe with the mMCD diet. In a choline-supplementation experiment, the levels of MDA, GSH, and GSSG (assay kits) were measured in the livers of mice exposed to 0.003% PFOS in their diet for 6 weeks. The levels of MDA significantly increased, whereas the GSH/GSSG levels significantly decreased in mice fed either the 0.003% PFOS-normal diet or the 0.003% PFOS-mMCD diet. Choline supplementation alleviated the oxidative stress effects of PFOS. No biomarker data were available for the other dosage groups.

Zhang et al. (2022a) studied the effects of PFOA on oocyte maturation in ICR mice dosed with PFOA at 1 or 5 mg/kg per day for 28 days. ROS production measured by chloromethyl derivative of DCFH-DA was significantly increased in oocytes in a dose-dependent manner. PFOA-mediated induction of oxidative stress was further supported by an increase in the DNA damage marker γH2AX.

Another study investigated the effects of PFOA on the mouse epididymis (Lu et al., 2016b). Male BALB/c mice (age 6–8 weeks) were dosed by oral gavage with PFOA at 0, 1.25, 5, or 20 mg/kg per day for 28 days. At necropsy, the epididymides were extracted, and SOD, MDA, and

GPx levels were measured using the nitroblue tetrazolium-illumination, TBARS, and benzoic acid methods, respectively. Because there was no available method for measuring ROS in the epididymis, the assessment of ROS production was performed in sperm cells isolated from the epididymides and treated in vitro with PFOA at 0, 100, or 400 µM for 1, 2, 4, or 8 hours. Rosup was used as a positive control. ROS production in the sperm cells was measured spectroscopically using the DCF-DA method. The study reported significantly increased ROS production in sperm cells after 1 and 2 hours of treatment with 100 µM PFOA and at all time points after treatment with 400 µM PFOA. In the epididymides of the mice, MDA levels were significantly elevated when the mice were treated with PFOA at concentrations of 1.25 or 5 mg/kg per day, but not 20 mg/kg per day. No significant changes in SOD levels were observed upon PFOA exposure. However, the SOD/MDA ratio significantly decreased in the epididymides of mice exposed to PFOA at 1.25 or 5 mg/kg per day. Additionally, GPx levels were significantly decreased when mice were treated with PFOA at 5 or 20 mg/kg per day. In this study, the authors also demonstrated that PFOA disturbed the profile of polyunsaturated fatty acids in the epididymides of PFOA-treated mice. This observation, coupled with the increased oxidative stress in the epididymis, may explain the negative effects of PFOA on the reproductive function of male mice.

Zhang et al. (2021b) studied the mechanisms of PFOA toxicity in the uterus and liver of Kunming mice during early pregnancy. PFOA (1, 5, 10, 20, or 40 mg/kg per day) was administered to pregnant mice by gavage from GD1 to GD7, and they were killed on GD9. Their liver MDA levels significantly increased with increasing PFOA concentration, and the SOD and GPx levels significantly decreased in a dose-dependent manner. [The Working Group noted that the findings of this study suggest that oxidative damage may be involved when PFOA induces liver toxicity and uterine cell apoptosis, leading to possible embryo loss or damage.]

Chen et al. (2017) also studied the effects of PFOA on pregnant mice. Adult female Kunming mice were dosed by oral gavage with PFOA at 2.5, 5, or 10 mg/kg per day from GD1 to GD7 or GD13. Significant dose-dependent elevations in MDA and H_2O_2 and significant dose-dependent decreases in CAT and SOD levels were observed in the ovaries of PFOA-exposed mice on GD13. On GD7, MDA levels also significantly increased in the ovaries of mice at all the tested PFOA concentrations; however, no dose-dependent effects were observed. The levels of H_2O_2, SOD, and CAT were not significantly changed on GD7. Biomarkers were measured using commercial kits.

In a recent study (Zhang et al., 2023a), female mice (strain not reported) were treated with PFOA at 4 mg/kg per day for 30 days. The levels of mt-ROS were significantly increased in granulosa cells upon treatment with PFOA, as assessed using the fluorescence ratio of MitoSOX Red/MitoTracker Green.

In another study on the reproductive effects of PFOA, male Sprague-Dawley rats were dosed by oral gavage with PFOA at 0.01 g/kg bw for 30 days (Zhang et al., 2023c). Levels of MDA, SOD, and GPx in the testes were measured by ELISA kits. Levels of MDA were significantly increased, whereas levels of SOD and GPx were significantly decreased upon PFOA exposure. In addition, in PFOA-treated rats that were subsequently treated with lipoic acid at a dose of 0.1 g/kg per day for 42 days, levels of the markers were restored to those in the control rats. Lipoic acid at the low daily dose of 0.05 g/kg was less effective than the higher dose. Shalaby et al. (2023) reported that when rats were dosed with the potassium salt of PFOS by oral gavage at 5 mg/kg per day for 28 days, jejunal MDA levels were significantly increased, whereas jejunal SOD and GPx levels were significantly decreased. When rats were co-treated with lemongrass

essential oil at 100 mg/kg, 30 minutes before the PFOS dose (5 mg/kg per day) every day for 28 days, the lemongrass essential oil restored the levels of oxidative stress biomarkers.

Du et al. (2023) reported that CBD partially alleviated PFOS-induced apoptosis via the NOX4/ROS/JNK pathway. In this study, one group of male C57BL/6 mice was dosed by oral gavage with PFOS alone at 5 mg/kg per day for 4 weeks. Another group of PFOS-dosed mice was co-treated with CBD at 5 mg/kg by oral gavage daily, 4 hours after the PFOS treatment. The levels of MDA were significantly increased, whereas the SOD and GPx levels were significantly decreased in the serum of PFOS-dosed mice, as was the expression of NOX4, assessed using immunofluorescence staining, in kidney tissue. Co-treatment with CBD partially restored the levels of these markers. In addition, the authors observed alterations of MDA, SOD and GPx (oxidative stress end-points) in mouse kidney HEK293 cells in vitro.

Intraperitoneal injections of PFOS (1 or 10 mg/kg bw) were given to male Sprague-Dawley rats every other day for 15 days (Tang et al., 2022). The kidney MDA levels significantly increased in all PFOS-dosed rats compared with the control group. A significant decrease in GPx level was only observed in the group dosed with 10 mg/kg bw.

Xu et al. (2016) proposed that PFOS induces adipogenesis and glucose uptake in association with an activation of the Nrf2 signalling pathway. Male C57BL/6 mice (age 21 weeks) were given PFOS (100 μg/kg bw) by oral gavage for 36 days. The expression of SOD and HO-1 did not increase, but *Nrf2* mRNA expression was significantly increased upon PFOS exposure, implying an upregulation of the Nrf2 signalling pathway and adipogenic gene expression in epididymal white adipose tissue. [The Working Group considered that the activation of the Nrf2 pathway suggested an oxidative stress-mediated effect of PFOS on adipogenesis.]

D'Amico et al. (2022) studied the oxidative effects of PFOS exposure on healthy DBA/1J mice and mice with rheumatoid arthritis (induced by type II chicken collagen and complete Freund's adjuvant administered intradermally on days 0 and 21). PFOS (10 mg/kg bw) and other endocrine disruptors were given by oral gavage from day 21 to 35. Paw tissue was analysed for oxidative stress biomarkers, including MDA, H_2O_2, SOD, and GPx. PFOS exposure significantly induced oxidative stress in healthy mice, and this effect was more severe in mice with rheumatoid arthritis.

The induction of metabolic disturbances by PFOS in different regions of the mouse gut was studied by Chen et al. (2023a). Male C57BL/6 mice (age 6 weeks) were exposed to PFOS at 500 μg/L in drinking-water for 28 days. CAT, GSH, and MDA levels were measured using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China). The MDA levels significantly increased in the ileum and colon of mice exposed to PFOS, and this effect was more marked in the ileum than in the colon. However, no significant changes in CAT or GSH levels were observed in the ileum and colon of PFOS-exposed mice compared with control mice. The levels of tumour necrosis factor alpha (TNF-α) and interleukin-1-beta (IL-1β) were also significantly increased in the ileum but not in the colon of mice treated with PFOS.

In a study by Huang et al. (2020) in mice, the authors investigated the protective effects of grape seed proanthocyanidin extract against PFOS-induced oxidative stress. Male Kunming mice were dosed with PFOS (10 mg/kg bw) via gastric gavage daily for 21 days. Levels of MDA, H_2O_2, and SOD were measured in the liver. PFOS-induced oxidative stress was indicated by an increase in MDA and H_2O_2 levels and a decrease in SOD level. The extract was shown to be protective against PFOS-induced oxidative stress.

In another study (Ma et al., 2021), gastrodin, a water-soluble organic compound isolated from the root of *Gastrodia elata* Blume, was shown to reduce the oxidative stress induced by PFOA. C57BL/6J mice were dosed with PFOA (5 mg/kg bw) by oral gavage daily for 6 weeks, which was followed by treatment with gastrodin (20 mg/kg bw) or vehicle (saline) daily for 8 weeks. After PFOA administration, the liver MDA levels significantly increased, whereas the CAT, SOD, and GPx levels significantly decreased. The administration of gastrodin significantly reduced PFOA-induced oxidative stress.

Ma et al. (2023) also investigated the potential for rutin to reduce the PFOA-induced oxidative effects in mice. As reported above, male ICR mice were dosed with PFOA (20 mg/kg bw) daily for 28 days in the presence or absence of rutin. The serum contents of MDA, GPx, and SOD were significantly increased after exposure to PFOA. Rutin was able to reduce the oxidative stress induced by PFOA.

Chen et al. (2012) studied the effects of prenatal PFOS exposure on oxidative stress in the lungs of rat offspring. Pregnant female rats were dosed with PFOS (0.1 or 2 mg/kg bw) between GD1 and GD21 daily by gavage. Fresh lung tissue was collected from two male and two female pups from each group for biomarker analysis on PND0 and PND21. Pups from the dams that received the higher dose (2 mg/kg bw) showed significantly increased levels of MDA and significantly decreased GSH level and SOD activity compared with the controls at both time points. The MDA level showed a small but significant increase in the neonatal lungs of pups from dams exposed to PFOS at 0.1 mg/kg.

Crebelli et al. (2019) conducted a subacute experiment by supplying male C56BL/6 mice (age 6–8 weeks) with drinking-water containing PFOA (28, 5.5, or 0.55 mg/L, corresponding to 5, 1, or 0.1 mg/kg bw per day) for 5 weeks. Liver MDA levels were measured using the TBARS method. The study reported no significant changes in liver lipid peroxidation in any of the PFOA-treated groups. However, CCl_4-treated mice (positive control) showed a significant increase in MDA level.

Salimi et al. (2019) studied the potential of PFOA to induce abortion and developmental toxicity in mice. Pregnant female NMRI mice (age 8–9 weeks) were dosed with PFOA at 1, 10, or 20 mg/kg between GD5 and GD9. mt-ROS production, measured using DCFH-DA, was significantly increased in the liver, brain, and heart of the fetuses, but not in the placenta.

Liu et al. (2009) studied the oxidative effects of PFOS in young mouse pups. The pups of Kunming mice were weaned on PND21, then dosed with PFOS at 50 mg/kg bw once by subcutaneous injection on PND7, PND14, PND21, PND28, or PND35. The levels of MDA, SOD, and TAC were determined in liver and brain 24 hours after exposure. No significant changes in MDA levels in the liver or brain were observed in pups of either sex. In the brain, significant decreases in SOD level were only observed in male pups after injections on PND7 and PND21. In the liver, a significantly decreased SOD level was only observed in female pups after injection on PND14. A significant decrease in TAC was only observed in male pup brains after injection on PND21. In the liver, a significant decrease in TAC was observed in female pups after injection on PND21 and in male pups after injection on PND7 and PND14. However, TAC was significantly increased in male pups after injection on PND21.

Wang et al. (2022c) orally dosed male Balb/c mice with PFOS at 0.2, 1, or 5 mg/kg bw daily for 28 days. GSH levels were significantly increased in the livers of mice exposed to PFOS at 5 mg/kg; however, a non-significant decrease was observed in the two lower-dosed groups. The level of GSR in the liver was increased in all the PFOS-exposed mice, but this only reached significance in the group at the highest dose.

Endirlik et al. (2022) examined the effects of PFOA exposure on the livers and brains of male Balb/c mice. The mice were orally dosed with PFOA at 15 or 30 mg/kg for 10 days. Levels of MDA and GSH were measured using the TBARS method and the 5,5′-dithiobis (2-nitrobenzoic acid) method, respectively. GPx, CAT, and Cu-Zn-SOD levels were measured using assay kits. In the liver, no changes in MDA level, but significantly increased GSH and CAT levels and significantly decreased Cu-Zn-SOD and GPx levels, were observed. In the brain, the MDA level significantly increased in mice exposed to PFOA at 30 mg/kg, whereas the GPx level significantly decreased, and no significant changes in the levels of the other biomarkers were observed. It was suggested that PFOA exposure results in a depletion of antioxidative systems and exhibits neurotoxic effects by inducing oxidative stress.

Badr and Birnbaum (2004) studied the oxidative effects of PFOA in male Fischer 344 rats ranging in age from juvenile (age 4 weeks) to post-puberty (age 10 weeks), mature adulthood (age 20 weeks), middle age (age 50 weeks), and senescence (age 100 weeks). The rats were dosed by gavage with PFOA (150 mg/kg bw) or vehicle. The rats were killed between days 0 and 28 post-dosing for the measurement of hepatic peroxisomal β-oxidation and CAT activity in the liver. Hepatic peroxisomal β-oxidation was increased 3–5-fold in all PFOA-dosed groups, with an increased recovery time in older rats. Liver CAT activity was also significantly lower in senescent livers compared with those from all other groups.

Liu et al. (1996) treated adult male Crl:CD BR (CD) rats with APFO (C8) (0.2, 2, 20, or 40 mg/kg per day) for 14 days. β-Oxidation in the liver was measured using [1-^{14}C]palmitoyl-CoA as the substrate, and potassium cyanide was used to inhibit mitochondrial β-oxidation. Hepatic β-oxidation was significantly increased upon PFOA exposure (≥ 2 mg/kg per day) in a dose-dependent manner.

Lv et al. (2018) showed the antioxidative properties of naringin (NAR, 4′,5,7-trihydroxyflavonone-7-rhamnoglucoside), a naturally occurring flavonoid glycoside isolated from citrus fruits, with respect to PFOS-induced oxidative stress. Male mice were exposed to PFOS by oral gavage (10 mg/kg bw) daily for 3 weeks. Levels of MDA, H_2O_2, SOD, and GSH were measured in the livers of the mice in the presence or absence of NAR (100 mg/kg bw). PFOS exposure significantly increased the MDA and H_2O_2 levels and reduced the SOD and GSH levels. Mice co-treated with NAR were protected from PFOS-induced oxidative stress.

[The Working Group noted that the studies described above reported the effects of acute, subacute, and subchronic repeated-dose treatment (up to 28 days) of PFOA or PFOS in vivo. The agents were mostly administered by oral gavage; the i.p. route, administration via drinking-water, or ad libitum feeding were used only in a few studies. Rodent studies dominated, and the dosing regime was typically 5–10 mg/kg per day. It was noted that such doses were much higher than exposures in humans. Despite this, PFOA and PFOS were shown to induce oxidative stress as shown also in studies in exposed humans and in human cells in vitro.]

(ii) Non-human mammalian systems in vitro

A wide range of experiments in vitro in non-human mammalian cells (primary and immortalized cells derived from different organ systems) have been used to explore the oxidative effects of PFOA and PFOS. The studies are briefly described below.

Lindeman et al. (2012) reported no significant increase in oxidatively damaged DNA (Fpg-modified comet assay) in freshly isolated rat testicular cells treated with 100 or 300 µM PFOA for 24 hours. 1,2-Dibromo-3-chloropropane was used as a positive control.

Liver cells of animal origin have been widely used to explore the oxidative stress-inducing

potential of PFOA and PFOS. Xu et al. (2019) isolated primary hepatocytes from young adult male C57BL/6J mice (age, 6 weeks). The liver cells were exposed to PFOA or PFOS (0, 10, 100, 500, or 1000 μM) for 24 hours. PFOA and PFOS significantly increased the ROS (PFOA at ≥ 500 μM; PFOS at 1000 μM), SOD (PFOA and PFOS at ≥ 500 μM), and GSH (PFOA at ≥ 500 μM; PFOS at 100 μM and 1000 μM) levels and significantly reduced CAT activity at all concentrations. [The Working Group noted that PFOA or PFOS induced cell death at a concentration of 1000 μM and that the increases in oxidative end-points are not relevant at this concentration.] Goines and Dick (2022) also reported that PFOS increased ROS production and reduced the GSH level in HepG2 cells that were treated with 100 μM PFOS for 16 hours.

PFOS was shown to induce ROS production in kidney cells. Wen et al. (2021) exposed rat proximal renal tubular cells (NRK-52E) to PFOS (100 μM) and measured ROS production after 1, 3, 6, and 24 hours of exposure. The exposed cells showed significantly increased ROS production after 1 hour, and the ROS levels remained significantly elevated at all the tested time points.

PFOA and PFOS were shown to induce ROS production in other organ systems. For example, Reistad et al. (2013) examined primary cultures of neurons isolated from rat cerebellum (age, 6–8 days; sex not reported). The cultured neurons were exposed to different concentrations (0, 6, 12, 25, 50, or 100 μM) of PFOA or PFOS for 24 hours. PFOA and PFOS significantly increased ROS production in a dose-dependent manner. The estimated half-maximal effective concentration (EC_{50}) values (± SD) were 25 ± 11 μM and 27 ± 9.0 μM for PFOA and PFOS, respectively. Increased ROS production was observed at concentrations close to the serum PFOA and PFOS concentrations found in occupationally exposed workers.

López-Arellano et al. (2019) studied the effects of PFOA on mouse oocytes in vitro. Ovary explants were dissected from 17-day post-coitus mouse CD-1 embryos and cultured for 7 days. Oocytes were then treated with PFOA at 1/4 LC_{50} (28.2 μM) or LC_{50} (112.8 μM) for 24 hours. ROS production was significantly increased in the treated oocytes compared with the controls. A similar study was conducted in porcine oocytes and cumulus cells (Mario et al., 2022). Cumulus cells are a group of granulosa cells that surround the oocytes and play a role in the process of oocyte development and maturation. The cells were treated with PFOA – 1/8 LC_{50} (20 μM) or 1/4 LC_{50} (40 μM) – during in vitro maturation for 44 hours. Exposure to 40 μM PFOA significantly increased ROS production in the cumulus cells. An earlier study (Chen et al., 2021) also showed that PFOS exposure during in vitro maturation (100 μM, 44–48 hours) significantly increased ROS levels in porcine oocytes.

Basini et al. (2022) studied the effects of PFOA on porcine ovarian granulosa cells that were exposed to PFOA (2, 20, or 200 ng/mL equal to 4.83, 48.3, or 483 μM) for 48 hours. RONS, including the O^{2-}, H_2O_2, and NO levels, were assessed, and in contrast to the above findings, these were found to be significantly decreased after treatment with all the tested PFOA concentrations. Non-enzymatic scavenging activity was assessed by the ferric-reducing ability of plasma and was found not to be affected by PFOA exposure at the tested concentrations.

PFOS was suggested to have an adverse effect on cerebellar granule cells by inducing apoptosis via a ROS-mediated alteration in protein kinase C (PKC) signalling. PKC is a pivotal messenger molecule that is involved in neuronal function and development. PFOS (3 or 30 μM) was found to increase ROS production in cerebellar granule cells from Sprague-Dawley rats aged 7 days. NAC (10 mM) pre-treatment 24 hours before PFOS exposure was shown to inhibit ROS production in the cells (Lee et al., 2012). In a study aiming to investigate the potential for PFOS to cause neurotoxicity (Li et al., 2017b),

PFOS (250 µM, 24 hours) significantly increased ROS production in PC12 cells, a cell line derived from a rat pheochromocytoma. When cells were pre-treated with taurine, an antioxidant (80 mM, 30 minutes), PFOS-induced ROS generation was inhibited.

Qian et al. (2010) studied ROS in mouse RAW 264.7 macrophages exposed to PFOS (100 µM, 5 minutes) using electron spin resonance spectroscopic measurement. PFOS significantly induced ROS (by 2.4-fold) in the cells. Electron spin resonance is considered to be a more robust technique for the detection of ROS because of its specificity and sensitivity. The study also showed that the production of ROS plays a role in PFOS-induced actin filament remodelling and the increase in endothelial permeability.

Elumalai et al. (2023) reported that PFOS-induced oxidative stress contributed to the apoptosis of rat insulinoma INS-1 cells. When cells were treated with 50 or 100 µM PFOS for 36 hours, mitochondrial ROS generation increased through NOX2–gp91Phox activation and the inhibition of cAMP-PKA.

Dos Santos et al. (2022) treated the rat β-cell line INS-1E with low concentrations of PFOA (1 nM or 1 µM) for 24 hours. No significant changes in ROS production, measured by fluorescence of DCF-DA, were observed.

Effects of PFOS (75–600 µM, 24 hours) and PFOA (400–1000 µM, 24 hours) on $Nrf2^{-/-}$ knockout and wildtype C57BL/6 mouse astrocytes have also been studied (Alharthy and Hardej, 2021). The study showed that exposure to 600 µM PFOS or 800 µM PFOA significantly increased ROS levels, lipid peroxidation, and apoptosis in both the wildtype and $Nrf2^{-/-}$ astrocytes. However, the effects of PFOA and PFOS were significantly larger in the $Nrf2^{-/-}$ astrocytes than in wildtype cells. The GSH/GSSG ratio was significantly decreased in both wildtype and $Nrf2^{-/-}$ astrocytes treated with PFOA or PFOS. However, the decrease was significantly larger in the $Nrf2^{-/-}$ astrocytes than in the wildtype cells. Pre-treatment with butylated hydroxytoluene before PFOA and PFOS exposure significantly reduced ROS production in wildtype astrocytes but not in $Nrf2^{-/-}$ astrocytes. In summary, the study showed that $Nrf2^{-/-}$ astrocytes were more susceptible to PFOA and PFOS toxicity.

[The Working Group noted that the literature in experimental systems in vitro regarding the potential effects of PFOA and PFOS to induce oxidative stress was quite extensive. Oxidative stress is typically measured by generation of ROS, and this is sometimes coupled with the measurement of antioxidant enzyme activities, including those of SOD, CAT, GSH, and/or GPx. The Working Group identified more studies reporting evidence of oxidative stress induction than those reporting marginal evidence or no evidence.]

Oxidative stress was induced by PFOS in embryonic stem cell-derived cardiomyocytes from 129 mice (Cheng et al., 2013), in rat hepatocytes (Khansari et al., 2017), in rat HAPI microglial cells (Wang et al., 2015c), and in a co-culture of Sertoli cells and gonocytes from male Sprague-Dawley rat pups (Zhang et al., 2013d).

PFOA was shown to induce oxidative stress in MC3T3 osteoblast cells (Choi et al., 2016), in mouse spermatogonial GC-1 cells (Lin et al., 2020a), and in rat brain and liver mitochondria (Mashayekhi et al., 2015). Both PFOA and PFOS induced oxidative stress in rat mesangial cells (Gong et al., 2019).

Studies showing marginal or no effects included a study by Berntsen et al. (2017), in which rat cerebellar granule neurons were exposed to PFOA or PFOS at 75, 150, 300, or 600 µM for 3 hours, and H_2O_2 was used as a positive control. No significant increase in ROS production upon exposure to PFOA or PFOS was observed after 3 hours. PFOA and PFOS exposure for 3 hours resulted in a significant decrease in lipid peroxidation at many of the tested concentrations.

PFOA- or PFOS-induced oxidative stress may also affect neuronal development. Slotkin et al.

(2008) used PC12 cells as an in vitro model to study the effects of PFOA and PFOS exposure on undifferentiated (24 hours) and differentiating (4 days) cells. PFOA (at 10 and 250 μM) and PFOS (at 50, 100, and 250 μM) significantly increased MDA levels in the differentiating cells; 50 μM chlorpyrifos was used as a positive control.

Other evidence that PFOA and PFOS induce oxidative stress in non-human cell lines was provided by several studies. Wang et al. (2015a) used *gpt delta* transgenic mouse embryonic fibroblast cells immortalized by the SV40 T antigen, Oseguera-López et al. (2020) performed a spermatozoa capacitation study using sperm from boars, and Wei et al. (2021) used mouse oocytes, and all three studies showed that PFOS induced oxidative stress in the respective cell types. A study by Suh et al. (2017) provided further evidence that PFOA induces oxidative stress in rat pancreatic β-cell-derived RIN-m5F cells.

In a rabbit renal proximal tubule model (Schnellmann, 1990), PFOS at 10, 20, 50, or 100 μM did not increase MDA levels. However, GSH levels were significantly decreased in cells exposed to 100 μM PFOS for 15 minutes.

Synopsis

[The Working Group noted that there is some evidence that PFOS induces oxidative stress in exposed humans, whereas the evidence for PFOA is mixed. Ten studies examined the effects of PFOS, of which six generated positive results, two marginal, and two negative results. Eleven studies in exposed humans examined the effects of PFOA; of these, only three studies showed a positive association of PFOA with oxidative stress, with one marginal and seven negative results.

In human primary cells in vitro, there is evidence that PFOA and PFOS induce oxidative stress. Similarly, PFOA and PFOS were found to induce ROS production in most in vitro studies using immortalized human cell lines, including HepG2 cells and other human cells, confirming the observations in both in vitro and in vivo studies in humans.

In experimental systems in vivo and in vitro, there is evidence that PFOA and PFOS induce oxidative stress.

The PFOA and PFOS concentrations used in exposure experiments in various non-human mammalian cells were similar to those used in human cell studies. The findings suggested that PFOA and PFOS can induce oxidative stress in vitro at concentrations similar to those used in human cells, in the micromolar concentration range. Some studies reported increased levels of 8-OHdG or 8-oxodG in cells or biological fluids, as measured by LC-MS/MS, which provided evidence for oxidative stress and potential oxidative damage to DNA. The generation of oxidative stress by PFOA and PFOS exposure is further supported by increased ROS production and decreased antioxidant defence end-points. Co-treatment with antioxidants has been shown to reduce oxidative stress, which provides additional evidence that oxidative stress may be involved when experimental systems are exposed to PFOA or PFOS.]

4.2.6 Induces chronic inflammation

(a) Humans

(i) Exposed humans

See Table 4.18.

Changes in inflammatory markers, including C-reactive protein (CRP) cytokines such as the interleukins (ILs) IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, and IL-10, TNF-α, interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), neutrophil count; lymphocyte count; leptin; and adiponectin; as well as parameters related to eczema and rhino-conjunctivitis, atopic dermatitis, and lung function; were examined as part of the KC "induces chronic inflammation".

In total, the Working Group had access to 18 studies that investigated associations between

Table 4.18 End-points relevant to chronic inflammation in humans exposed to PFOA or PFOS

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Inflammatory markers in general population exposure							
35 inflammatory proteins	Serum/ plasma	Europe HELIX project; in 1101 mother–child pairs, PFAS measured in blood collected during pregnancy (prenatal exposure) and in children of age 8 years (postnatal exposure) (range, 6–12 years). 35 inflammatory proteins measured in the children's plasma Cross-sectional design.	Maternal PFOS: median, 6.2 ng/mL Child PFOS: median, 1.5 ng/mL *n* = 1101 Maternal PFOA: median, 2.2 ng/mL Child PFOA: median, 1.9 ng/mL *n* = 1101	↓ MIG, MIP1-β (prenatal PFOS) No association with adiponectin, BAFF, C-peptide, CRP, EGF, eotaxin, FGF-2, G-CSF, HGF, IFN-α, IFN-γ, IL-10, IL-12, IL-13, IL-15, IL-17, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IP10/CXCL10, leptin, MCP-1/CCL2, MIP-1α/CCL3, PAI-1, or TNF-α ↑ IL-1β (prenatal PFOA) ↓ IL-8, HPG (postnatal PFOA) No association with adiponectin, BAFF, C-peptide, CRP, EGF, eotaxin, FGF-2, G-CSF, HGF, IFN-α, IFN-γ, IL-10, IL-12, IL-13, IL-15, IL-17, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IP10/CXCL10, leptin, MCP-1/CCL2, MIP-1α/CCL3, PAI-1, or TNF-α	Maternal age and education, pre-pregnancy BMI, parity, cohort, child ethnicity, age at examination, and sex	Potential divergence of PFAS exposure profiles in childhood from gestational exposure profiles because of factors such as transplacental transfer, breastfeeding, and dietary exposure. Sample size relatively large. Possibility of loss to follow-up and selection bias in longitudinal birth cohorts, children of mean age 8 years. Both mixture-approach statistical analysis and linear regression analysis were performed. All significance levels were set to 0.05 in this study, but it is unclear whether the linear analysis involved multiple correction.	Papadopoulou et al. (2021)

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
HbA1c, adiponectin, leptin, CRP, SHBG, and IL-6	Plasma	USA Prospective prebirth cohort with 3 years of follow-up. Measurements in mothers during and after pregnancy	PFOS: median, 24.8 ng/mL, n = 450–454, depending on the marker, during pregnancy	No association with HbA1c, adiponectin, leptin, CRP, SHBG, or IL-6	Age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity, and breastfeeding history	Loss to follow-up between pregnancy and 3 years postpartum, requiring correction for potential selection bias. Limited power to detect subtle effects, potentially resulting in false negatives; small effect sizes with wide confidence intervals; and no adjustment for multiple testing.	Mitro et al. (2020)
			PFOA: median, 5.6 ng/mL, n = 450–454, depending on the marker during pregnancy	No association with HbA1c, adiponectin, leptin, CRP, SHBG, or IL-6			
IFN-γ, IL-6, IL-10, TNF-α, CRP	Serum	Atlanta, USA African American Maternal–Child Cohort Longitudinal assessment	PFOS: median, 2.19 ng/mL; n = 425	↑ TNF-α and IFN-γ at both 8–14 wk and 24–30 wk of gestation ↑ IL-6 and CRP at 24–30 wk of gestation	Models were adjusted for gestational age at sample collection, maternal age, education, prenatal BMI, and parity	PFOS and PFOA were detected in > 98% of samples at 8–14 wk of gestation; inflammatory markers measured at up to two time points (8–14 wk and 24–30 wk of gestation).	Tan et al. (2023)
			PFOA: median, 0.72 ng/mL; n = 425	No associations with IFN-γ, IL-6, IL-10, TNF-α, or CRP at either 8–14 wk or 24–30 wk of gestation			

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
87 inflammatory proteins	Plasma	Sweden Prospective adult population-based cohort Cross-sectional design	PFOS: median, 13.4 ng/mL; *n* = 965 PFOA: median, 3.3 ng/mL; *n* = 965	No association with CRP or other inflammatory markers ↓ CRP	Sex, sample storage time in freezer, smoking, exercise habits, education, energy and alcohol intake, BMI, glomerular filtration rate, glucocorticoid and COX-inhibitor treatment	Sample size was moderate, multiple covariate adjustments and correction for multiple testing were applied. Older individuals, sensitivity analysis included. CRP assessed using ELISA, Older Swedish participants, limiting the generalizability of the findings to other age and ethnic groups.	Salihović et al. (2020b)
CRP, IL-6, IL-1β, adiponectin, and leptin	Serum	Taiwan, China Young adults (age 12–30years) Cross-sectional design	PFOS: median, 8.93 ng/mL; *n* = 287 PFOA: median, 2.39 ng/mL; *n* = 287	No associations No association with CRP, IL-6, IL-1β, adiponectin, or leptin	Adjustment for age, sex, additional lifestyle factors, and measured parameters; a significant association was only identified when it remained consistent across all three models, avoiding model-dependent results	Sample size was relatively small.	Lin et al. (2011)
IL-6, IL-10, and TNF-α	Serum of women with overweight or obesity during pregnancy or afterwards	San Francisco Bay Area, USA Participation in the MAMAS Cross-sectional design	PFOS: median, 2.83 ng/mL; *n* = 103 PFOA: median, 1.4 ng/mL; *n* = 103	↑ IL-6 No association with IL-10 or TNF-α ↑ IL-6 No association with IL-10 or TNF-α	Age, race/ethnicity, time-varying BMI, parity, education, smoking status, number of gestational weeks at baseline, and visit	Sample size was small, which may have limited the ability to detect associations (*n* = 103), lack of data on breastfeeding	Zota et al. (2018)
CRP, absolute neutrophil count, and lymphocyte count	Serum	USA. NHANES 2005–2006. Adults aged ≥ 20 years. Cross-sectional design	PFOS: median, 11.4 ng/mL; *n* = 6652	↓ Neutrophil count ↑ Lymphocyte count No association with CRP	Age, sex, race/ethnicity, education, poverty income ratio, BMI, and serum cotinine	Sample size was relatively large, potential bias from participants taking anti-inflammatory or immunomodulatory medication.	Omoike et al. (2021)

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
			PFOA: median, 3.2 ng/mL; *n* = 6652	↓ Neutrophil count ↑ Lymphocyte count No association with CRP			Omoike et al. (2021) (cont.)
MCP-1, HO-1, and IL-8	Maternal plasma or serum	China Prospective, nested case–control study Cross-sectional assessment	PFOS: median, 1.79 ng/mL *n* = 519 (144 cases and 375 controls)	↑ MCP-1 No association with IL-8 or HO-1	Crude models were adjusted for gestational age. Full models included covariates that were significantly associated with the levels of the biomarkers or PFOS and PFOA	Case–control study, sample size was small (144 cases and 375 controls). Participants were recruited only during the first and second trimesters (4–22 wk of gestation).	Liu et al. (2020a)
			PFOA: median, 0.79 ng/mL; *n* = 519 (144 cases and 375 controls)	↓ IL-8 No association with MCP-1 or HO-1			
Leptin, adiponectin	Umbilical cord serum	China SMBCS Prospective birth cohort Cross-sectional assessment	PFOS: median, 1.94 µg/L; *n* = 1111	↑ Adiponectin ↑ Leptin (women)	Maternal factors (age, occupation type, education level, family annual income, pre-pregnancy BMI, gestational weight gain, weekly physical activity, smoking during pregnancy, parity); infant factors (sex and gestational age); and an interaction term for PFAS and sex	Sample size was relatively large, biomarkers not specific for chronic inflammation.	Ding et al. (2023)
			PFOA: median, 3.76 µg/L; *n* = 1111	↑ Adiponectin ↑ Leptin (women)			

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Inflammatory markers in communities with elevated exposure							
IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, GM-CSF, TNF-α	Serum	USA Population-based cohort study, adults with median age 61 years Cross-sectional design	PFOS: median, 8.2 ng/mL; *n* = 212 PFOA: median, 3.3 ng/mL; *n* = 212	No association with IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, GM-CSF, or TNF-α ↓ IL-1β No association with IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, GM-CSF, or TNF-α	Age, BMI, sex, smoking history, and race/ethnicity	Sample size was small (*n* = 212), may not be generalizable to non-AFFF-exposed populations; the biomarkers were not all measured in the entire sample, which further reduced the power. Limitations of the study included low detection frequency of cytokines and non-fasting samples, suggesting that cytokine concentrations may have been influenced by recent food intake.	Barton et al. (2022)
TNFα, IL-6, IL-8, IFN-γ, adiponectin, and leptin	Serum	Mid-Ohio Valley, USA The C8 Health Study of adults Cross-sectional design	PFOS: mean, 26.9 ng/mL; *n* = 200 PFOA: mean, 94.6 ng/mL; *n* = 200	↑ Adiponectin ↓ TNF-α, IL-8 No association with IL-6, IFN-γ, or leptin ↑ IFN-γ ↓ TNF-α No association with IL-6, IL-8, adiponectin, or leptin	Age, alcohol consumption, BMI, sex, and eGFR	Serum samples were randomly selected from a larger cohort; pilot study with no multiple comparisons.	Bassler et al. (2019)
End-points of disease outcome							
Eczema and rhino-conjunctivitis	Maternal plasma	Hokkaido, Japan Prospective birth cohort with 7 years of follow-up Longitudinal design	PFOS: median, 5.1 ng/mL; *n* = 2689 PFOA: median, 1.9 ng/mL; *n* = 2689	↓ Eczema No association with rhino-conjunctivitis ↓ Eczema No association with rhino-conjunctivitis	Sex parity, maternal age at delivery, maternal smoking during pregnancy, BMI pre-pregnancy, and annual household income during pregnancy	Large sample size, loss to follow-up, self-reported questionnaires, with the possibility of recall bias affecting outcomes, lack of inflammatory biomarker measurement.	Ait Bamai et al. (2020)

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Lung function, asthma, atopic dermatitis, rhinitis, LRTIs	Serum at age 10 years	Norway ECA prospective birth cohort with 10–16 years of follow up. Longitudinal design	PFOS: median, 5.2 ng/mL; *n* = 378 PFOA: median, 1.6 ng/mL; *n* = 378	↑ LRTIs (entire sample and boys aged 10–16 years) No association with reduced lung function, asthma, atopic dermatitis, or rhinitis ↑ Rhinitis (PFOA) ↑ LRTIs (entire sample and girls aged 10–16 years) No association with lung function, asthma, or atopic dermatitis	BMI at age 16 years, puberty status, maternal education, and physical activity level at age 16 years (frequency of activities leading to breaking sweat and shortness of breath); after 10 years of follow up: physical activity and maternal education	Sample size was small, potential selection bias related to socioeconomic factors and loss to follow-up.	Kvalem et al. (2020)

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Childhood atopic dermatitis	Fetal umbilical cord blood	Shanghai, China Prospective birth cohort with 2 years of follow-up	PFOS: median, 2.5 ng/mL; $n = 687$	No association with atopic dermatitis	Infant sex, parity (nulliparous and parous), birth weight, gestational age at delivery, mode of delivery, maternal pre-pregnancy BMI, maternal age, maternal education, maternal ethnicity, paternal age, paternal education, parental history of allergic disorders, paternal smoking during pregnancy, family income, and breastfeeding during the first 6 months; as only a few women consumed alcohol (1.98%) or smoked (0.5%), maternal alcohol consumption and smoking were not included in the fully adjusted model	Diagnosis of atopic dermatitis confirmed by two dermatologists. Adjustment for a large number of potential confounders. Loss to follow-up resulted in a substantial proportion (23%) of participants being excluded from the analysis. Limited sample size for some types of exposure.	Chen et al. (2018a)
			PFOA: median, 7.0 ng/mL; $n = 687$	↑ Atopic dermatitis in girls			

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Lung function, asthma, eczema	Maternal plasma	Spain. Prospective birth cohort with 7 years of follow-up	PFOS: median, 5.8 ng/mL; *n* = 1243	Trends toward associations with lower risks of asthma and eczema; no association with reduced lung function	Maternal age at delivery, parity, previous breastfeeding, pre-pregnancy BMI, region of residence, and country of birth	Large sample size. Lack of correction for multiple comparisons and small effect estimates for the relationships of PFAS with lung function necessitate careful interpretation. The potential for chance findings because of multiple comparisons was acknowledged. Lack of information about *P*-value criteria and statistical significance, reliance on maternal PFAS concentrations as proxies for fetal exposure, and self-reported questionnaires for outcome assessment introduced potential for recall and misclassification bias.	Manzano-Salgado et al. (2019)
			PFOA: median, 2.3 ng/mL; *n* = 1243	Trend toward a positive association with reduced lung function (FVC and FEV1) at age 4 years, no association at age 7 years; no association with asthma or eczema			
Childhood atopic eczema, food allergy, allergic rhinitis, and asthma	Maternal plasma	Norway MoBA study, prospective population-based pregnancy sub-cohort with 7 years of follow-up	PFOS: median, 12.9 ng/mL; *n* = 1943	↓ Common cold, ear infection, urinary tract infection ↑ Bronchitis/ pneumonia No association with atopic eczema, food allergy, allergic rhinitis, asthma, throat infection, pseudocroup, or diarrhoea/gastric flu	Maternal age, maternal BMI, maternal education, parity, and smoking during the pregnancy; correction for multiple testing using false discovery rate	7 years of follow-up, a relatively large number of participants, loss to follow-up and reliance on questionnaire-based outcomes. All samples had PFOS and PFOA concentrations above the LOQ.	Impinen et al. (2019)

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Childhood atopic eczema, food allergy, allergic rhinitis, and asthma (cont.)			PFOA: median, 2.5 ng/mL; *n* = 1943	↓ Common cold, urinary tract infection ↑ Bronchitis/ pneumonia, pseudocroup No association with atopic eczema, food allergy, allergic rhinitis, asthma, throat infection, ear infection, or diarrhoea/gastric flu			Impinen et al. (2019) (cont.)
Reduced lung function at birth, asthma, allergic rhinitis, atopic dermatitis	Fetal umbilical cord blood	Norway ECA prospective birth cohort with 2–10 years of follow-up	PFOS: median, 5.2 ng/mL; *n* = 641 PFOA: median, 1.6 ng/mL	No association with reduced lung function at birth, asthma, allergic rhinitis, or atopic dermatitis	Confounders examined: sex; birth weight; birth month; breastfeeding at 6 and 12 months; maternal smoking during pregnancy; household smoking at birth, at preschool age and at school age; parental asthma; atopic dermatitis and allergic rhinitis; parental education; and household income Regression models were adjusted for sex only and correction for multiple testing was performed using Bonferroni	High follow-up rate after 10 years and representativeness of the study sample. Potential recall bias, especially in questionnaires and interviews, lack of data on important confounding factors, such as parity and previous breastfeeding.	Impinen et al. (2018)
			PFOA: median, 1.6 ng/mL; *n* = 641	No association with reduced lung function at birth, asthma, allergic rhinitis, or atopic dermatitis			

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Lung function	Umbilical cord blood plasma and serum from children	Taiwan, China Prospective birth cohort with 7 years of follow-up	Cord blood PFOS: median, 6.4 ng/mL Children's serum PFOS: median, 5.9 ng/mL; *n* = 165	Positive association with reduced lung function in children	Sex, child height, child BMI, birth weight, maternal education, eating habits, prenatal smoking history, history of environmental tobacco smoke exposure, maternal cotinine concentration, gestational age, family income, use of pesticide or incense at home	Sample size was small, lack of multiple testing correction, differences in demographic characteristics of the participants at follow-up compared with the original cohort might have introduced selection bias, reliance on cord blood and blood for the assessment of associations with PFAS exposure, which may not have fully captured the changing concentrations of these compounds over time, particularly during early life and childhood.	Kung et al. (2021)
			Cord blood PFOA: median, 2.4 ng/mL Children's serum PFOA: median, 2.7 ng/mL; *n* = 165	Positive association with reduced lung function in children			

AFFF, aqueous film-forming foam; BMI, body mass index; COX, cyclooxygenase; CRP, C-reactive protein; ECA, Environment and Childhood Asthma study; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; FEV1, forced expiratory volume in 1 second: FVC, forced vital capacity; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; HbA1c, glycated haemoglobin; HELIX, Human Early Life Exposome; HO-1, haem oxygenase 1; IFN-γ, interferon-gamma; IL, interleukin; LOQ, limit of quantification; LRTIs, lower respiratory tract infections; MAMAS, Maternal Adiposity, Metabolism, and Stress Study; MCP-1, monocyte chemoattractant protein-1; MoBA, Norwegian Mother, Father and Child Cohort Study; NHANES, National Health and Nutrition Examination Survey; PFAS, perfluoroalkyl and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; SHGB, sex hormone-binding globulin; SMBCS, Sheyang Mini Birth Cohort Study; TNF, tumour necrosis factor; USA, United States of America; wk, week(s).

[a] ↑, increase; ↓, decrease.

human exposure to PFOA and PFOS and end-points relevant to chronic inflammation. Of these, 11 studies examined the relations between PFOS and/or PFOA concentration and circulating markers of inflammation. Furthermore, seven of the studies explored the associations between the concentrations of PFOS and/or PFOA in maternal or umbilical cord blood and health outcomes (atopic dermatitis, eczema, rhinitis, asthma, and lung function) in children.

Measurement of inflammatory end-points

Altogether, 11 studies addressed associations of PFOA and PFOS concentrations with inflammatory markers, including CRP, MCP-1, IL-8, IL-6, neutrophil count, lymphocyte count, IL-10, TNF-α, leptin, and adiponectin. Of these, nine assessed associations either in cross-sectional (n = 7) or prospective (n = 2) settings, involving both children and adults from the general population (study size range, n = 103–6652). In these settings, the primary focus was on examining associations with inflammatory proteins, considering background exposure as the predominant source. Of the 11 studies, two cross-sectional studies examined potential associations in individuals exposed to elevated levels of PFAS: Bassler et al. (2019) focused on participants with elevated exposure due to PFOA-contaminated drinking-water in the C8 Health Study; and Barton et al. (2022) examined a population exposed to drinking-water contaminated with aqueous film-forming foam (AFFF).

Studies of exposure in the general population

The nine studies have been organized in descending order of informativeness, taking into account factors such as study size, prospective versus cross-sectional design, and the specificity of the assessed markers of inflammation for chronic inflammation. Prospective cohort studies are more informative because they enable longitudinal assessments.

In a study of the effects of prenatal (during pregnancy) and childhood (in children aged 8 years) exposure to PFOA and PFOS on cardiometabolic factors and inflammatory status in children, the authors assessed 1101 mother–child pairs from the Human Early Life Exposome project (the children had an average age of 8 years) (Papadopoulou et al., 2021). The covariates included maternal age and education, pre-pregnancy BMI, parity, cohort, child ethnicity, age, and sex. The study reported that prenatal PFOA concentration was positively correlated with that of the pro-inflammatory cytokine IL-1β. Postnatal PFOA concentration was negatively correlated with those of IL-8 and HGF. Prenatal PFOS concentration was negatively associated with those of MIG/CXCL9 and MIP-1β/CCL4. No association was observed between PFOA and/or PFOS concentrations and several end-points, including adiponectin, BAFF, C-peptide, CRP, EGF, eotaxin, FGF-2, G-CSF, IFN-α, IFN-γ, IL-10, IL-12, IL-13, IL-15, IL-17, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IP10/CXCL10, leptin, MCP-1/CCL2, MIP-1α/CCL3, PAI-1, or TNF-α (Papadopoulou et al., 2021). [The Working Group noted that the strengths of this study included the assessment of both prenatal and postnatal exposure to PFOA and PFOS and multiple markers of relevance for chronic inflammation in a large sample of participants from six European cohorts.]

Mitro et al. (2020) conducted a study involving 450 pregnant women recruited between 1999 and 2002 in Massachusetts, USA, to investigate the potential links between PFOA and PFOS plasma concentrations and the markers CRP, IL-6, HbA1c, adiponectin, leptin, and sex hormone-binding globulin (SHBG) during pregnancy and in plasma samples collected 3 years postpartum. The covariates included age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity, and breastfeeding history. No significant associations between PFOA or PFOS exposure and CRP, IL-6, HbA1c, adiponectin,

leptin, or SHBG were reported (Mitro et al., 2020).

In the Atlanta African American Maternal–Child Cohort (n = 425), associations between serum concentrations of a PFAS mixture (including PFOA and PFOS) and serum concentrations of IFN-γ, IL-6, IL-10, TNF-α, and CRP at two different time points (8–14 weeks and 24–30 weeks of gestation) were examined. Exposure to PFOS was considered to be the main driver of the effect of the mixture, according to the four different statistical methods used for analysis. When examining associations of the PFOA and PFOS concentrations with various markers, distinctions were observed between the two. PFOS concentration displayed positive associations with IFN-γ and TNF-α during both 8–14 weeks and 24–30 weeks of gestation and were positively associated with IL-6 and CRP only during the 24–30-week period. In contrast, there were no significant associations between PFOA concentration and CRP, IL-6, IFN-γ, IL-10, and TNF-α during both gestation periods (Tan et al., 2023). [The Working Group noted that the findings from this longitudinal assessment indicate disparities in the associations of PFOA and PFOS concentrations with circulating markers of inflammation, suggesting differences between the two agents' induction of circulating markers of chronic inflammation.]

In a cross-sectional study of 965 elderly individuals (age, 70 years; 50% women) from Sweden, associations of PFOA and PFOS with 86 inflammatory protein markers, including multiple cytokines, such as IL-6 and TNF-α, measured using a proximity extension assay, were assessed (Salihović et al., 2020b). Examination of the associations of the PFOA and PFOS concentrations with CRP, measured using a different assay, was included as a sensitivity analysis. The concentration of PFOA was consistently inversely associated with circulating CRP after multiple adjustments (see Table 4.18) and correction for multiple testing (Salihović et al., 2020b). [The Working Group observed that the cross-sectional findings suggest that PFOA concentrations were mainly associated with decreased levels of circulating markers of chronic inflammation, including CRP.]

In a cross-sectional study involving 287 participants (age, 12–30 years) from Taiwan, China, no significant associations of PFOA or PFOS exposure with CRP, IL-6, IL-1β, adiponectin, or leptin were reported (Lin et al., 2011).

In a cross-sectional study of pregnant women with overweight or obesity from the San Francisco Bay area, USA, the associations of prenatal exposure to PFOA and PFOS with levels of inflammatory markers, such as IL-6, IL-10 and TNF-α, in pregnant and postpartum women were assessed (Zota et al., 2018). The study reported a positive association between PFOS and IL-6 ($P < 0.05$). Additionally, the PFOA concentration showed a positive association with the pro-inflammatory cytokine IL-6 ($P < 0.05$). These findings suggest that exposure to PFOA and PFOS is cross-sectionally associated with elevated levels of IL-6 in pregnant and postpartum women.

Using cross-sectional data (n = 6652) from the NHANES 2005–2012, PFOA and PFOS concentrations were found to be significantly associated with decreased neutrophil count and increased lymphocyte count in linear models. However, when stratified by quintiles, the associations of PFOA concentration with the neutrophil and lymphocyte counts diminished. No significant associations between PFOA or PFOS and CRP were found (Omoike et al., 2021). The study could not account for potential bias introduced by participants taking anti-inflammatory or immune-modulatory medication. [The Working Group noted that the percentage changes in markers of chronic inflammation were of small magnitude and that the finding of decreased neutrophil count versus increased leukocyte count makes the interpretation of the findings challenging.]

In a nested case–control study comprising 144 women experiencing spontaneous preterm birth and 375 control participants (total, $n = 519$), the associations of serum PFOA and PFOS concentrations (both as continuous variables and after division into quartiles) with inflammatory markers, specifically MCP-1 and IL-8, were examined during weeks 4–22 of gestation. The study reported that in the full cohort, the PFOS concentration was positively associated with that of MCP-1, whereas the concentration of PFOA was inversely associated with that of IL-8, after adjustment for major confounders, including maternal age at delivery, parity, pre-pregnancy BMI, occupation, spontaneous abortion history, medication use, folic acid use, fasting status, pre-pregnancy passive smoking status, the gestational week of sampling (≤ 12 weeks or 12–27 weeks), and the sex of the child (Liu et al., 2020a). [The Working Group observed that the assessed markers may lack specificity for chronic inflammation.]

In 1111 mother–infant pairs from the Sheyang Mini Birth Cohort Study (SMBCS), associations of prenatal exposure to PFOA and PFOS with the leptin and adiponectin concentrations in umbilical cord serum were assessed, and sex-stratification was performed in each model to assess potential sexually dimorphic effects. The PFOA concentration was positively associated with leptin concentration in women only ($P < 0.05$) and positively associated with adiponectin concentration ($P < 0.05$) both in the entire sample and in men. The PFOS concentration was found to be positively associated with that of adiponectin ($P < 0.05$), both in the entire sample and in men, and positively associated with the leptin concentration in women (Ding et al., 2023). [The Working Group noted that the evaluated markers are not specific for chronic inflammation and that use of the findings of this study to draw conclusions regarding chronic inflammation may require additional context and complementary markers to be assessed, in order to provide a comprehensive assessment of the inflammatory state.]

Studies in communities with elevated exposure

Two studies investigated associations between elevated exposure to PFOA and PFOS in contaminated drinking-water and inflammation end-points (see Table 4.18).

Barton et al. (2022) evaluated associations of PFAS exposure via AFFF-contaminated drinking-water with IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, granulocyte–macrophage colony-stimulating factor (GM-CSF), and TNF-α in the PFAS Assessment of Water and Resident Exposure (PFAS-AWARE) study ($n = 212$). All the models were adjusted for age, race/ethnicity, smoking history, sex, and BMI. The authors reported an inverse association between serum PFOA concentration and the probability of detecting IL-1β. No significant associations were observed with IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, GM-CSF, or TNF-α. The authors recognized certain constraints, such as the infrequent detection of specific cytokines, including IL-1β, and the possibility that cytokine levels could have been affected by recent food consumption, because of non-fasted samples (Barton et al., 2022).

Bassler et al. (2019) included 200 adult C8 Health Study participants with an elevated exposure to PFOA (PFOA mean concentration, 94.6 ng/mL). Associations of PFOA and PFOS concentrations with the inflammatory markers TNF-α, IL-6, IL-8, IFN-γ, adiponectin, and leptin were assessed. The PFOA concentration was positively associated with that of IFN-γ and inversely associated with that of TNF-α. The PFOS concentration was positively associated with that of adiponectin and inversely associated with those of TNF-α and IL-8. No significant associations were observed between the PFOS concentration and those of IL-6, IFN-γ, and leptin (Bassler et al., 2019). [The Working Group

noted that in the context of elevated community PFOA exposure, both elevated and decreased concentrations of chronic inflammatory markers were reported.]

Measurement of end-points of disease outcome

Studies of exposure in the general population

In a prospective cohort study including 2689 children in Japan in which maternal enrolment occurred during the first trimester of pregnancy, with follow-up questionnaires administered when the children reached age 7 years, higher maternal PFOS concentrations were associated with a decreased risk of eczema. No significant association was reported with rhino-conjunctivitis. Similarly, PFOA concentration was also inversely associated with eczema, and no significant association was found for rhino-conjunctivitis (Ait Bamai et al., 2020).

The Environment and Childhood Asthma (ECA) Study was a prospective birth cohort including 378 participants with PFOA and PFOS measurements made at age 10 years and follow-up data collected at age 10 years (cross-sectional data) and age 16 years (longitudinal data). The cross-sectional data collected at age 10 years showed associations of the PFOA and PFOS concentrations with asthma. The longitudinal data showed no associations of the PFOA or PFOS concentration with atopic dermatitis. No associations were observed between PFOA or PFOS and lung function. Notably, PFOA concentration was positively associated with rhinitis in all participants at age 16 years. For airway infections, the longitudinal data showed that PFOA and PFOS concentrations were positively associated with lower respiratory tract infections (LRTIs) between ages 10 and 16 years (Kvalem et al., 2020). [The Working Group noted that the study's longitudinal approach minimized selection bias and the chance of misclassifying health outcomes. The asthma diagnosis was based on medication use and clinical diagnosis, further enhancing its accuracy and reducing misclassifications.]

The association of prenatal exposure to PFOA and PFOS with childhood atopic dermatitis was investigated in a prospective birth cohort study including 687 children who had completed a 2-year follow-up. Only in female children (n = 328), a log-unit increase in PFOA concentration was found to be associated with an increase of 2.1-fold in atopic dermatitis risk (adjusted odds ratio, 2.07; 95% CI, 1.13–3.80) after adjusting for potential confounders (Chen et al., 2018a).

Four studies reported no significant associations of PFOS and/or PFOA concentrations with atopic dermatitis/eczema, food allergy, rhinitis, asthma, and/or lung function (Impinen et al., 2018, 2019; Manzano-Salgado et al., 2019; Kung et al., 2021). More specifically, in a prospective cohort study conducted in Spain involving 1243 mother–child pairs, the associations of the prenatal concentrations of PFOA and PFOS with asthma, eczema, and lung function were investigated (see Section 1.6.2 for details). Although the findings were not significant, the study showed that the PFOA and PFOS concentrations were associated with asthma and eczema during childhood (Manzano-Salgado et al., 2019). [The Working Group noted that the interpretation of the findings was hampered since the study presented trends in associations rather than statistically significant comparisons, requiring cautious interpretation.]

In a prospective cohort study conducted in Norway, the associations of prenatal exposure to PFOA and PFOS with the development of asthma, allergies, and common infectious diseases in early childhood was assessed. The study involved a prospective cohort of 1943 mother–child pairs and followed participants up to age 7 years. Health outcomes were assessed at age 7 years, including asthma, allergies, and common infections. Questionnaires were completed at ages 3 and 7 years. PFOS was inversely associated with

the common cold, ear infection, and urinary tract infection between the age of 0–3 years (n = 1207). In addition, PFOS was positively associated with bronchitis/pneumonia, whereas no significant associations were observed with atopic eczema, food allergy, allergic rhinitis, asthma, throat infections, pseudocroup, or diarrhoea/gastric flu at ages 0–3 years and 6–7 years. The PFOA concentration was inversely associated with the common cold and urinary tract infection at age 0–3 years. PFOA was also positively associated with bronchitis/pneumonia and pseudocroup. No associations with atopic eczema, food allergy, allergic rhinitis, asthma, throat infections, ear infections, or diarrhoea/gastric flu were identified at ages 0–3 years and 6–7 years (Impinen et al., 2019).

In a study of the 0–2- and 2–10-year intervals obtained from the 2- and 10-year follow-up investigations of a prospective general population birth cohort, the ECA study, performed in Norway, associations of PFOA and PFOS with physician-diagnosed asthma, wheeze, obstructive airway disease severity, reduced lung function, atopic dermatitis, rhinitis, rhino-conjunctivitis, allergic sensitization, common cold episodes, and LRTIs were assessed. Adjustment for maternal age, maternal BMI, maternal education, parity, and smoking during pregnancy; and correction for multiple testing using the FDR were performed. No associations between PFOA or PFOS and lung function at birth, asthma, allergic rhinitis, or atopic dermatitis were observed (Impinen et al., 2018).

Kung et al. (2021) conducted a study examining the potential link between prenatal or childhood exposure to PFOA and PFOS and the development of lung function during childhood. This investigation included 165 children from Taiwan, China. In the entire cohort, the PFOA and PFOS levels in cord blood were inversely associated with lung function. In particular, intrauterine PFOS exposure was inversely correlated with mean FEV1 (forced expiratory volume in the first second), FVC (forced vital capacity), and PEF (peak expiratory flow) in childhood, especially in subgroups with lower birth weight and allergic rhinitis (Kung et al., 2021).

(ii) Human cells in vitro

See Table 4.19.

Human primary cells

Overall, five studies were available: four studies evaluated the effects of PFOA and PFOS exposure on cytokines (IL-6, IL-8, TNF-α, IL-4, IL-10, and IFN-γ) in peripheral blood leukocytes (Brieger et al., 2011; Corsini et al., 2011, 2012; Maddalon et al., 2023a) and the fifth focused on the IL-6 and IL-1β responses to PFOS exposure in human primary decidual stromal cells during early pregnancy (Yang et al., 2016).

Brieger et al. (2011) reported a significant association between PFOS exposure and decreased TNF-α release by human peripheral blood mononuclear cells in response to lipopolysaccharide (LPS)-stimulation at both the 4-hour and 24-hour time points. Notably, there was no significant correlation of IL-6 with either PFOA or TNF-α.

Corsini et al. (2011) measured the release of pro-inflammatory cytokines (IL-6, IL-8, TNF-α, IL-4, IL-10, and IFN-γ) in LPS-stimulated human peripheral blood leukocytes and in the human promyelocytic cell line THP-1. The study showed that both PFOA and PFOS reduced the release of TNF-α in peripheral blood leukocytes and in THP-1 cells, but only PFOS reduced the release of IL-6 in peripheral blood leukocytes. PFOA reduced the release of IL-8 at 100 μg/mL, and PFOS reduced IL-8 release at 1–100 μg/mL in a dose-dependent manner in THP-1 cells. Both PFOA and PFOS reduced the release of T-cell derived cytokines (IL-4 and IL-10), whereas IFN-γ release was reduced only by PFOS in peripheral blood leukocytes (Corsini et al., 2011).

Table 4.19 End-points relevant to chronic inflammation in human cells in vitro exposed to PFOA or PFOS

End-point	Assay	Tissue, cell line	Result[a]	Concentration (LEC or HIC) or range	Comments	Reference
Primary cells						
IL-6 and TNF-α	ELISA	PBMCs	↓ TNF-α after 4 and 24 h No change in IL-6 No change in IL-6 or TNF-α	PFOS, 100 μg/mL (LEC) PFOS, 1–100 μg/mL PFOA, 1–100 μg/mL		Brieger et al. (2011)
IL-6, IL-8, TNF-α, IL-4, IL-10, IFN-γ	ELISA	Peripheral blood leukocytes	↓ IL-6, TNF-α, IL-4, IL-10, IFN-γ No change in IL-8 ↓ TNF-α (LEC, 1 μg/mL), IL-4, IL-10 (LEC, 10 μg/mL) No change in IL-6, IFN-γ, or IL-8	PFOS, 0.1 μg/mL (LEC) PFOS, 0.1–10 μg/mL PFOA, 10 μg/mL (LEC) PFOA, 0.1–10 μg/mL	Relevance to human exposure and concentration effects	Corsini et al. (2011)
IL-6, TNF-α, IL-10, IFN-γ	ELISA	Peripheral blood leukocytes	↓ IL-6, IL-10, IFN-γ after PFOS treatment in blood leukocytes obtained from a female donor ↓ TNF-α after PFOS treatment of female and male donor or after PFOA in female donor	PFOS, 10 μg/mL (LEC) PFOS, 0.1 μg/mL (LEC) PFOA, 1 μg/mL (LEC)	Sex of the donor considered	Corsini et al. (2012)
IL-8 and TNF-α	ELISA	PBMCs from male and female donor	↓ IL-8 and TNF-α (male donor only)	PFOS, 0.2 μM	Sex of the donor considered	Maddalon et al. (2023a)
IL-6 and IL-1β	qPCR	Primary human decidual stromal cells of early pregnancy	No change	PFOS, 0.1 μM		Yang et al. (2016)
Cell lines						
IL-8, TNF-α	ELISA	Promyelocytic cell line THP-1	↓ IL-8, TNF-α ↓ TNF-α ↓ IL-8	PFOS, 1 μg/mL (LEC) PFOA, 10 μg/mL (LEC) PFOA, 100 μg/mL (LEC)	Relevance to human exposure and concentration effects	Corsini et al. (2011)
TNF-α	ELISA	Promyelocytic cell line THP-1	↓ TNF-α	PFOS, 0.1 μg/mL PFOA, 10 μg/mL (LECs)		Corsini et al. (2012)

Table 4.19 (continued)

End-point	Assay	Tissue, cell line	Result[a]	Concentration (LEC or HIC) or range	Comments	Reference
IL-4, GATA-3, IFN-γ (Th1 and Th2 responses)	ELISA, western blot, qPCR	Human Jurkat cells	↓ IFN-γ, ↑ IL-4 ↓ IFN-γ, ↑ IL-4	PFOS, 1 μM (LEC) PFOA, 1 μM (LEC)		Yang et al. (2021)
IL-6, IL-8	qPCR, flow cytometry	HCEpiC, HCEC, and RPE cells	↑ IL-6, IL-8	PFOA, 400 ppm for 16 h	Only PFOA was detected in all the indoor carpet samples, and had the highest (37 458 ng/g) concentrations of all the perfluorinated compounds	Tien et al. (2020)
IL-6, TNF-α, and IL-10; mRNA expression of *IL6*, *TNFA*, and *IL10*	ELISA and mRNA by PCR	Human placental trophoblast (HTR-8/ Svneo) cells	↓ IL-6, ↑ TNF-α ↑ IL-10 ↓ mRNA expression of *IL10* at low concentrations ↑ *IL6* mRNA expression at high concentrations	PFOS, 0.01 mg/L (LEC) PFOS, 0.1 mg/L (LEC) PFOS, 0.01 mg/L (LEC) PFOS, 0.1 mg/L (LEC)		Zhu et al. (2020)
IL-6	ELISA	CCD-18Co myofibroblasts	↓ IL-1β-induced IL-6 production ↓ IL-1β-induced IL-6 production	PFOS, 0.6 μM (300 ng/mL) every 24 h to 96 h PFOA, 0.36 μM (0.15 ng/mL) every 24 h to 96 h	PFOS showed greater cytotoxicity than PFOA in CCD-18Co myofibroblasts	Giménez-Bastida et al. (2015)
CXCL-8, CXCL-10, IL-1α, IL-1β, IL-6	ELISA	Bronchial epithelial cells (HBEC3-TK)	↑ IL-1α, ↑ IL-1β at a PFOS concentration of 10 μM with, or ≥ 30 μM without, immune stimulation via TLR3 No change in IL-6, ↓ CXCL-10 and CXCL-8 (with TLR3 stimulation)	PFOS concentrations ranged from 0.13 to 60 μM and exposure lasted for 48 h		Sørli et al. (2020)

Table 4.19 (continued)

End-point	Assay	Tissue, cell line	Result[a]	Concentration (LEC or HIC) or range	Comments	Reference
			No significant changes in cytokine release were observed at non-cytotoxic PFOA concentrations in unstimulated cells; ↑ IL-1β in TLR3-stimulated cells	PFOA concentrations ranged from 0.13 to 10 μM and exposure lasted for 48 h		Sørli et al. (2020) (cont.)
mRNA expression of *IL6*, *IL8*, *TNFA*, *NFKB1*, *MAPK8*	qPCR	Human pancreatic cell line (PANC-1)	No changes in *IL8*, *TNFA*, *NFKB1*, *MAPK8*. ↓ IL-6 at 100 μM	PFOA, 10, 50, or 100 μM	LD_{50} was 195.74 μM	Abudayyak et al. (2021a)
IL-6, IL-8	ELISA	Human hepatocarcinoma cell line (HepG2)	↑ IL-6, ↓ IL-8 (at both 25 and 50 μM)	PFOA, 10, 25, or 50 μM		Abudayyak et al. (2021b)
Tachykinin	LC-MS/MS	Astroglial SVG p12 cells	↑ tachykinin at 80 μM	PFOA, 10 or 80 μM for 48 h	No positive control	Osemwegie et al. (2021)
Mast cell-mediated inflammatory response, eicosanoids		Human mast cells (HMC-1)	↑ eicosanoids (PFOS)	PFOS or PFOA, 30 μM		Park et al. (2021)
IL6, *TNFA*	qPCR	BV2 microglial cells	↑ *IL6* at all time points ↑ *TNFA* after 1 and 3 h ↑ *IL6* at 1 or 10 μM, after 6 h ↑ *TNFA* at 10 μM after 6 h	PFOS, 1 μM for 1, 3, or 6 h PFOS: 0.1, 1.0, or 10 μM for 6 h		Zhu et al. (2015)
IL-6, and TNF-α	ELISA		↑ IL-6 and TNF-α at all time points ↑ IL-6 ↑ TNF-α	PFOS: 1 μM for 6, 12, or 24 h 0.1 μM (LEC) for 24 h 1 μM (LEC) for 24 h		

Table 4.19 (continued)

End-point	Assay	Tissue, cell line	Result[a]	Concentration (LEC or HIC) or range	Comments	Reference
IL6, *IL1B*, and *TNFA*	qPCR	Human placental cell lines (HTR-8/SVneo and JEG-3 cells)	↑ *IL6*, *IL1B*, and *TNFA*	PFOS, 0–100 µM 50 µM (LEC) in HTR-8/SVneo 20 µM (LEC) in JEG-3 cells	Primarily focused on the immediate effects of PFOS exposure; the study did not include an extensive exploration of concentration–response relationships for the observed outcomes	Li et al. (2021a)
IL-8, TNF-α	ELISA	Human monocytic cell line THP-1	↓ IL-8 at 20 µM ↓TNF-α at all time points	PFOS, 0.2, 2, or 20 µM 0.2 µM (LEC)	LPS 10 ng/mL was added for 24 h	Masi et al. (2022)
TNFA, *IL1B*, *IL6*, *IL8*, *COX2*	RT-PCR and qPCR	Human mast cells (HMC-1)	↑ *TNFA* and *IL8* ↑ *IL1B* and *IL6* ↑ *COX2* ↑ histamine ≥ 25 µM	PFOA, 50–400 µM for 12 h 200 µM (LEC) 50 µM (LEC) 100 µM (LEC) PFOA, 25–100 µM for 24 h 25 µM (LEC)		Singh et al. (2012)

CXCL, CXC motif chemokine ligand; ELISA, enzyme-linked immunosorbent assay; h, hour(s); HCEC, human corneal endothelial cells; HCEpiC, human corneal epithelial cells; HIC, highest ineffective concentration; HMC, human mast cells; IFN, interferon; IL, interleukin; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LD_{50}, median lethal dose; LEC, lowest effective concentration; LPS, lipopolysaccharide; mRNA, messenger RNA; PCR, polymerase chain reaction; PBMC, peripheral blood mononuclear cell; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; ppm, parts per million; qPCR, quantitative polymerase chain reaction; RPE, retinal pigment epithelial; RT, reverse transcription; Th, T-helper; TLR, toll-like receptor; TNF, tumour necrosis factor.

[a] ↑, increase; ↓, decrease.

In another study, similar results were obtained using human leukocytes and the human promyelocytic cell line THP-1, and the authors further showed that PFOA and PFOS affect nuclear factor kappa B (NF-κB) activation, thereby suppressing cytokine secretion by immune cells (Corsini et al., 2012).

More recently, Maddalon et al. (2023a) investigated male and female peripheral blood mononuclear cells that were treated with PFOS at 0.2 μM. The authors observed that PFOS reduced both IL-8 and TNF-α release for male donors only, and in case of TNF-α, a statistically significant difference between male and female donors was observed. [The Working Group noted that the results of these studies suggested that PFOA and PFOS exposure was associated with an attenuated inflammatory response in peripheral blood leukocytes.]

Yang et al. (2016) investigated the impact of PFOS exposure on decidual stromal cell function. PFOS alone had no effects on the pro-inflammatory cytokines IL-6 or IL-1β (Yang et al., 2016).

Human cell lines

One study conducted in vitro experiments using Jurkat cells to investigate the mechanisms of PFOA- and PFOS-mediated T-helper (Th) Th1 and Th2 responses. The results showed that both PFOA and PFOS enhanced the Th2 response (IL-4) in Jurkat cells at 1.0–50 μM via STAT6 activation, but inhibited Th1-related gene expression (IFN-γ). The effects of PFOS on IL-4 and IFN-γ were reversed in Jurkat cells that did not express STAT6, suggesting that PFOA and PFOS exposure aggravate inflammation (Yang et al., 2021).

Tien et al. (2020) investigated whether PFOA in indoor particulate matter induced inflammation in corneal and retinal cells. Exposure to PFOA was found to promote the secretion of IL-6 and IL-8. The authors suggested that exposure to PFOA in particulate matter may increase the risk of age-related macular degeneration by inducing oxidative stress and inflammation in the retina. Only PFOA was detected in all the indoor dust samples and it was present at the highest concentrations, of the substances investigated (Tien et al., 2020). [The Working Group noted that the observed response might be attributed to both dust particles and the presence of PFOA in the dust.]

Zhu et al. (2020) investigated the effects of PFOS on the secretion of IL-6, TNF-α, and IL-10, as well as the mRNA expression of *IL6*, *TNFA*, and *IL10*, in human placental trophoblast (HTR-8/Svneo) cells. The cells were exposed to PFOS at 0, 0.01, 0.1, or 1.0 mg/L for 24 hours, and the survival rates, secretion levels, and mRNA expression were measured. PFOS exposure reduced the survival rate of the cells at higher concentrations and induced changes in the secretion of inflammatory cytokines. Specifically, PFOS reduced IL-6 secretion at lower concentrations but increased TNF-α secretion and *IL6* mRNA expression at higher concentrations. In addition, PFOS increased IL-10 secretion at higher concentrations but reduced *IL10* mRNA expression at lower concentrations (Zhu et al., 2020).

Giménez-Bastida et al. (2015) selected the CCD-18Co myofibroblast cell model to investigate the ability of PFOA and PFOS to modulate inflammatory mechanisms. IL-1β, an essential pro-inflammatory cytokine, was added to the cells to induce an inflammatory response. The study showed that PFOA and PFOS individually possess the ability to impair cell viability, induce cell proliferation, and reduce cell inflammation by reducing IL-1β-induced IL-6 production by subepithelial myofibroblasts of the colonic CCD-18Co line at non-cytotoxic concentrations (Giménez-Bastida et al., 2015) (see also Section 4.2.10).

Another study assessed the effects of PFOA and PFOS on the respiratory system using bronchial epithelial cells (HBEC3-TK) (Sørli et al., 2020). PFOA and PFOS inhibited lung

surfactant function at 0.5 mM in an acellular test and induced a pro-inflammatory response at micromolar concentrations. PFOA and PFOS were not associated with reduced cell viability at 10 μM. PFOS increased the release of the pro-inflammatory cytokine IL-1α, and also increased that of IL-1β at ≥ 30 μM without immunostimulation by the toll-like receptor 3 (TLR3) and at 10 μM with TLR3 stimulation. PFOS reduced the release of the chemokine CXCL8 at 3.3 μM and reduced that of CXCL10 at 10 μM when cells were immunostimulated. In contrast, PFOA at 10 μM increased only the release of IL-1β in HBEC3-TK that were immunostimulated by TLR3. The authors acknowledged that the concentrations of PFOA and PFOS tested in the assay (0.5 mM) were considerably higher than what would be deposited in the alveoli of a person inhaling contaminated air, which may affect the study's relevance to human exposure (Sørli et al., 2020).

The role of inflammation in PFOA-induced pancreatic toxicity was investigated by measuring the mRNA expression levels of *IL6*, *IL8*, *TNFA*, *NFKB1*, and *MAPK8* in a human pancreatic cell line (PANC-1). PFOA was reported to induce dose-dependent cytotoxicity (IC_{50}, 195.74 μM), with apoptosis being the main cell-death pathway induced. PFOA did not significantly increase the mRNA expression of the analysed immune response-related biomarkers (Abudayyak et al., 2021a).

Abudayyak et al. (2021b) also investigated the effects of PFOA on human hepatocarcinoma (HepG2) cells and found that PFOA significantly increased IL-6 levels (≤ 1.8-fold; $P \leq 0.05$) but reduced IL-8 levels at 25 μmol/L (40% decrease) and 50 μmol/L (35% decrease) (Abudayyak et al., 2021b).

Osemwegie et al. (2021) investigated the effects of the in vitro exposure to PFOA of the astroglial SVG p12 cell line. The study measured the gene expression of the inflammatory marker tachykinin, which was increased by 80 μM PFOA treatment for 48 hours (Osemwegie et al., 2021).

The effects of PFOA and PFOS on human mast cells have also been examined. The results showed that PFOS had the greatest impact, increasing degranulation and the production of inflammatory eicosanoids in mast cells (Park et al., 2021).

PFOS had effects on BV2 microglia, and its potential contribution to inflammation in the central nervous system was assessed (Zhu et al., 2015). The results showed that PFOS increased BV2 cell activation after 12 hours of treatment at 0.1–10 μM. Treatment with PFOS at 1 μM for 1–6 hours significantly increased *IL6* mRNA expression and increased *TNFA* mRNA expression at 1 μM after treatments of 1 and 3 hours, but not longer. In addition, cells treated with a higher concentration of PFOS (10 μM) increased the expression of both *IL6* and *TNFA* after 6 hours of treatment (Zhu et al., 2015).

A study by Li and colleagues aimed to examine the potential impact of PFOS on the development of the human placental cell lines HTR-8/SVneo and JEG-3 cells, mediated through the PPARγ pathway. The mRNA expression of *IL6*, *IL1B*, and *TNFA* were significantly increased by PFOS exposure in HTR-8/SVneo cells at 50 and 100 μM. In JEG-3 cells, PFOS increased the mRNA expression of *IL6* when present at 10 μM to 30 μM, and of *IL1B* and *TNFA* at 20 and 30 μM (Li et al., 2021a).

Masi et al. (2022) investigated the effect of exposure to PFOS on the expression of receptor for activated C kinase 1 (RACK1) receptor and immune cell activation. The results showed that PFOS exposure downregulated RACK1 expression when treated with low doses (0.2–20 μM) for 18 or 24 hours, and reduced LPS-induced IL-8 and TNF-α production at 20 μM and 0.2 μM, respectively.

In another study, the role of PFOA in human mast cell (HMC-1)-mediated allergic inflammation and the underlying mechanisms was investigated (Singh et al., 2012). The HMC-1 cells were treated with PFOA (25–400 μM) for 12 or

24 hours. PFOA exposure was found to increase histamine release after 24 hours at concentrations of ≥ 25 µM and to increase the gene expression of *TNFA*, *IL1B*, *IL6*, *IL8*, and *COX2* in the mast cells after 12 hours (Singh et al., 2012).

(b) Experimental systems

(i) Non-human mammalian systems in vivo

Pro-inflammatory effects

See Table 4.20.

Quist et al. (2015) examined adverse hepatic changes at both the microscopic and ultrastructural levels in CD-1 mice exposed prenatally to low doses of PFOA that were within the higher bound of the reference interval for human exposure. Pregnant CD-1 mice were exposed orally by gavage to PFOA at doses ranging from 0.01 to 1 mg/kg on GD1 to GD17, and histopathological changes in the livers of the offspring were observed on PND21 and PND91. Prenatal exposure to low doses of PFOA induced histopathological changes in the liver of offspring on PND21 and PND91, including chronic active periportal inflammation, which primarily involved lymphocytes and macrophages, with fewer plasma cells and occasional neutrophils. These effects were dose-dependent (Quist et al., 2015).

In a 2-year feeding study in rats exposed to PFOA (NTP, 2020; see also Section 3.1.2), the incidence of chronic active inflammation in the glandular stomach and of focal inflammation in the liver of male rats was increased in post-weaning exposure groups at 150 or 300 ppm at age 16 weeks, compared with controls. The study in male rats was stopped at the interim time point, and a second study was started at lower exposures (up to 80 ppm). Glandular stomach chronic inflammation in female rats was not increased at the 16-week time point, but it was at the 2-year time point (NTP, 2020).

Filgo et al. (2015) (see also Section 3.1.1) investigated the severity of chronic liver inflammation in three mouse strains – CD-1, 129/SV wildtype (WT), and 129/SV knockout (KO) – that were exposed to PFOA from GD1 to GD17. Chronic inflammation increased in severity only in CD-1 mice and, notably, severity was increased at the two highest doses, but incidence was not increased. Indeed, no increase in the incidence of chronic inflammation was observed in any of the three strains. [The Working Group noted that the study investigated only female mice, which are recognized as being less responsive to PFOA than are males. Among the three strains, the evidence of chronic inflammation in two 129/SV strains (WT and KO) was inconclusive; only CD-1 exhibited a positive but not significant response.]

In a study by Kamendulis et al. (2022), mice (age 8 weeks) were exposed to drinking-water containing PFOA at 5 ppm for 4 or 7 months (see Sections 3.1.2, 4.2.5). The chronic inflammation score was investigated at age 6 and 9 months, and was based on histopathological staining of sections of the pancreas. The study reported an increased inflammation score at 9 months compared with controls. [The Working Group noted that the evidence supporting a connection between PFOA and chronic pancreatic inflammation in the experimental model was inconclusive.]

Son et al. (2009) investigated the effect of PFOA exposure on immune organs (the spleen and thymus) of male ICR mice. The ICR mice were exposed to drinking-water containing PFOA at various doses for 21 days. The study showed that PFOA increased the expression of TNF-α, IL-1β, and IL-6 in the spleen, which also showed enlargement, with hyperplasia of the white pulp in the groups at 250 ppm. No changes were observed in the thymus (Son et al., 2009).

Guo et al. (2021b) also investigated the potential of PFOA to induce splenic atrophy in male mice. Mice were exposed to different

Table 4.20 End-points relevant to chronic inflammation effects in non-human mammalian systems in vivo exposed to PFOA or PFOS

End-point, assay	Species, strain, sex	Tissue	Result[a]	Dose (LED or HID) or range	Route, duration, dosing regimen	Comments	Reference
Pro-inflammatory effects							
Chronic active periportal inflammation, by histology	Pregnant mouse, CD-1, female	Livers of offspring	↑ in offspring at PND91 and PND21, dose-related	PFOA, 0.01, 0.1, 0.3, or 1 mg/kg	Oral gavage from GD1 to GD17, daily	PFOA ammonium salt	Quist et al. (2015)
Chronic inflammation (active or focal), by histology	Rat, Sprague-Dawley, both sexes	Glandular stomach	+ active inflammation in males after 16 weeks	PFOA, 0/150, 0/300 ppm (only postweaning exposure) in study 1	Feed, 16-week interim time point of 2-year feeding study (study 1)	PFOA	NTP (2020)
		Liver	+ focal inflammation in males after 2 years	PFOA, 0/40, 0/80, 300/80 ppm in study 2	Feed, 2-year study (study 2)		
		Forestomach	+ active inflammation in females after 2 years	PFOA, 0/1000 ppm (only postweaning) or 300/1000 ppm (both exposures) in study 1			
Chronic inflammation, by histology	Pregnant mouse, CD-1, 129/SV WT, and 129/SV KO, female	Livers of offspring	+ only in CD-1 mice at 1 and 5 mg/kg bw	PFOA, 0.01–5 mg/kg per day	Oral gavage daily from GD1 to GD17	PFOA ammonium salt; dose-related increases in severity scores in PFOA-exposed livers	Filgo et. al. (2015)
Chronic inflammation, by histology	Mouse, Pdx-1 (KC), both sexes	Pancreas	(↑) at 9-month time point	PFOA, 5 ppm	Drinking-water, from age 8 weeks until 6 or 9 months (time points of analysis)	Inconclusive evidence	Kamendulis et al. (2022)

Table 4.20 (continued)

End-point, assay	Species, strain, sex	Tissue	Result[a]	Dose (LED or HID) or range	Route, duration, dosing regimen	Comments	Reference
Tnf, *Il1b*, *Il6*, by RT-PCR	Mouse, ICR, male	Spleen Thymus	↑ *Tnf*, *Il6* at 250 ppm ↑ *Il1b* at 50 and 250 ppm No changes	PFOA, 0, 2, 10, 50, or 250 ppm	Oral (drinking-water) for 21 days	PFOA ammonium salt; spleen hyperplasia	Son et al. (2009)
TNF-α, IL-1β, IL-6, IRF5, IL12p70, macrophages, by ELISA	Mouse, BALB/c, male	Spleen and serum	↑ TNF-α, IL-1β, IL-6, and IRF5 at 2 and 10 mg/kg per day (spleen and serum) ↑ IL-1β, IL-6, and IL12p70 at ≥ 0.4 mg/kg per day (serum) ↑ TNF-α at ≥ 2 mg/kg per day (serum) ↑ IL12p70 at 10 mg/kg per day (serum) ↑ macrophage ratio at all doses with dose dependence	PFOA, 0, 0.4, 2, or 10 mg/kg per day	Oral (gavage) for 28 days, daily		Guo et al. (2021b)
IL-6, TNF-α, IFN-γ, IL-4, by ELISA	Mouse, C57BL/6, male	Liver	↓ TNF-α, IFN-γ, IL-4, IL-6	PFOA, 0.002% w/w, 3 ± 0.7 mg/kg per day	Oral (dietary exposure), 10 days		Qazi et al. (2013)
IL-5, IL-13, IL-4, IL-12, IL-2, IFN-γ, IL-17α, IL-1α, IL-6, and TNF-α, by Luminex	Mouse, $B6C3F_1$, female	Serum	↓ IL-5, IL-13, IL-12 at high dose ↓ IL-17α, IL-1α at both doses ↑ TNF-α at high dose No significant effects on IL-4, IL-2, IFN-γ, or IL-6	PFOA, 1.88 (low) or 7.5 (high) mg/kg per day	Oral (in drinking-water), for 4 weeks. 5 days prior the end of the exposure, one single intraperitoneal injection (300 mg/mouse in 0.5 mL) of keyhole limpet hemocyanin	Effects of PFOA alone not investigated	De Guise and Levin (2021)
IL-6, COX-2, CRP, by ELISA Inflammatory cells, by histology	Mouse, Kunming, male	Liver	↑ IL-6, COX-2, and CRP at 10 mg/kg per day ↓ IL-6, COX-2, CRP at 2.5 and 5 mg/kg per day ↑ infiltration of inflammatory cells (and hypertrophy), dose-dependent	PFOA, 2.5, 5, or 10 mg/kg per day	Oral for 14 consecutive days		Yang et al. (2014)

Table 4.20 (continued)

End-point, assay	Species, strain, sex	Tissue	Result[a]	Dose (LED or HID) or range	Route, duration, dosing regimen	Comments	Reference
Liver inflammation, steatosis, IL-1β, by histology, immunohistochemistry, ELISA	Mouse, C57BL/6J, male	Liver and serum	↑ steatosis after both diets ↑ liver inflammation in HFD-fed mice ↑ IL-1β (serum) after both diets	PFOS, 5 mg/kg per day	Oral for 4 weeks; HFD or chow diet ± PFOS		Qin et al. (2022b)
Tnf, *Il1b*, *Cox2*, *Il10*, by qPCR	Mouse, C57BL/6J, male	Colon, serum, cerebral cortex, hippocampus	↑ *Tnf* and *Cox2* at 3 mg/kg ↑ *Il1* at 1 and 3 mg/kg ↓ *Il10* at 1 and 3 mg/kg in colon ↓ *Il10* and ↑ *Tnf* in serum ↑ *Tnf* at 1 mg/kg and *Cox2* at 1 and 3 mg/kg in cortex and at 3 mg/kg in hippocampus ↓ *Il10* in hippocampus at 3 mg/kg	PFOA, 0.5, 1, or 3 mg/kg per day	Oral for 5 weeks		Shi et al. (2020)
TNF-α, IL-1β, IL-6, macrophages ($CD11b^+$ cells), by ELISA, RT-PCR (cytokines); flow cytometry (CD11b)	Mouse, C57BL/6, male	Peritoneal cavity, spleen	↑ TNF-α and IL-6 at ≥ 25 mg/kg, ↑ IL-1β at ≥ 5 mg/kg ↑ % macrophages in peritoneal cavity at ≥ 1 mg/kg ↑ TNF-α and IL-6 at ≥ 125 mg/kg, ↑ IL-1β at ≥ 50 mg/kg ↑ macrophages at ≥ 50 mg/kg in spleen	PFOS, cumulative doses of 0.5, 1, 5, 25, or 50 mg/kg; max 2.08 mg/kg per day	Oral for 60 days	PFOS concentrations were measured in the serum PFOS was the potassium salt	Dong et al. (2012a)
TNF-α and IL-6, by ELISA	Mouse, $B6C3F_1$, female	Serum, peritoneal lavage fluid	Serum cytokines: ↓ TNF-α at 1 mg/kg (↑ at 300 mg/kg), ↑ IL-6 at 1 mg/kg (↓ at 3 mg/kg) Peritoneal macrophage cytokines: ↑ TNF-α at 300 mg/kg, no change in IL-6 Peritoneal lavage fluid: ↓ TNF-α and IL-6	PFOS, 1, 3, or 300 mg/kg total administered dose, corresponding to 0, 0.0331, 0.0993, or 9.93 mg/kg per day	Oral for 28 days	PFOS potassium salt	Mollenhauer et al. (2011)

Table 4.20 (continued)

End-point, assay	Species, strain, sex	Tissue	Result[a]	Dose (LED or HID) or range	Route, duration, dosing regimen	Comments	Reference
Il1b, *Tnf*, *Nfkb1*, *Creb1*, AP-1(mRNA expression), by qPCR	Rat, Sprague-Dawley, pregnant female	Hippocampus, cortex	Hippocampus of offspring: ↑ *Il1b*, ↑ *Tnf* on PND0 and PN21 ↑ AP-1 at all doses on PND0 and at 0.6 and 2 mg/kg per day on PND21 ↑ *Nfkb1* at 0.6 and 2 mg/kg per day on PND0 and at 2 mg/kg on PND21 ↑ *Creb1* at 0.6 and 2 mg/kg per day on PND0 and PND21 Cortex: ↑ Il1b at 0.6 mg/kg per day on PND0 and PND21, at 2 mg/kg per day on PND21 ↑ *Tnf*, AP-1, *Nfkb1* at 2 mg/kg per day on PND0 and PND21 ↑ CREB at 0.6 and 2 mg/kg per day on PND0 and PND21	PFOS: 0.1, 0.6, or 2.0 mg/kg per day	Oral, from GD2 to GD21	PFOS potassium salt	Zeng et al. (2011)
TNF-α and TGFβ3, by ELISA, western blot	Mouse, BALB/c, male	Testicular homogenates	↑ TNF-α, TGFβ3	PFOA, 0–20 mg/kg per day	28 consecutive days		Lu et al. (2016c)
IL-6, IFN-γ, TNF-α, HGF, by ELISA	Mouse, C57BL/6J, male	Serum	↑ IL-6, IFN-γ, TNF-α, HGF	PFOA, 1, 5, 10, or 20 mg/kg per day	Oral gavage up to 28 days		Soltani et al. (2023)
IL-1β, IL-6, TNF-α, by ELISA	Rat, Sprague-Dawley, female	Serum	↑ IL-6, TNF-α No change in IL-1β	PFOS, 0.3 mg/kg per day	Oral daily gavage of pregnant (GD1 to birth) and lactating (PND1 to PND21) mothers		Liu et al. (2023b)
Tnf, *Il6*, *Nfkb1*, and *Mapk8* gene expression, by qRT-PCR	Rat, Wistar, male	Liver	↑ *Tnf*, *Il6*, *Nfkb1*, and *Mapk8* gene expression	PFOA, 10 mg/kg per day	Oral daily gavage for 4 weeks		Naderi et al. (2023)

Table 4.20 (continued)

End-point, assay	Species, strain, sex	Tissue	Result[a]	Dose (LED or HID) or range	Route, duration, dosing regimen	Comments	Reference
Crp, TNF-α, IL-6, and *Nfkb1* gene expression, by RT-PCR, histopathology, ELISA	Rat, albino, male	Jejenum	↑ CRP, TNF-α, IL-6, *Nfkb1*	PFOS, 5 mg/kg per day	Oral daily gavage for 28 days		Shalaby et al. (2023)
CRP, IL-6, COX-2, inflammatory cell infiltration, oedema, by ELISA, histopathology	Mouse, Kunming, male	Liver	↑ CRP, IL-6, COX-2, inflammatory cell infiltration, oedema	PFOA, 10 mg/kg per day	Intragastrically administered for 14 days		Zou et al. (2015)
Anti-inflammatory effects							
Oedema, dorsal-to-ventral paw thickness measured with micro-callipers	Rat, Sprague-Dawley, male	Paw	↓ oedema	PFOA, 20, 50, or 100 mg/kg	Injection into paw 30 min before induction of inflammation	Carrageenan-induced oedema; dose–response relation	Taylor et al. (2002)
Leukocyte activation, PGE2 synthesis, by ELISA	Rat, Sprague-Dawley, female	Serum	↓ leukocytes and PGE2	PFOA, 150 mg/kg	SC	Acute pancreatitis induced by cerulein	Griesbacher et al. (2008)
Inflammatory oedema formation, by histology		Pancreas	No change				

AP, activator protein; bw, body weight; COX, cyclooxygenase; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; GD, gestational day; HFD, high-fat diet; HID, highest effective dose; IFN, interferon; IL, interleukin; IRF, interferon regulatory factor; KO, knock-out; LED, lowest effective dose; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PG, prostaglandin; PND, postnatal day; ppm, parts per million; qRT-PCR,quantitative reverse transcription-polymerase chain reaction; SC, subcutaneous; TGF, transforming growth factor; TNF, tumour necrosis factor; WT, wildtype; w/w, weight per weight.

[a] ↑, increase; ↓, decrease; (↑), decrease in a study with limitations; +, occurrence.

concentrations of PFOA (0, 0.4, 2, or 10 mg/kg per day) for 28 days. PFOA induced splenic atrophy and increased levels of TNF-α, IL-1β, IL-6, and IRF5 in the spleen at the two highest doses (2 and 10 mg/kg). In the serum, levels of the cytokines IL-1β, IL-6, and IL12p70 were increased by 0.4 mg/kg at higher doses, whereas TNF-α was significantly increased at doses ≥ 2 mg/kg. In addition, dose-dependent increases in activated macrophages were found in the spleen (Guo et al., 2021b).

The effects of exposure to PFOA on immune-mediated liver damage in mice was assessed by Qazi et al. (2013). PFOA exposure at 3 ± 0.7 mg/kg per day for 10 days was associated with decreased hepatic levels of IL-6, TNF-α, IFN-γ, and IL-4, and PFOA caused marked hypertrophy of hepatocytes.

In the study conducted by De Guise and Levin (2021), an investigation of levels of pro-inflammatory markers (IL-5, IL-13, IL-4, IL-12, IL-2, IFN-γ, IL-17α, IL-1α, IL-6, and TNF-α) was performed in mice exposed to low (1.88 mg/kg per day) or high (7.5 mg/kg per day) doses of PFOA in drinking-water for 4 weeks. The results showed a significant reduction in the serum concentrations of the Th2 cytokines IL-5 and IL-13, the Th1 cytokine IL-12, a non-significant dose-dependent increase in IL-2, and an increase in IFN-γ at the highest PFOA exposure. Both doses of PFOA reduced IL-17α and IL-1α, whereas TNF-α was increased at the highest dose of PFOA (De Guise and Levin, 2021).

The hepatic effects of PFOA exposure were also assessed in a study of mice orally dosed with different concentrations of PFOA for 14 days (Yang et al., 2014). The results showed that only high doses of PFOA (10 mg/kg per day) were associated with higher levels of IL-6, COX-2, and CRP (Yang et al., 2014). In addition, hepatic hypertrophy was induced, as indicated by a significant increase in relative liver weight; in addition, a significant increase in inflammatory cells was observed on histological examination.

Qin et al. (2022b) investigated the effects of chronic PFOS exposure (5 mg/kg per day) on liver disease progression by exploring the initiation of NLRP3 inflammasome activation and its potential role in liver inflammation. The study assessed several end-points, including the activation of liver inflammation and steatosis, as well as the release of the pro-inflammatory cytokine IL-1β under a high-fat diet (HFD) or chow diet. The study reported significant findings, including the activation of the NLRP3 inflammasome, an increase in IL-1β secretion, and hepatocyte steatosis in both chow- and HFD-fed animals exposed to PFOS, but exacerbation of liver inflammation around the portal vein and steatosis was also observed in HFD-fed mice exposed to PFOS (Qin et al., 2022b).

Shi et al. (2020) investigated the potential effects of PFOA exposure on the gut and brain in male C57BL/6J mice. Mice were exposed to different concentrations of PFOA (0, 0.5, 1, or 3 mg/kg bw per day) for 35 days. PFOA exposure resulted in increased TNF-α, IL-1 β, and COX-2 and decreased IL-10 levels in the colon. Different results were observed in serum and the brain (see Table 4.20).

Dong et al. (2012a) investigated the effect of 60-day oral exposure to the potassium salt of PFOS on the inflammatory response in peritoneal macrophages, splenic macrophages, and serum of adult male C57BL/6 mice. The mice were dosed daily by oral gavage with PFOS at various doses, ranging from 0.0083 to 2.0833 mg/kg per day, to achieve total administered doses (TADs) of 0.5 to 125 mg/kg over the treatment period. The study reported that PFOS exposure significantly increased the percentage of peritoneal macrophages ($CD11b^+$ cells) at concentrations of ≥ 1 mg/kg TAD, and PFOS exposure elevated the ex vivo production of IL-1β in the peritoneal cavity at concentrations of ≥ 5 mg/kg TAD. Furthermore, PFOS exposure markedly enhanced the ex vivo production of TNF-α, IL-1β, and IL-6 by peritoneal and splenic

macrophages when stimulated with LPS. In addition, the serum levels of TNF-α, IL-1β, and IL-6 were also significantly elevated in response to LPS stimulation (Dong et al., 2012a).

Mollenhauer et al. (2011) investigated the effects of PFOS exposure on the inflammatory markers TNF-α and IL-6 in both serum and peritoneal lavage fluid in adult female $B6C3F_1$ mice. The mice were orally exposed to PFOS at 0, 1, 3, or 300 mg/kg TAD, corresponding to daily doses of 0, 0.0331, 0.0993, or 9.93 mg/kg per day, for 28 days. The study reported that the serum TNF-α levels were significantly decreased by PFOS at 1 mg/kg TAD compared with controls, whereas the serum IL-6 levels were increased. The IL-6 concentrations in peritoneal lavage fluid decreased with increasing PFOS dose. Additionally, the number of splenocytes expressing intracellular IL-6 was significantly decreased in the 3 mg/kg treatment group compared with controls (Mollenhauer et al., 2011).

The pro-inflammatory effects of PFOS on glial activation in the hippocampus and cortex were investigated in a study of rat offspring. The dams received different doses of PFOS via gavage (0.1, 0.6, or 2.0 mg/kg bw) from GD2 to GD21. The study reported increased levels of IL-1β and TNF-α, and elevated mRNA levels of *Jun*, *Nfkb1*, and *Creb1* in the hippocampus at lower doses than in the cortex at PND0 or PND21 (Zeng et al., 2011).

Lu et al. (2016c) investigated the effect of PFOA exposure (0–20 mg/kg per day) on the cytokines TNF-α and TGFβ3 in BALB/c male mice. The authors observed that PFOA increased the TNF-α and TGFβ3-based inflammatory response in testes.

Soltani et al. (2023) investigated the effects of PFOA in C57BL/6 J male mice exposed at 1, 5, 10, or 20 mg/kg. The study reported that PFOA increased the circulating levels of cytokines such as IL-6, IFN-γ, TNF-α, and HGF.

Liu et al. (2023b) investigated the effects of PFOS exposure during pregnancy and lactation in rats. The study reported that PFOS increased the levels of circulating TNF-α and IL-6 on PND14 in the pups.

Naderi et al. (2023) investigated the effects of PFOA exposure on the expression of *Tnfa*, *Il6*, *Nfkb1*, and JNK genes in Wistar male rats exposed to 10 mg/kg. The study reported that PFOA increased the expression of *Il6*, *Tnfa*, *Nfkb1*, and JNK genes in the liver.

Shalaby et al. (2023) investigated *Crp*, *Tnfa*, *Il6*, and *Nfkb1* gene expression in response to PFOS exposure at a dose of 5 mg/kg per day. The study showed increased expression of *Crp*, *Tnfa*, *Il6*, and *Nfkb1* genes.

Zou et al. (2015) investigated the potential of quercetin to offset the adverse effects of PFOA in mice treated intragastrically with PFOA at a dose of 10 mg/kg per day alone or in combination with quercetin at a dose of 75 mg/kg per day for 14 consecutive days. The study showed that PFOA changes the liver morphology by disrupting liver architecture and inducing marked oedema, vacuolar degeneration, hepatocellular necrosis, and inflammatory cell infiltration. In addition, PFOA significantly increased levels of CRP, IL-6, and COX-2, but supplementation with quercetin reduced these levels (Zou et al., 2015).

Overall, when assessing data from experimental animal models, the aggregated findings from the evaluated studies displayed a mixture of outcomes. In a broader context, there is a notable tendency towards a positive correlation of PFAS exposure with chronic inflammation, as indicated by the histological data, and this connection is particularly robust for PFOA, in contrast to PFOS.

Anti-inflammatory effects

See Table 4.20.

Taylor et al. (2002) investigated the effects of PFOA on the inflammation and hyperalgesia induced by intraplantar injection of carrageenan in male Sprague-Dawley rats, with the aim of better understanding the contribution

Table 4.21 End-points relevant to chronic inflammation in non-human mammalian cells in vitro exposed to PFOA or PFOS

End-point	Assay	Tissue, cell line	Result[a]	Concentrations, range	Reference
TNF-α, IL-1β, IL-6, and IL-8	ELISA, western blot	Rat RBL-2H3 mast cells	↑ IL-1β, IL-6, and IL-8 at 100 μM ↑ TNF-α, IL-1β, IL-6, and IL-8 ≥ 10 μM in IgE-stimulated cells	PFOA, 10, 50, 100, or 500 μM	Lee et al. (2017)
TNF-α, IL-6	ELISA	Rat Kupffer cells and primary hepatocytes	↑ TNF-α, IL-6 in Kupffer cells at all time points No change in hepatocytes	PFOS, 100 μM for 0 or 48 h	Han et al. (2018b)
TNF-α	ELISA	Rat C6 glioma cells	↑ TNF-α	PFOS, 0, 0.1, 1, 5, 10, 20, 50, or 100 nM for 12 h or 20 nM for 0, 1, 3, 6, 12, 24, or 48 h	Chen et al. (2018b)
TNF-α	ELISA	Rat HAPI microglial cells	↑ TNF-α > 1 nM and following 3 h of exposure	PFOS, 0–200 nM for 6 h or 20 nM for 0, 1, 3, 6, 8, 12 or 24 h. 0.1% DMSO as the control	Yang et al. (2015)

DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; h, hour(s); IgE, immunoglobulin E; IL, interleukin; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; TNF, tumour necrosis factor.
[a] ↑, increase.

of PPARs to the inflammatory process. The oedema (swelling) and thermal hypersensitivity were assessed by measuring the dorsal-to-ventral paw thickness. The results showed that PFOA inhibited carrageenan-induced oedema and thermal hypersensitivity. The authors concluded that PFOA exhibits anti-inflammatory and/or anti-hyperalgesic effects in vivo by interfering with the initiation of inflammation (Taylor et al., 2002). In another study, the potential anti-inflammatory effects of PFOA in cerulein-induced acute pancreatitis in female rats was investigated (Griesbacher et al., 2008). The study showed that PFOA significantly reduced leukocyte accumulation and prostanoid synthesis, and thereby displayed a potential for anti-inflammatory effects in cerulein-induced acute pancreatitis (Griesbacher et al., 2008).

(ii) Non-human mammalian systems in vitro

See Table 4.21.

The immunological effects of PFOA and PFOS exposure on macrophages via sirtuin regulation was assessed using the murine macrophage cell line RAW 264.7 (Park et al., 2019). The results showed that both PFOA and PFOS had effects on sirtuins. More specifically, PFOA reduced *Sirt7* and *Sirt4* expression at 0.5 μM, but increased *Sirt1*, *Sirt3*, *Sirt4*, *Sirt5*, and *Sirt6* gene expression at 5 μM. In contrast, PFOS increased *Sirt2*, *Sirt3*, *Sirt5*, and *Sirt6* gene expression (Park et al., 2019).

Lee et al. (2017) investigated the correlation between PFOA exposure and allergic inflammation by examining its effect on RBL-2H3 mast cell degranulation and allergic symptoms. The results showed that PFOA activated NF-κB, leading to enhanced expression of TNF-α, IL-1β, IL-6, and IL-8 in IgE-stimulated mast cells (Lee et al., 2017).

Han et al. (2018b) investigated the effects of PFOS exposure on the inflammatory responses of Kupffer cells (liver macrophages). Rat Kupffer cells and primary hepatocytes were exposed to 100 μM PFOS for various time periods (0–48 hours). The study demonstrated that PFOS

induced the production of TNF-α and IL-6 in Kupffer cells (Han et al., 2018b).

Chen et al. (2018b) investigated the effects of PFOS exposure on the TNF-α inflammatory response in rat C6 glioma cells. The C6 cells were exposed to different concentrations (0–100 nM) of PFOS for 12 h or 20 nM PFOS for different periods of time (0, 1, 3, 6, 12, 24, or 48 hours). The study reported that PFOS increased the TNF-α inflammatory response (Chen et al., 2018b).

Yang et al. (2015) investigated the effect of PFOS exposure on the TNF-α inflammatory response in rat HAPI microglia. The cells were treated with PFOS at different concentrations for 6 hours (0–200 nM) or at 20 nM for 0, 1, 3, 6, 8, 12, or 24 hours. The study reported that PFOS increased the TNF-α inflammatory response at ≥ 5 nM for 6 hours and at 20 nM after 3 hours or longer (Yang et al., 2015). Overall, exposure to PFOA and PFOS consistently increased chronic inflammatory responses, as demonstrated by increased IL-6 and TNF-α, in various cell types in vitro.

Synopsis

[Overall, the Working Group noted that the findings of studies of human populations, both cross-sectional and longitudinal, that examined inflammatory markers (cytokines and proteins), neutrophil and leukocyte counts, and inflammatory disease outcomes (eczema, atopic dermatitis, asthma, and allergic rhinitis) in relation to PFOA and PFOS exposure yielded mixed results. Moreover, the associations varied by the biomarker investigated, sex, and exposure source, highlighting the complexity of these relationships. The evidence is not informative enough to conclude that PFOA and PFOS induce chronic inflammation in exposed humans.

In human primary cell studies, a trend emerged for decreases in the levels of chronic inflammatory markers in response to PFOA and PFOS treatment, although the outcomes varied depending on the specific model and assay used. In experimental systems in vivo, the findings were also mixed, but in a broader context, there was a discernible inclination towards a positive association with chronic inflammation, which was more pronounced for PFOA than for PFOS. While the evidence from the in vitro studies is clearer, the results from human and experimental systems in vivo collectively support a nuanced relationship of PFOA and PFOS exposure with chronic inflammation.]

4.2.7 Is immunosuppressive

See Table 4.22.

(a) Humans

(i) Exposed humans

The evaluation of the evidence on whether PFOA and PFOS exhibit the key characteristic of carcinogens “is immunosuppressive” was prompted by observations of clinical end-points related to immunosuppression (e.g. by infections), the effects on related immune end-points (vaccination, immune cell subpopulation monitoring, performance of functional assays on immune cells collected from exposed humans), or the measurement of biomarkers in fluids from exposed humans (cytokines or other mediators of inflammation). The most informative and relevant studies are reported in Table 4.22.

The Working Group first described the clinical outcomes observed in humans, mostly children exposed to the two agents. The strengths of the studies were: serum levels representing combined exposure from all sources and routes, including contributions from metabolites of precursors, the monitoring of diverse types of infections (respiratory tract and digestive tract), and/or responses to diverse vaccine antigens (tetanus, diphtheria, influenza, COVID-19, etc.), and the combination of longitudinal and cross-sectional studies including a large number of individuals. Confounding factors, such as smoking, BMI, maternal education, sex of the

Table 4.22 End-points relevant to immunosuppression in humans exposed to PFOA or PFOS

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Infectious diseases	Maternal plasma	Hokkaido, Japan Study on the environment and children's health to age 4 years Cross-sectional study	PFOS, 4.92 ng/mL; PFOA, 2.01 ng/mL (median) 1558 mother–child pairs	+ (increased OR of total infectious diseases for PFOS) Q4 versus Q1, OR, 1.61; 95% CI, 1.18–2.21; *P* for trend, 0.008	Maternal age, number of older siblings, maternal smoking during pregnancy, maternal education, infant sex, breastfeeding period	Cross-sectional study with a large number of mother–child pairs recruited. Limitations were: infectious disease incidences were based on maternal reports that were not corroborated by medical records. No studies on the validity of self-reported physician-diagnosed infections. These could have resulted in some level of outcome misclassification.	Goudarzi et al. (2017a)
Infectious diseases	Maternal plasma	Hokkaido, Japan Cohort for the study of the environment and children's health Prospective birth cohort with 7 years of follow-up	PFOS, 5.12 ng/mL; PFOA, 1.94 ng/mL (median) 2689 mother–child pairs; finally analysed 2206 children	+ PFOA, increased risk of pneumonia: OR, 1.17 (95% CI, 1.01–1.37); $P = 0.043$ for the children as a whole ($n = 2689$) RSV infection: OR, 1.58 (95% CI, 1.13–2.22); $P = 0.008$, P for trend = 0.038, for children with no siblings ($n = 379$) PFOS was inversely associated with increased risks of pneumonia and RSV infection	Sex, parity, maternal age at delivery, maternal smoking during pregnancy, BMI pre-pregnancy, annual household income during pregnancy	Longitudinal birth cohort design with a large sample size. Limitations were: loss to follow-up, reliance on questionnaire-based outcomes, possibility of recall bias affecting, health outcomes recorded using parental self-reported questionnaires.	Ait-Bamai et al. (2020)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Respiratory tract infection	Fetal umbilical cord blood	Oslo, Norway Environment and Childhood Asthma study Prospective birth cohort with 2–10 years of follow-up	PFOS, 5.2 ng/mL; PFOA, 1.6 ng/mL (median) *n* = 641	+ positive associations of the cord blood concentrations of PFOS and PFOA with airway infections; significant association of lower respiratory tract infection in infants aged 0–10 years with PFOS (β = 0.50; 95% CI, 0.42–0.57) and PFOA (β = 0.28; 95% CI, 0.22–0.35)	Confounders examined were sex; birth weight; birth month; breastfeeding at age 6 and 12 months; maternal smoking during pregnancy; household smoking at birth, at preschool age, and at school age; parental asthma, atopic dermatitis and allergic rhinitis; parental education; and household income	Strengths of the study: high follow-up rate at 10 years; highly representative study sample; prospective longitudinal design, extensive characterization of the children from birth to age 2 years, as well as at 10 years; use of questionnaires, structured interviews, and clinical examinations of the children. Limitations: potential recall bias, especially in questionnaires and interviews, lack of data on important confounding factors, such as parity and previous breastfeeding.	Impinen et al. (2018)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Infectious diseases	Maternal plasma	Norway, MoBA nationwide cohort. Prospective population-based pregnancy sub-cohort with 7 years of follow-up	PFOS, 12.9 ng/mL, PFOA, 2.5 ng/mL (medians) *n* = 1943 *n* = 1270 (questionnaire after 3 years) *n* = 972 (questionnaire after 7 years)	+ positive associations between bronchitis and/or pneumonia with PFOS (RR, 1.20;95% CI, 1.07–1.34; *P* = 0.001) and PFOA (RR, 1.27, 95% CI, 1.12–1.43; *P* < 0.001) (age 0–3 years) Positive association between viral pseudocroup and PFOA (RR, 1.22; 95% CI, 1.07–1.38; *P* = 0.002) (age 0–3 years) Positive association between gastric flu/ diarrhoea and PFOA (RR, 1.48; 95% CI, 1.31–1.67; *P* < 0.001) (age 6 –7 years)	Maternal age, maternal BMI, maternal education, parity, and smoking during the pregnancy Correction for multiple testing using false discovery rate	Longitudinal prospective cohort design with 7 years of follow-up, sample size was relatively large. All samples had PFOS and PFOA concentrations above the LOQ. Loss to follow-up at 7 years and reliance on questionnaire-based outcomes. Possible that a single illness episode could be reported with more than one of the symptoms of infection, making the number of episodes more uncertain.	Impinen et al. (2019)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Infectious diseases	Maternal plasma	Odense, Denmark Odense Child Cohort; data collected 2010–2012 Prospective cohort	PFOS, 7.52 ng/mL; PFOA, 1.68 ng/mL (medians) 1503 mother–child pairs	+ PFOS doubling in mothers associated (adjusted HR, 1.23) with a 23% increase in the risk of hospitalization because of any infection + PFOA and PFOS doubling increased the risk of LRTI by 27% (adjusted HR, 1.27; 95% CI, 1.01–1.59) and 54% (adjusted HR, 1.54; 95% CI, 1.11–2.15), respectively	Maternal educational, maternal, parity, BMI, smoking during pregnancy	Prospective cohort study in 1503 mother–child pairs. Estimation of the number of severe infections may be imprecise. Analyses of some specific types of infections based on a small number of events (gastrointestinal tract, 40 events).	Dalsager et al. (2021)
Response to vaccination (tetanus and diphtheria)	Maternal and infant plasma	Faroe Islands, Denmark National Hospital Faroe Island Birth cohort Prospective study	Maternal PFOS GM, 27.3 ng/mL; age 5 years children PFOS GM, 16.7 ng/mL Maternal PFOA GM, 3.20 ng/mL; age 5 years children PFOS GM, 4.06 ng/mL 656 recruited 1997–2000; *n* = 587, follow-up to 2008	+ Prenatal exposure to PFOS and PFOA negatively associated with anti-diphtheria antibody concentrations, at age 5 and 7 years for PFOS and 7 years for PFOA – (negative) association between anti-tetanus titre and PFOA at age 7 years	Pairwise; possible effect of PCB exposure, birth weight, maternal smoking during pregnancy, duration of breastfeeding	Prospective cohort study addressing the response to vaccination as the end-point. Average sample size. Closed population studied (fishing community in the Faroe Islands).	Grandjean et al. (2012)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Response to vaccination (tetanus, diphtheria)	Infant blood collected at birth and at ages 18 months and 5 years (clinical examinations)	Faroe Islands, Denmark Birth Cohort 5, born in 2007–2009 Prospective birth cohort	Age 18 months, PFOS GM, 7.1 ng/mL; age 5 years, PFOS GM, 4.7 ng/mL Age 18 months, PFOA GM, 2.8 ng/mL; age 5 years, PFOA GM, 2.2 ng/mL Total, $n = 490$; age 18 months, $n = 275$; and age 5 years, $n = 349$	+ (at age 5 years, 152 (44%) children had antibody concentrations lower than the protective level of 0.1 IU/mL for diphtheria and 126 (36%) for tetanus)	Breastfeeding duration, maternal serum PCB concentrations, caesarean section; others not mentioned	Clear vaccination schema, prospective study with follow-up.	Grandjean et al. (2017)
Response to vaccination (*Haemophilus influenzae*, diphtheria, tetanus) and IFN-γ production by PBMCs	Infant blood collected at age 1 year	Berlin, Germany Cross-sectional study	Data expressed as means Formula-fed children, PFOS, 6.8 ± 3.4 ng/mL; PFOA, 3.8 ± 1.1 ng/mL Breastfed children, PFOS, 15.2 ± 6.9 ng/mL; PFOA, 16.8 ± 6.6 ng/mL 101 healthy children age 1 year (21 formula-fed, 80 breastfed for at least 4 months)	+ PFOA associations of adjusted PFOA level with antibody levels for Hib, tetanus IgG1, and diphtheria, $P < 0.05$) No significant associations of PFOS with Hib, or tetanus or diphtheria antibodies + association of PFOA level with a lower production of IFN-γ by PBMCs stimulated with tetanus and diphtheria toxoid found in 55 children) No effect of PFOA or PFOS on infections during the first year of life	Children (age 341–369 days) of German parents, either breastfed for a maximum of 2 weeks ("formula-fed" children) or breastfed (exclusively breastfed for ≥ 4 months) Healthy infants with no acute or chronic diseases, including atopic eczema, no medication, vaccinated	Strengths: children breastfed for a long time with relatively high levels of internal exposure to persistent organic pollutants. High stability of the associations identified using different methods of evaluation, broad spectrum of other contaminants also measured as possible confounders. Limitation: small sample size: 101 children (51 boys, 50 girls) examined; 21 formula-fed, and 80 breastfed for at least 4 months.	Abraham et al. (2020)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Response to vaccination (rubella)	Blood from participants	US NHANES for years 1999–2000 and 2003–2004 Cross-sectional study	Mean PFOA concentration 6 ng/mL in men, 4.3 ng/mL in women, 4.8 ng/mL in youth Mean PFOS concentrations were 28.1 ng/mL, 22.1 ng/mL, and 25.1 ng/mL, respectively; 581 women, 621 men, and 1012 young participants (aged 12–18 years)	+ associations of both PFOA (*P* = 0.0016) and PFOS (*P* = 0.0295) quartiles with rubella titres after adjusting for covariates	Age, race/ethnicity, educational level (high school), BMI, parity, live births	Limitations: cross-sectional study, temporal nature of the associations between PFAS and humoral immune response could not be determined. No young children in the cohort. No information on vaccination status or recent rubella infection. No information on age when vaccination was performed, other infections, or variation in vaccination rates and schedules.	Pilkerton et al. (2018)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Response to vaccination (mumps, rubella, and measles antibodies)	Blood from participants	US NHANES 1999–2000 and 2003–2004 Cross-sectional study	GM PFOS, 20.8 ng/mL; PFOA, 4.13 ng/mL; 1191 children	+ (doubling of PFOS associated with a 7.4% decrease in mumps antibodies; doubling of PFOS and PFOA associated with 5.9% and 6.6% decreases in mumps antibodies if analysis restricted to seropositive children) + (doubling of PFOS was associated with a 13.3% decrease in rubella antibodies; for PFOA decrease not significant) No associations between PFOS or PFOA with measles antibody level	No information on vaccination status in NHANES; however, in early 1990s, most US states have required a measles/mumps/rubella booster before starting school; parity	Limitations: possible exposure misclassification, although less risk than in a typical cross-sectional study, because these compounds have long serum half-lives. Lack of information on receipt and/or timing of measles/mumps/rubella vaccination.	Stein et al. (2016)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Response to vaccination (rubella, measles, and mumps antibodies)	Blood from participants	US NHANES 2003–2004 and 2009–2010 cycles Cross-sectional study	GM PFOA in entire sample, 3.33 ng/mL; 3.42 ng/mL in those with lower folate; 3.15 ng/mL in those with higher folate PFOS in entire sample, 12.44 ng/mL; 12.88 ng/mL in those with lower folate; 11.57 ng/mL for those with higher folate 819 adolescents aged 12–19 years	+ inverse associations of serum PFOS ($P = 0.22$) and PFOA ($P = 0.03$) with rubella antibodies, and between PFOA ($P = 0.01$) and mumps antibodies, only in adolescents with blood folate concentrations < 66th percentile (lower folate group) No associations in adolescents with higher RBC folate levels (higher folate group)	Demographic characteristics, including age (continuous), sex (dichotomous), race, household income, using self-reported questionnaires; income-to-poverty ratio, weight, height, dietary information	NHANES study collected high quality data, good sample size. Seropositivity for rubella and measles was used as a proxy for rubella/measles/mumps vaccination, because of lack of information on vaccination or boosting for the study sample.	Zhang et al. (2023d)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Response to vaccination (influenza virus, A/H3N2 strain)	Blood from participants	Mid-Ohio Valley region of Ohio and West Virginia, USA Cross-sectional study (pre-post measurements)	PFOA: first quartile: 0.25–13.7 ng/mL; second quartile: 13.8–31.5 ng/mL; third quartile: 31.6–90 ng/mL; fourth quartile: 90.4–2140 ng/mL. PFOS: first quartile: 0.1–5.8 ng/mL; second quartile: 5.9–9.2 ng/mL; third quartile:9.3–14.5ng/mL; fourth quartile: 14.7–42.3 ng/mL *n* = 411	+ PFOA (serum concentrations between 13.7 and 90 ng/mL) associated with a reduced response to A/H3N2 influenza virus + PFOA associated with an increased risk of not attaining the antibody threshold considered to offer long-term protection) – (no evidence that PFOS serum concentration was associated with reduced vaccine responses; no associations of self-reported colds or influenza with PFAA concentrations)	Age, medical comorbidities, and medications	Serum samples collected pre-vaccination and 21 ± 3 days post-vaccination in 2010. Number of participants was only 411. Analysis revealed a strong effect of previous influenza vaccination on the immune response, based on self-reported information.	Looker et al. (2014)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Response to vaccination against COVID-19	Blood from participants	Ronneby, Sweden Adults (age 20–60 years) from the Biomarker Cohort and a background-exposure group from Karlshamn Observational study	Ronneby group, median PFOS serum level, 47 ng/mL (adults, *n* = 309) Background group, median exposure PFOS serum level, 4 ng/mL (*n* = 47) 2 doses of mRNA vaccine Spikevax	– (PFOA and PFOS levels were not associated with antibody levels after 5 weeks and 6 months)	Sex, age, smoking Exclusion of individuals with previous SARS-CoV-2-infection Others mentioned in the questionnaire, but analysis not provided	PFAS levels were measured before vaccination and 5 weeks (*n* = 350) and 6 months (*n* = 329) after the second vaccine dose. Prospective design with measurement of antibody responses at defined time points after vaccination. Number of participants used to assess background exposure was small. Possibility that there was an effect of PFAS within the background exposure levels cannot be excluded.	Andersson et al. (2023)
Response to vaccination against COVID-19	Blood from participants	Factories in Alabama and Wisconsin, USA Employees and retirees from two facilities (Spring 2021) Observational study	415 participants (757 observations, repeated measures analyses)	+ fully adjusted IgG concentration was −3.45% (95% CI, −7.03 to 0.26%) per 14.5 ng/mL IQR increase in PFOS + For PFOA, −1.95% (95% CI, −4.35 to 0.51) per 3.59 ng/mL IQR increase in PFOA	Age, sex, race/ethnicity, site, BMI, smoking, immunocompromising conditions Antigenic stimulus group and time since last antigenic stimulus used as precision variables	SARS-CoV-2 neutralizing antibodies were measured to assess the polyclonal response to SARS-CoV-2. Wide distribution of serum concentrations of PFOS and PFOA (increased statistical power to detect an association). Potential longer-term associations with PFAS not assessed.	Porter et al. (2022)

β, adjusted Poisson regression estimate; BMI, body mass index; CI, confidence interval; COVID-19, disease caused by SARS-CoV-2-infection; GM, geometric mean; Hib, *Haemophilus influenzae* type b; HR, hazard ratio; IFN-γ, interferon-gamma; IgG, immunoglobulin G; IQR, interquartile range; IU, international unit; LOQ, limit of quantification; LRTI, lower respiratory tract infection; MoBA, Norwegian Mother, Father and Child Cohort Study; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; PBMCs, peripheral blood mononuclear cells; PCB, polychlorinated biphenyl; PFAA, perfluoroalkyl acids; PFAS, perfluoroalkyl and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; Q, quartile; RR, Poisson regression rate ratio; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; US, United States; USA, United States of America.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study).

infant, breastfeeding period, and others were considered in the analyses. The weaknesses of the studies were mainly restricted to the less detailed self-reported questionnaires used in some of the studies.

Increased risk of infections

The Hokkaido Study on the relation between environment and children's health recruited 20 787 pregnant women from February 2003 to March 2012. Using this cohort, Goudarzi et al. (2017a) examined the relation between prenatal exposure to various perfluoroalkyl acids (PFAA), including PFOS, and the prevalence of infectious diseases in children aged up to 4 years (Goudarzi et al., 2017a). A total of 1558 mother–child pairs were included in this data analysis. PFAA concentrations were measured in maternal plasma samples collected at 28–32 weeks of gestation. Medical history of common infectious diseases, based on physicians' diagnoses, was extracted from the questionnaires completed by the mothers. The median serum concentrations were 4.92 ng/mL for PFOS and 2.01 ng/mL for PFOA, and 67.1% of the children (n = 1046) were diagnosed with at least one of the diseases defined as infectious. After adjusting for appropriate confounders (maternal age, number of older siblings, maternal smoking during pregnancy, maternal education, infant sex, and breastfeeding period), the highest quartile of PFOS concentration was associated with an increased OR for infectious diseases as a whole in all the children. No associations between infectious diseases and the other PFAAs examined, including PFOA, were found (Goudarzi et al., 2017a).

[The Working Group noted that prenatal exposure to PFOS seemed to be associated with infectious disease occurrence in early life. As in other studies, the classification of infectious diseases was based on maternal reports that were not corroborated by medical records. No studies on the validity of self-reported physician-diagnosed infections were conducted.]

In a subsequent study conducted on the same Hokkaido cohort, Ait Bamai et al. (2020) investigated the effects of prenatal exposure to PFAS on the prevalence of infectious diseases in children aged up to 7 years (Ait Bamai et al., 2020). Of the children diagnosed with infectious diseases between ages 2 and 4 years, the analysis was limited to 2206 children (out of a total 2689) for whom data on PFAS measurements and questionnaires, administered to mothers during the first trimester of pregnancy and when children were 7 years old, were available. Median concentrations in the blood were 1.94 ng/mL for PFOA and 5.12 ng/mL for PFOS. Prenatal exposure to PFOA was associated with increased risk of infections in all the children (n = 2689) and with increased risk of respiratory syncytial virus (RSV) infection among children without any siblings (n = 379), and PFOS was inversely associated with increased risks of pneumonia and RSV infection.

In the study by Impinen et al. (2018), the objective was to determine whether prenatal exposure to PFAS was associated with asthma or other allergic diseases, or respiratory tract infections, in childhood. PFAS were measured in cord blood from 641 infants in the ECA prospective birth cohort study cohort in Oslo, Norway. The results showed that reported airway infections were significantly associated with cord blood concentrations of PFOA and PFOS (median concentrations of PFOA and PFOS were 1.6 and 5.2 ng/mL, respectively) and, specifically, that LRTIs in infants aged 0–10 years were associated with PFOA (β = 0.28; 95% CI, 0.22–0.35) and PFOS (β = 0.50; 95% CI, 0.42–0.57). Associations were also found for perfluorooctanesulfonamide, perfluorononanoic acid (PFNA), and perfluoroundecanoic acid. [The Working Group considered that, although it is not known how cord blood concentration is linked to overall future exposure, it represents a good marker of gestational exposure. These observations suggested that PFOA and PFOS were associated with an increased number of

respiratory tract infections in the first 10 years of life, suggesting immunosuppressive effects of these two molecules. The common cold, asthma, allergic rhinitis, atopic dermatitis, and allergic sensitization were not significantly associated with PFOA or PFOS.]

The potential association between PFAS plasma level and the risk of infectious diseases was also investigated over a 7-year follow-up in mothers and children recruited in Norway between 1999 and 2008 in the Norwegian Mother and Child Cohort Study (MoBa) (Impinen et al., 2019). Blood samples were obtained from the mothers during mid-pregnancy (n = 1943). The median blood concentrations were 12.9 ng/mL for PFOS and 2.5 ng/mL for PFOA. From age 0 to 3 years, positive associations were found between bronchitis and/or pneumonia with both PFOA and PFOS, and between pseudocroup and PFOA only. A positive association between PFOA and gastric flu/diarrhoea from age 6 to 7 years was also found.

Dalsager et al. (2021) investigated the association between the maternal serum concentrations of PFAS during pregnancy and the rate of hospitalization of the children due to common infectious diseases between birth and age 4 years (1503 mother–child pairs were included). Serum samples collected from first-trimester pregnant women from the Odense Child Cohort (OCC) in 2010–2012 were analysed for concentrations of PFOS, PFOA, and other PFAS. Hazard ratios (HRs) expressing the relative change in the instantaneous risk of hospitalization associated with a doubling in maternal PFAS concentration were used. A doubling in maternal PFOS concentration was associated with a 23% increase in the risk of hospitalization due to any infection (HR, 1.23; 95% CI, 1.05–1.44). Every doubling of PFOA or PFOS increased the risk of LRTI by 27% (HR, 1.27; 95% CI, 1.01–1.59) or 54% (HR, 1.54; 95% CI, 1.11–2.15), respectively.

[The Working Group considered that these studies thoroughly addressed the effects of exposure during pregnancy or early life on infectious diseases in children. An association was found between PFOS and the overall risk of infectious disease, and of PFOA and PFOS exposure with the risk of LRTIs, including RSV.]

The objective of the study conducted by Granum et al. (2013) was to investigate the effect of prenatal exposure to PFAS on the responses to paediatric vaccines and immune-related health outcomes in children up to age 3 years (Granum et al., 2013). In the prospective BraMat birth cohort (n = 99), a subcohort of MoBa, pregnant women from Oslo and Akershus were recruited during 2007–2008. Maternal PFAS levels in the blood were measured at delivery (median concentrations for PFOA and PFOS were 1.1 and 5.5 ng/mL, respectively), and the children had their levels measured at age 3 years (not reported by the authors). There was a positive association between the maternal concentration of PFOA and the number of episodes of common cold in the children, and between PFOA and the number of episodes of gastroenteritis.

Zhang et al. (2022b) studied data from the NHANES, including for 517 children aged 3–11 years (2013–2014 cycle) and 2732 adolescents aged 12–19 years (2003–2016 cycles) (Zhang et al., 2022b). PFAS concentrations were measured in serum samples from random subsamples (one third of the total population in that age group of the cycle) for children (age 3–11 years) and adolescents (age 12–19 years). The common cold was self-reported by the participant or parent on the basis of having had a head cold or chest cold during the last month. In children, serum PFOA concentrations were related to the common cold, although the confidence intervals were wide (OR, 1.32; 95% CI, 0.67–2.62). No association was seen for PFOS and the common cold among the children. For adolescents, serum PFOS concentration showed a positive relationship with the study outcome (OR, 1.13; 95% CI, 0.96–1.32), although

with limited precision. No positive relation was found for PFOA. The Bayesian kernel machine regression model was used to study the effect of the mixture. The results showed a clear trend for common cold estimates to increase across quantiles of the total PFAS concentration in the children, whereas no clear pattern was found for the adolescents. [The Working Group considered the above two studies to be of low informativeness, because they were based on a small number of people and because the self-reported questionnaire completed by the participant or parent only described having a head cold or chest cold during the last month.]

The aim of the prospective cohort study conducted by Okada et al. (2012) was to evaluate possible associations between maternal PFOA and PFOS levels in pregnant women and infectious diseases in their infants during the first 18 months of life. Pregnant women were recruited between 2002 and 2005 from the Sapporo region of Japan. Maternal serum PFOA and PFOS levels in blood taken from a peripheral vein after the second trimester, or in certain cases immediately after delivery, were measured and related to the IgE levels in cord blood, reflecting the prenatal exposure of the fetus ($n = 231$), and to infectious diseases ($n = 343$) during the following 18 months. The median PFOA and PFOS concentrations were 1.3 ng/mL and 5.2 ng/mL, respectively. The development of infectious diseases at age 18 months was determined from questionnaires to mothers. The results showed no association between maternal PFOA and PFOS levels and infant infectious diseases at age 18 months. [The Working Group noted that this study had some limitations, with a relatively small sample size that was probably insufficient for the identification of significant relationships of PFOA and PFOS exposure with infectious diseases.]

Wang et al. (2022d) investigated the associations of prenatal exposure to PFAS with acute infectious diseases, including the common cold, bronchitis/pneumonia, and diarrhoea, during early childhood. A prospective cohort in Shandong, China, of 235 mother–infant pairs recruited between September 2010 and 2013 from the Laizhou Wan (Bay) birth cohort (LWBC) was studied. Ten selected PFAS were measured in maternal serum, including PFOA and PFOS. The geometric mean concentrations of PFOA and PFOS were and 44.88 ng/mL and 4.39 ng/mL, respectively. Questionnaires completed by parents were used to collect detailed information on acute infectious diseases after 1 year of follow-up. The questionnaire data were confirmed by the medical records. The OR for diarrhoea was 4.99 (95% CI, 1.86–13.39) per log-unit increase in PFOA. When stratified by breastfeeding duration (at least 4 months, or not), the relation between PFAS exposure and diarrhoea was more pronounced among the breastfed infants. However, there were no associations between prenatal PFAS exposure and the common cold or bronchitis/pneumonia (Wang et al., 2022d). [The Working Group noted that the sample size of the study was small, and the results should be interpreted with caution.]

Effects on responses to vaccination

In the following paragraphs, the potential effects of PFOA and PFOS on the response to vaccination are evaluated. The immune response to vaccination is normally dependent on the adaptive immune system, which involves antigen presenting cells and T and B lymphocytes. The response to common vaccines represents a relevant end-point to address the consequences for the immune system of the exposure to chemicals. Indeed, the concentrations of antigen-specific antibodies in serum represent a useful and clinically relevant indicator of immune function in humans. The studies are reported in descending order of informativeness.

Grandjean et al. (2012) studied the relationship between PFAS exposure and vaccination with tetanus and diphtheria toxoids in infants using the serum antibody concentrations at ages

5 and 7 years as a marker. ELISA was used for tetanus antibody and Vero cell-based neutralization assay was used for diphtheria antibody. The study was a prospective study of a birth cohort from the National Hospital in the Faroe Islands, Denmark, with a total of 656 consecutive single-child births recruited during 1997–2000, 587 of which had follow-up until 2008. Maternal serum PFAS concentrations were measured during the third trimester. Maternal geometric mean serum concentrations were 3.20 and 27.3 ng/mL for PFOA and PFOS, respectively. Geometric mean serum concentrations at age 5 years were 4.06 and 16.7 ng/mL for PFOA and PFOS, respectively. Prenatal exposure to both PFOA and PFOS, as indicated by the maternal serum concentrations, was negatively associated with the anti-diphtheria antibody concentrations. The strongest negative correlations were for the association between maternal PFOS and pre-booster diphtheria antibody concentration at age 5 years, with a two-fold higher exposure being associated with a difference of −39% (95% CI, −55% to −17%) in the diphtheria antibody concentration. For the antibody concentrations at age 7 years, an increase of two-fold in PFOA exposure associated with differences of −36% (95% CI, −52% to −14%) and −25% (95% CI, −43% to −2%) for tetanus and diphtheria, respectively. PFOS exposure was associated with a difference in diphtheria antibody of −28% (95% CI, −46% to −3%) and a non-significant difference of −23.8% (95% CI, −44.3% to 4.2%) for tetanus. These results showed that elevated exposures to PFAS were associated with reduced humoral immune responses to routine childhood immunizations in children aged 5 and 7 years. [The Working Group noted the following strengths: the serum levels represented the combined exposure to all exposure sources and routes, including metabolites of precursors, over a relatively long period of time, due to the long half-lives of PFOA and PFOS. In addition, the study considered co-exposure to smoking and polychlorinated biphenyls (PCBs).]

In a subsequent study, Grandjean et al. (2017) attempted to link the PFAS-associated decreases in antibody concentrations to past exposure during infancy using a new prospective birth cohort in the Faroe Islands (Cohort 5, born in 2007–2009). A total of 381 children participated in the examinations at age 5 years, and 370 of these had also participated at age 18 months. To increase the statistical power of the study, joint statistical analyses were conducted with a previous birth cohort in the Faroe Islands (Grandjean et al., 2012), in which the PFAS exposure was higher. The geometric mean PFOS concentration at age 5 years was 16.7 ng/mL in the 2012 study versus 4.7 ng/mL in the 2017 study. The geometric mean PFOA concentration at age 5 years was 4.06 ng/mL in the 2012 study versus 2.2 ng/mL in the 2017 study. The serum concentrations of antibodies against the tetanus and diphtheria vaccines were determined at age 5 years. This study confirmed the inverse associations of prenatal exposure with the antibodies against both the tetanus and diphtheria toxoids. A doubling of the exposure to PFOA led to a decrease of about 30% in the antibody concentration for tetanus at age 5 years, whereas the association with diphtheria was weaker. Using the same cohorts of people recruited in the Faroe Islands, Mogensen et al. (2015b) estimated the combined (analysis of multiple exposures) change in antibody concentrations for tetanus and diphtheria. In this study, it was not possible to attribute causality to any single PFAS compound. Hence, the three 7-year concentrations, i.e. the concentrations of PFOA, PFOS, and perfluorohexanesulfonic acid (PFHxS), were combined, and the study showed that an increase of two-fold in PFAS was associated with a decrease of 54.4% (95% CI, 22.0–73.3%) in the antibody concentration after vaccination (Mogensen et al., 2015b).

A cross-sectional study in 101 healthy children (aged 1 year) from Berlin, Germany (21 formula-fed, and 80 breastfed for at least 4 months), which included measurements of

internal levels of POPs and a broad panel of biological parameters, was performed at the end of the 1990s (Abraham et al., 2020). The plasma levels (mean ± SD) of PFOA and PFOS were 3.8 ± 1.1 and 6.8 ± 3.4 μg/L in the 21 formula-fed children, and 16.8 ± 6.6 and 15.2 ± 6.9 μg/L in the 80 children who were exclusively breastfed for at least 4 months, respectively. This study showed significant correlations of the level of PFOA, but not of PFOS, with the adjusted levels of vaccine antibodies against *Haemophilus influenza* type b (Hib; $r = 0.32$), tetanus ($r = 0.25$), and diphtheria ($r = 0.23$). These results showed an association between exposure to PFOA and lower levels of antibodies. Moreover, significant associations of PFOA level with lower production of IFN-γ after the stimulation of peripheral blood lymphocytes with tetanus ($r = -0.33$; $P = 0.01$) or diphtheria ($r = -0.24$; $P = 0.08$) toxoid were found in 55 children, suggesting alterations in the cell-mediated immune system. However, no relevant associations were observed between PFOA or PFOS and lymphocyte proliferation after specific or nonspecific stimulation with vaccine components (Abraham et al., 2020). [The Working Group noted that the strengths of the study were that studies on children in their first year are especially relevant because infants have a relatively high exposure if breastfed and may have a higher susceptibility, because their immune system is developing; and the use of a sensitive analytical method to measure PFOA, PFOS, and PFHxS in almost all samples. The plasma concentrations represented the combined exposure from all exposure sources and routes, including metabolites of precursors, and although measured at a single time point, represent exposure over a relatively long period of time, because of the long half-lives of PFOA and PFOS. Also, co-exposures to other substances, including several carcinogens, were considered in the analysis.]

To examine whether serum PFOA and PFOS were associated with a reduced immunity-response to rubella immunization, and whether interactions with sex or ethnicity warranted data stratification, Pilkerton et al. (2018) analysed a nationally representative sample of individuals aged 12 years in NHANES, USA, for the years 1999–2000 and 2003–2004. In total, 581 adult women, 621 adult men (age, 19–49 years), and 1012 young participants (age, 12–18 years) were included. The average serum PFOA concentrations were 6 ± 0.3 ng/mL in men, 4.3 ng/mL in women, and 4.8 ng/mL in the young participants. The average serum PFOS concentrations were 28.1 ng/mL in men, 22.1 ng/mL in women, and 25.1 ng/mL in young participants. Whole-group linear regression analyses of the young participants showed no significant associations of the rubella titre with either PFOA or PFOS, after adjusting for covariates (sex, age, race/ethnicity, educational level, and BMI), nor were there interactions of these PFAS with sex or ethnicity. In adults, there were significant associations of both PFOA ($P = 0.0016$) and PFOS ($P = 0.0295$) quartiles with the rubella titre after adjusting for covariates. In adults, when the effect size analyses were stratified by sex, a significant negative association was observed only for PFOA in men; the association for PFOS was borderline.

Stein et al. (2016), in a cross-sectional study, examined the relations between PFAS serum concentration and measles, mumps, and rubella antibody concentrations in the NHANES cohorts of 1999–2000 and 2003–2004 ($n = 1191$). The study included 1191 children. The geometric means for serum concentrations were 20.8 ng/mL (95% CI, 19.1–22.7 ng/mL) for PFOS and 4.13 ng/mL for PFOA (95% CI, 3.76–4.53 ng/mL). A doubling of PFOS was associated with a 7.4% (95% CI, −12.8% to −1.7%) decrease in mumps antibodies. When restricted to seropositive children, a 6.6% (95% CI, −11.7% to −1.5%) decrease in mumps antibodies was associated with a doubling of PFOA and a 5.9% decrease with a doubling of PFOS.

The largest decrease in antibody concentration (13%) was observed for the association of rubella antibodies with a doubling of PFOS, although only in the seropositive subsets.

Crawford et al. (2023) with financial support by the industry, conducted a systematic review, using PROSPERO to determine the closeness of the association between a doubling in serum PFAS concentration and the difference in $\log_e$[antibody concentration] after vaccination (Crawford et al., 2023). The literature retrieved from PubMed and Web of Science searches was evaluated, and five PFAS, including PFOA and PFOS, were assessed across 14 reports deemed eligible and published between 2012 and 2022. The evaluation of the responses to diphtheria, rubella, and tetanus infections, including a meta-analysis, were supportive of an association with PFAS, with stronger associations identified for PFOA, PFOS, and PFHxS than for PFNA or perfluorodecanoic acid.

In a study conducted in 819 adolescents aged 12–19 years in the NHANES 2003–2004 and 2009–2010 cycles who had detectable serum levels of rubella and measles antibody, Zhang et al. (2023d) found inverse associations of the serum concentrations of PFOA ($P = 0.03$), and to a lesser extent PFOS ($P = 0.22$), with rubella antibodies. An inverse correlation was also found between the serum concentrations of PFOA ($P = 0.01$) and mumps antibodies. However, these associations were present only in adolescents with lower folate levels (measured as a ratio between whole-blood and serum folate levels) and not in adolescents with higher levels. [The Working Group noted that the study did not investigate the potential mechanism or the effect of folate.]

An investigation of the relation between the antibody response after vaccination with an inactivated trivalent influenza vaccine and the circulating levels of PFOA and PFOS was performed in the USA (Looker et al., 2014). The study participants comprised 411 adults who lived for at least 1 year between 1950 and 2004 in one of six water districts in the Mid-Ohio Valley region of the USA, where the inhabitants had elevated PFOA exposure from contaminated drinking-water. To assess the response to influenza vaccination, haemagglutination-inhibition tests were conducted on serum samples collected pre-vaccination and 21 ± 3 days post-vaccination in 2010. Serum samples were also analysed for PFOA and PFOS concentrations. The concentrations of PFOA were as follows: first quartile: 0.25–13.7 ng/mL; second quartile: 13.8–31.5 ng/mL; third quartile: 31.6–90 ng/mL; and fourth quartile: 90.4–2140 ng/mL. The concentrations of PFOS were as follows: first quartile: 0.1–5.8 ng/mL; second quartile: 5.9–9.2 ng/mL; third quartile: 9.3–14.5 ng/mL; and fourth quartile: 14.7–42.3 ng/mL. Questionnaires were completed regarding the occurrence and frequency of recent (during the last 12 months) respiratory infections. The results showed that PFOA at serum concentrations between 13.7 and 90 ng/mL was associated with a reduced response to influenza virus A/H3N2. The authors also found an increased risk of not attaining the antibody threshold considered to offer long-term protection. However, there was no evidence that the PFOS serum concentration was associated with the vaccine response. The authors also found no evidence of an association between self-reported colds or influenza and PFOA or PFOS concentration, suggesting that the extent of suppression of the vaccine response and the associations with disease outcomes are difficult to assess, particularly in a small study sample (Looker et al., 2014).

Andersson et al. (2023) performed an observational study with prospective design of 309 adults from the Ronneby Biomarker Cohort in Ronneby, Sweden, who had a median PFOS serum concentration of 47 ng/mL, and a group from another town with background exposure ($n = 47$) who had a median PFOS serum concentration of 4 ng/mL. These groups received two doses of the Spikevax mRNA vaccine against

COVID-19 (SARS-CoV-2). The serum levels of seven PFAS were measured before vaccination. IgG against the SARS-CoV-2 spike antigen was measured before vaccination and 5 weeks ($n = 350$) and 6 months ($n = 329$) after the second vaccine dose. PFAS exposure, regardless of how it was estimated, was not negatively associated with antibody levels after COVID-19 vaccination.

In a study by Porter et al. (2022), a total of 415 participants were included in repeated measures analyses (757 observations) of antibodies against SARS-CoV-2. The participants were current and retired workers from two manufacturing facilities with historical use of PFAS in Alabama and Wisconsin, USA; the study was sponsored by the company. The log-transformed concentrations of anti-spike IgG and neutralizing antibodies were modelled in relation to the concentrations of PFAS at enrolment, after adjusting for antigenic stimulus. The median concentration of PFOS was 121.50 ng/mL and that of PFOA was 31.7 ng/mL. The fully adjusted IgG concentration was 3.45% lower (95% CI, –7.03% to 0.26%) for each 14.5 ng/mL (interquartile range) increase in PFOS. For PFOA, the results were comparable to those for PFOS: the concentration was 1.95% lower (95% CI, −4.35% to 0.51%) for each 3.59 ng/mL (IQR) increase in PFOA.

One of the objectives of the study conducted by Granum et al. (2013) was to investigate the effect of prenatal exposure to PFAS on the responses to paediatric vaccines. To this end, the antibody levels specific for four vaccines in the Norwegian Childhood Vaccination Program were measured: measles, rubella, tetanus, and Hib (Granum et al., 2013). Pregnant women in the prospective BraMat birth cohort ($n = 99$), a subcohort of MoBa, were recruited during 2007–2008 in Oslo and Arkershus, Norway. Blood samples were collected from the mothers at the time of delivery and from the children at age 3 years. In multivariate models, increased concentrations of all four PFAS, including PFOA (median, 1.1 ng/mL; interquartile range, 0.2–2.7 ng/mL) and PFOS (median, 5.5 ng/mL; interquartile range, 1.4–11.0 ng/mL), in maternal blood (50 samples tested) were significantly associated with reduced levels of anti-rubella antibodies in the children at age 3 years (PFOA bivariate $\beta = -0.40$; 95% CI, −0.64 to −0.17; $P = 0.001$; and PFOS bivariate $\beta = -0.08$; 95% CI, 0.14 to −0.02; $P = 0.007$). However, no significant associations were found between the concentrations of PFAS, including PFOA and PFOS, and antibody levels developed in response to the other vaccines, including tetanus toxoid (Granum et al., 2013). [The Working Group noted that a major limitation of the study was the very small study sample.]

In a randomized controlled trial conducted in rural regions close to Bissau, Guinea-Bissau, where exposure to PFAS is generally low compared with that in all other parts of the world (237 infants; median serum PFOS concentration, 0.77 ng/mL; median serum PFOA concentration, 0.68 ng/mL), a doubling of serum PFOS concentration in children vaccinated at age 4–7 months was associated with a 21% lower concentration of measles antibodies (95% CI, –37% to –2%) measured at age 9 months for each 1 ng/mL increment in blood concentration (Timmermann et al., 2020).

Kaur et al. (2023) investigated the relation between maternal plasma PFAS concentration ($n = 72$) and SARS-CoV-2 anti-spike IgG antibody protein levels in a New York City, USA-based pregnancy cohort, the Gen C cohort, established in April 2020. Of all the congeners tested, the nine congeners that were measurable in the maternal plasma negatively correlated with the SARS-CoV-2 anti-spike IgG antibody level ($P < 0.05$) in multivariable analyses. There was a significant association for PFOA ($\beta = -0.62$; 95% CI, −1.11 to −0.12; $P = 0.017$), but not for PFOS ($\beta = -0.33$; 95% CI, −0.85 to 0.20; $P = 0.209$). The mean maternal serum levels were 1.10 ng/mL for PFOA and 1.84 ng/mL for PFOS.

In Greenlandic children who participated in the INUENDO cohort and the IVAAQ cohort,

Timmermann et al. (2022) examined associations between exposure to PFAS, PCBs, and mercury in all forms and blood concentrations of diphtheria and tetanus vaccine antibodies after vaccination. The study included cross-sectional data for children aged 7–12 years, collected in Greenland during 2012–2015. A total of 338 children were eligible for the study and had blood samples available, and 175 of these had available vaccination records. In the children, the median concentration of PFOS was 8.68 ng/mL and that of PFOA was 2.28 ng/mL. The authors found that higher childhood exposure to environmental chemicals was associated with lower antibody concentrations after vaccination and with higher odds of not having a sufficient antibody concentration to be protected against diphtheria. The ORs for not being protected against diphtheria were 1.14 (95% CI, 1.04–1.26) for PFOS and 1.41 (95% CI, 0.91–2.19) for PFOA (Timmermann et al., 2022). [The Working Group noted that the concentrations of the specific diphtheria antibodies were quite low, probably due to the time interval since the most recent vaccination or booster.]

End-points of immunity

Associations between neonatal serum concentrations of PFOA and PFOS and immunoglobulin (Ig) isotype profiles in dried blood spots from newborns were assessed in a prospective cohort of infants (3175 infants in the Upstate KIDS prospective birth cohort study, 2008–2010) (Jones et al., 2022). After correction for multiple comparisons, higher PFOA levels in the neonates' blood samples were associated with higher IgA, IgM, and IgG2 levels, and lower levels of IgE, in single-pollutant models, with P values ranging from 0.002 to < 0.00001. However, PFOS showed no significant association with the Ig isotype profile. When PFOA and PFOS were examined as a mixture, with estimates adjusted for infant sex, maternal BMI, race, parity, age, and infertility treatment, small (but significant) to marginal joint effects of the mixture on the outcome isotypes, consistent with single-pollutant PFAS models, were found (Jones et al., 2022). [The Working Group considered that the clinical relevance of these observations remains to be established.]

Zhu et al. (2016) assessed the polarization of T-helper lymphocytes in 231 asthmatic children and 225 non-asthmatic children (controls) from the Genetic and Biomarkers study for Childhood Asthma (GBCA) in northern Taiwan, China. The levels of Th1 (IFN-γ and IL-2) and Th2 (IL-4 and IL-5) cytokines were measured using ELISA. The Th2/Th1 cytokine concentration ratio and the IgE levels were in accordance with the asthmatic status of the patients. The results showed that asthmatic children had significantly higher serum PFAA concentrations, but not higher concentrations of specific PFAS, compared with the healthy controls. In male asthmatic children, significant positive associations were found between the IL-4/IFN-γ ratio and PFOS and PFOA (correlation coefficients, Spearman $\rho = 0.210$; $P = 0.008$; and Spearman $\rho = 0.290$; $P < 0.001$, respectively), and between the IL-5/IFN-γ ratio and PFOS and PFOA (Spearman $\rho = 0.198$; $P = 0.017$; and Spearman $\rho = 0.189$; $P = 0.017$, respectively). In female asthmatic children, significant associations were found only of the IL-4/IFN-γ ratio with PFOS (Spearman $\rho = 0.291$; $P = 0.013$), and of the IL-5/IFN-γ ratio with PFOS (Spearman $\rho = 0.245$; $P = 0.037$). [The Working Group considered that the limitation of these analyses should be noted, with only single serum samples being collected for cytokine measurement.]

Lopez-Espinosa et al. (2021) studied the associations of PFHxS, PFOA, PFOS, and PFNA with leukocyte counts (neutrophils, monocytes, eosinophils, lymphocytes, and basophils). Serum PFAS concentrations were measured, and leukocyte types were counted in 42 782 (between 2005 and 2006) and 526 (during 2010) adults living in an area with PFOA contamination of

drinking-water in the Mid-Ohio Valley, USA. The circulating numbers of immune cells (CD3+ T-cells, CD3+ CD4+ T-helper cells, CD3+ CD8+ T-cytotoxic cells, CD3+ CD4+ CD8+ double-positive T-cells, CD16+ CD56+ natural killer (NK) cells, and CD19+ B cells) were measured only in the 2010 adults. Data on the study participants were collected through questionnaires, and the covariates sex, race/ethnicity, age, education, BMI (available for the 2005–2006 survey), tobacco consumption, alcohol intake, a present or past diagnosis of an immune disease and/or cancer, and the use of anti-inflammatory medication (available for the 2010 survey) and other medication were considered. A weak consistent association was shown for both PFOA and PFOS with the total lymphocyte count, but no clear associations with the various lymphocyte subtypes. No clear associations were found between the total leukocyte count and PFAS level, with statistical significance only being reached for PFHxS and PFOS in the 2005–2006 survey (Lopez-Espinosa et al., 2021). [The Working Group noted that the small differences in the overall lymphocyte count observed in the study cannot be considered clinically meaningful.]

(ii) Human cells in vitro

Mechanisms of PFOA- and PFOS-induced immunosuppression were evaluated in vitro using human primary immune cells (lymphocytes and monocytes) and human immune cell lines. In a recent study, Kasten-Jolly and Lawrence (2022) showed a significant reduction in the proliferation of human T-cells (from peripheral blood mononuclear cells (PBMCs) isolated from healthy staff of the New York State Department of Health) in response to phytohaemagglutinin (PHA; polyclonal activation) or influenza antigen (memory T-cell activation) in the presence of 100 μM PFOA or PFOS (cells were exposed to increasing concentrations of the two chemicals at 1, 10, and 100 μM). Moreover, both PFOA and PFOS at the concentration of 100 μM significantly ($P < 0.05$) suppressed the release of IFN-γ, a cytokine known to be important for the immune response to viruses. The production of other cytokines and chemokines by human PBMCs was also affected by PFOA (MIP3α/CCL20, ITAC, IL-10, IL1-β, IL-6, and IL-7) and PFOS (same as for PFOA, plus GM-CSF, fractalkine, IL-17, IL-13, IL-21, and TNF-α). PFOS was more potent than PFOA. However, for both molecules, IL-2 production was significantly augmented, despite an inhibition of proliferation. These effects were significant with 100 μM of PFOA or PFOS, except for CCL20, the production of which was increased by 10 μM of either chemical.

The cytokines released by T lymphocytes were evaluated using a whole-blood assay and peripheral blood from healthy donors after stimulation with the polyclonal activator PHA. Both PFOA and PFOS reduced PHA-induced IL-4 and IL-10 release, whereas IFN-γ release was affected only by PFOS (Corsini et al., 2011). In all the tests performed, PFOS was a more potent inhibitor of cytokine production than PFOA. At the concentration of 0.1 μg/mL (equivalent to 241 nM PFOA and 200 nM PFOS), only PFOS was able to reduce the secretion of TNF-α, IL-4, IL-6, IL-10, and IFN-γ. The same assay was used to assess the release of pro-inflammatory cytokines after LPS stimulation. Both PFOA and PFOS induced a dose-related decrease in TNF-α production, IL-8 release was unaffected, and the release of IL-6 was reduced by PFOS, but not by PFOA (Corsini et al., 2011). Similar findings were reported in the human promyelocytic cell line THP-1, with PFOS causing significant decreases in the LPS-induced release of TNF-α and IL-8, starting from a concentration of 1 μg/mL. In these models, the suppression of cytokine production by PFOA was dependent upon PPARα activation, whereas the effects of PFOS were independent of PPARα activation. On the other hand, Midgett et al. (2015) demonstrated that PFOA did not have an impact on IL-2 production, but PFOS

at a concentration of 100 µg/mL suppressed IL-2 production in both the Jurkat T-cell line and human primary CD4+ T cells isolated from leukocytes of healthy donors (n = 11).

Using human primary lymphocytes (6 donors) as a model, together with integrative omics analyses, including of the transcriptome and lipidome, and bioinformatics analysis, Li et al. (2020c) observed that PFOS moderately altered the production of interleukins (IL-1, IL-4, IL-6, and IL-8) in human lymphocytes and dysregulated clusters of genes and lipids that play important roles in immune function, such as in lymphocyte differentiation, the inflammatory response, and the immune response. The same group performed a metabolomic analysis on human lymphocytes and highlighted alterations in organic acids, including taurine and NG-dimethyl-l-arginine; organooxygen molecules, such as d-ribulose 5-phosphate; and lipid compounds, such as carnitines; in lymphocytes treated with PFOA (Li et al., 2020d).

Corsini et al. (2012), using a human whole-blood assay and LPS stimulation, confirmed the inhibitory effect of PFOS, and to a lesser extent PFOA, on TNF-α production. The concentrations used were 0.1, 1, and 10 µg/mL. These results were confirmed using the human promyelocytic cell line THP-1.

In another study that used the monocytic THP-1 cell line under LPS stimulation, Masi et al. (2022) also observed that PFOS (0.2–20 µM) induced a dose-dependent downregulation of RACK-1 receptor promoter transcriptional activity, mRNA expression, and protein levels, which was mirrored by reductions in IL-8 and TNF-α production and CD86 expression.

Houck et al. (2023) tested 147 PFAS using a phenotypic screening platform of human primary cell co-culture systems, the BioMAP Diversity PLUS panel. This platform models tissues and the disease biology of organs (vasculature, immune system, skin, and lung). The platform includes 12 assays that use 12 different human primary cell systems and includes 148 mechanistic end-points, of which several are modulators and effectors of vascular and immune biology. The authors included four known immunosuppressants (azathioprine, methotrexate, dexamethasone, and cyclosporine A), covering different mechanisms of immunosuppressive action, in the test set, along with the 147 PFAS (Houck et al., 2023). PFOA and PFOS reduced the level of IL-10 in the co-culture system, and their mechanisms of action were different from those of other known immunosuppressants.

Human NK-cell activity has recently been shown to be slightly inhibited after 24 hours of exposure of human primary PBMCs from male donors to 0.2 µM PFOS (Maddalon et al., 2023b).

[The Working Group noted that PFOS and, to a lesser extent, PFOA reduced the production of some cytokines, with the possible involvement of PPARα and lipid metabolism, and also altered lymphocyte proliferation and NK-cell activity. The latter effect was only in cells isolated from male donors.]

(b) Experimental systems

(i) PFOA in non-human mammalian systems in vivo

Effects on immune end-points

PFOA was evaluated using standard assays of immune function (Yang et al., 2002a; De Witt et al., 2008; McDonough et al., 2020; De Guise and Levin, 2021).

In the study by De Witt et al. (2008), sheep red blood cell (SRBC) IgM antibody titres were suppressed in C57BL/6J and C57BL/6N mice in a dose-dependent manner at a PFOA exposure of ≥ 3.75 mg/kg per day (ammonium salt; purity, ≥ 98%) for 10–15 days given via the drinking-water. However, the IgG titres were elevated at PFOA exposure levels of 3.75 and 7.5 mg/kg per day (De Witt et al., 2008).

Using a diet supplemented with PFOA at 0.02% (w/w), Yang et al. (2002a) showed a clear decrease in the production of antibodies of both IgM and IgG isotypes against horse erythrocytes injected intravenously (5–10 × 10^7 cells in 200 mL of Earle balanced solution) into C57BL/6 mice.

A significant decrease in the IgM antibody response to the T-cell-dependent antigen keyhole limpet haemocyanin (KLH) was observed with PFOA at a dose of 1.88 mg/kg per day given to $B6C3F_1$ mice in their drinking-water for 4 weeks (De Guise and Levin, 2021).

Altered T-lymphocyte populations were observed in male ICR mice exposed to 0, 2, 10, 50, or 250 ppm (0, 0.49 ± 0.04, 2.64 ± 0.15, 12.63 ± 1.15, or 47.21 ± 3.57 mg/kg per day) PFOA (free acid; purity, 98%) in drinking-water for 21 days. In the spleen, all the doses of PFOA reduced CD8+ lymphocytes, and CD4+ lymphocytes were increased by PFOA at 50 and 250 ppm. Exposure to PFOA at 250 ppm increased CD8+ lymphocytes in the thymus. However, PFOA induced a significant decrease in body weight from the initial value at the two highest doses (Son et al., 2009) (see also Section 4.2.7(c) and Section 4.2.6(b)(i)).

Iwai and Yamashita (2006) treated male Crj:CD(SD)IGS rats orally for 14 days with PFOA (ammonium salt, 10% aqueous solution). The doses of PFOA were 0, 0.5, 5, and 50 mg/kg per day. Blood parameters (haematocrit, erythrocyte count, and haemoglobin) were reduced by PFOA at 50 mg/kg, but the reticulocyte count was not affected. No influence on lymphocyte subsets in blood (T-cells and NK cells) was observed at any of the tested doses. [The Working Group noted that the circulating levels of PFOA were not measured in this study.]

Loveless et al. (2008) conducted a study with the objective of comparing the effects of PFOA (ammonium salt, 19.5% aqueous solution) in male CD rats and CD-1 mice. Both species were dosed by oral gavage with *n*-PFOA at 0.3, 1, 10, or 30 mg/kg per day for 29 days. In the rats, the production of anti-IgM SRBC antibodies was not affected; however, systemic toxicity (reduced body-weight gain and increased corticosterone levels) was observed at the 10 and 30 mg/kg doses. Systemic toxicity was also observed in mice at the 10 and 30 mg/kg doses. In mice, reduced IgM antibody production after 10 mg/kg was observed (20% suppression), along with reduced spleen and thymus weights and cell numbers, as well as microscopic depletion/atrophy of lymphoid tissue (thymus and spleen). [The Working Group noted that non-specific alteration of the immune response to SRBCs (a T-cell-dependent antigen) because of systemic toxicity of PFOA in mice cannot be excluded.]

The proliferation in response to both T-cell (concanavalin A, ConA) and B-cell (LPS) activators of ex vivo spleen cells derived from male C57BL/6 mice fed for 10 days with a diet containing 0.02% (w/w) PFOA was attenuated by the PFOA treatment (Yang et al., 2002a).

Because of concerns regarding skin exposure, the immunotoxicity of PFOA (free acid; purity, 96%) after dermal exposure (0.5–2% w/v, or 12.5–50 mg/kg per dose administered topically on the dorsal portion of the ear) has been also evaluated in mice. The IgM antibody response to SRBCs was significantly reduced in the spleen after 4 days of dermal exposure in $B6C3F_1$ mice (Shane et al., 2020). In addition, PFOA exposure caused a significant decrease in thymus and spleen weights after 4 and 14 days of exposure. Immune-cell phenotyping identified a reduction in the frequency of splenic B-cells (Shane et al., 2020).

Effects on the developing immune system in mice

Experiments have also been conducted in mice to study the effects of PFOA and PFOS on the developing immune system (Keil et al., 2008; Hu et al., 2010, 2012; Zhong et al., 2016; Torres et al., 2021). The mean SRBC-specific IgM antibody titres in the female offspring (PND48) of C57BL/6 mouse dams exposed to

PFOA (ammonium salt; purity, ≥ 98%) from GD6 to GD17 were not affected by doses of up to 1 mg/kg bw given in the drinking-water (Hu et al., 2010). The adult offspring of dams exposed to PFOA at 2 mg/kg during gestation and lactation showed a reduction in the percentage of splenic CD4+CD25+Foxp3+ T-cells and in IL-10 production, suggesting alterations to regulatory T cells (Hu et al., 2012).

Effects in models of infection in mice

One study showed that PFOA (purity, 96%) can accumulate in the lungs, making this organ more susceptible to viral infections (Ahmad et al., 2021). Ahmad et al. (2021) observed in CD-1 mice orally exposed to 5 or 20 mg/kg per day PFOA for 10 days that it accumulated in the lungs, with mean concentrations of 14.14 ± 2.95 and 36.41 ± 15.09 µg/g, respectively, measured in the lung tissue. Changes in mRNA expression of the DNA methylation regulator genes encoding DNA methyltransferases (*Dnmts*) and tet methylcytosine dioxygenases (*Tets*) were observed, along with the genes encoding the membrane proteins angiotensin converting enzyme 2 (*Ace2*) and transmembrane serine protease 2 (*Tmprss2*), which are involved in SARS-CoV-2 virus infection (see also Section 4.2.4).

(ii) PFOS in non-human mammalian systems in vivo

Effects on immune end-points

Experimental data derived mainly from in vivo experiments in rodents (rats or mice) exposed for 7–60 days using the oral route (gavage or food intake). In vivo antibody production was evaluated after immunization with a specific antigen, and ex vivo experiments measured lymphocyte proliferation, cytokine production, and NK cell activity. Doses of PFOS above 20 mg/kg (total administered dose, TAD) were found to be toxic (reduced body weight) and were considered not to be relevant in general.

In vivo daily exposure of B6C3F$_1$ mice for 28 days to PFOS (potassium salt; stated purity, > 98%) at 0, 0.166, 1.66, 3.31, 16.6, 33.1, or 166 µg/kg bw per day, given by gavage, significantly reduced the T-cell-dependent production of antibodies against SRBCs in cells isolated from the spleen, assessed using a plaque-forming cell assay. The lowest observed effect level was 0.05 mg/kg TAD over 28 days in male mice and 0.5 mg/kg TAD in female mice, corresponding to 1.66 and 3.31 µg/kg per day, respectively (Peden-Adams et al., 2008). The measured PFOS serum concentrations at these doses were 91.5 ± 22.2 ng/mL and 666 ± 108 ng/mL (mean ± SD), respectively. NK-cell activity was significantly increased in male mice at the doses of 0.5, 1, and 5 mg/kg TAD, but not in females. The serum trinitrophenyl (TNP)-specific IgM titres were also reduced by PFOS after TNP-LPS (TNP conjugated with LPS) challenge, suggesting that the humoral immune effects may be attributed to B-cells rather than T cells, because both T-dependent (SRBC) and T-independent (TI) (TNP-LPS) antigen administration resulted in suppressed IgM production (Peden-Adams et al., 2008).

Dong et al. (2009) also observed a significant reduction in anti-SRBC IgM antibodies in male C57BL/6 mice daily exposed for 60 days by gavage to PFOS (potassium salt; purity, > 98%) at 0, 8.33, 83.33, 416.67, 833.33, or 2083.33 µg/kg bw per day, with a no observed adverse effect level (NOAEL) of 8.33 µg/kg per day (0.5 mg/kg TAD) and a lowest observed adverse effect level of 83.33 µg/kg per day (5 mg/kg TAD). The measured PFOS serum concentration for 0.5 mg/kg TAD was 0.674 ± 0.166 mg/L.

In a follow-up study, Dong et al. (2011) used a similar protocol but slightly different doses. The NOAEL for a decrease in IgM was 16.7 µg/kg per day and the lowest observed adverse effect level was 83.3 µg/kg per day (TAD, 5 mg/kg per day).

Antibodies to SRBC were also found to decrease after 7 days' exposure to PFOS (potassium salt; purity, > 98%) by gavage at 5 mg/kg per

day, and the PFOS level was 110.46 ± 6.18 mg/L (Zheng et al., 2009).

Delayed-type hypersensitivity (DTH), a marker of cell-mediated immunity, in adult male C57BL/6 mice treated with PFOS orally for 60 days was not altered by doses up to 50 mg/kg, corresponding to PFOS at 833.3 µg/kg bw per day and a serum level of 51.71 ± 3.81 mg/L (Dong et al., 2011).

In a study by McDonough et al. (2020), a formulation containing several PFAS (a commercial brand of electrochemically fluorinated AFFF) was administered for 10 days via gavage to female and male C57BL/6 mice (PFOS + PFOA, at 0, 1.88, 3.75, 7.5, or 10 mg/kg bw). The results showed that antigen-specific antibody production was suppressed, on average, by 13% in male mice and by 12.4% in female mice across all the doses (McDonough et al., 2020). [The Working Group considered that these results obtained in vivo in mice might suggest that T-cell-dependent antibody production was the main target of PFOS.]

Splenic T-cell immunophenotypes (expressing CD4 and CD8 markers or not) were minimally altered in $B6C3F_1$ female mice (age 7–8 weeks), but all the T-cell subpopulations were slightly but significantly modulated in male mice by PFOS at 0.1–0.5 mg/kg TAD for 28 days by the oral route. The PFOS doses corresponded to 131 ± 15.2 ng/mL for male mice and 123 ± 18.7 ng/mL for female mice (Peden-Adams et al., 2008).

Also, the absolute numbers of splenic cells expressing CD19/CD21 or major histocompatibility complex (MHC) II markers in adult female $B6C3F_1$ mice treated orally with PFOS for 28 days were not altered at doses up to 5 mg/kg TAD (Fair et al., 2011). The splenic and thymic cellularity of adult male C57BL/6 mice was significantly reduced at oral doses of PFOS from 25 mg/kg for 60 days. At this same dose, splenic and thymic CD4+ lymphocytes were altered in adult male C57BL/6 mice. The NOAEL was 5 mg/kg TAD. Splenic CD8+ and B-cell numbers were also affected at 50 mg/kg and above (Dong et al., 2009). Splenic and thymic cellularity, including CD4+, CD8+, and B-cells, were also decreased in adult male C57BL/6 mice after oral exposure to PFOS for 7 days at doses from 20 mg/kg **(**Zheng et al., 2009). [The Working Group considered that PFOS mostly altered lymphocyte function, but also induced a decrease in the number of cells at doses of 25 mg/kg and above.]

Other immune parameters (lymphoproliferation, NK cell activity, and cytokines) have been measured ex vivo in mice using splenocytes. NK cells are an important component of the immune surveillance for cancer and provoke the lysis of cells not expressing MHC class I molecules, such as cancer and virally infected cells.

NK cell activity, measured with a chromium assay and expressed in lytic units, was increased in male $B6C3F_1$ mice at 0.5, 1, and 5 mg/kg TAD compared with controls, but was not altered in females after 28 days' exposure to PFOS by gavage (Peden-Adams et al., 2008). An augmentation of splenic NK cell activity, determined by lactate dehydrogenase release, was also observed in adult male C57BL/6 mice after oral exposure to PFOS at 5 mg/kg TAD for 60 days (Dong et al., 2009).

T-lymphocyte and B-lymphocyte proliferation, measured after polyclonal activation using mitogens (ConA or LPS), was not affected in $B6C3F_1$ mice exposed by gavage to PFOS for 28 days at doses up to 5 mg/kg TAD, and in male C57BL/6 mice after oral exposure to PFOS for 60 days at doses up to 25 mg/kg TAD (Peden-Adams et al., 2008; Dong et al., 2009).

T-cell proliferation in response to ConA stimulation in PFOA-exposed Balb/c mice was inhibited after PFOS (potassium salt; purity, > 98%) exposure, and the T-cell receptor signalling, calcium signalling, and p38 MAPK signalling pathways, as measured by microarray data analysis, and calcium ion influx were augmented in mouse splenocytes (Lv et al., 2015). However, splenic T-lymphocyte proliferation was slightly

decreased in male C57BL/6 mice after oral exposure to PFOS for 7 days at 5 mg/kg per day (Zheng et al., 2009).

Ex vivo IL-6 production by B-cells was significantly increased by in vitro stimulation with either anti-CD40 or LPS in female B6C3F$_1$ mice exposed orally for 28 days to PFOS (potassium salt; stated purity, > 98%) at 1 mg/kg TAD (Fair et al., 2011). IL-4 secretion was increased by exposure to PFOS at ≥ 5 mg/kg TAD administered to male C57BL/6 mice daily via gavage for 60 days (Dong et al., 2011) and also after oral exposure to PFOS for 7 days at 5 mg/kg per day (Zheng et al., 2011).

Pierpont et al. (2023) measured immune parameters after chronic exposure of C57BL/6 mice to PFOS (potassium salt; purity, 88.9%). Mice were exposed to PFOS at 0.15, 1.5, 15, or 50 μg/kg for 28 days, after which B cells, T cells, and granulocytes from the bone marrow, liver, spleen, lymph nodes, and thymus were assessed. No effects of PFOS on the major T- or B-cell populations, macrophages, dendritic cells, basophils, mast cells, eosinophils, neutrophils, serum antibodies, or selected serum cytokines were observed. All the experimental groups had elevated serum PFOS levels, with the highest levels obtained at 50 μg/kg per day (females, 2792.0 ± 295.3 ng/mL, and males, 2159.2 ± 164.6 ng/mL).

Interestingly, when male B6C3F$_1$ mice were exposed to a PFOS tetraethylammonium salt at a TAD of 7 mg/kg bw (equivalent to a TAD for PFOS anion of 5.55 mg/kg) in the diet for 28 days, a serum concentration of 11 μg/mL was yielded. However, no effects were found on the cellular compositions of the thymus and spleen, the number of splenic cells secreting IgM antibodies against SRBCs or TNP-LPS (Qazi et al., 2010). [The Working Group noted that only a single dose administered via the diet was used in the study.]

Lefebvre et al. (2008) also addressed the effects of PFOS on immune system function (potassium salt; stated purity, ≥ 98%) using exposure via the diet, but in Sprague-Dawley rats. The rats were exposed for 28 days to PFOS at 2–100 mg/kg diet (corresponding to approximately 0.14–7.58 mg/kg per day). The body-weight reductions induced were significant in male and female rats exposed to PFOS at 50 or 100 mg/kg diet. There were no effects on the DTH response to KLH, KLH-specific IgG in the serum, or splenic T- and B-cell proliferation in response to a mitogen ex vivo (Lefebvre et al., 2008).

Effects on the developing immune system in mice

In the B6C3F$_1$ pups of dams exposed orally to PFOS (potassium salt; purity, 91%) during GD1–GD17, NK cell function and IgM production were significantly decreased at age 8 weeks. The NOAEL was 0.1 mg/kg per day (Keil et al., 2008). C57BL/6 mouse pups were evaluated for developmental immunotoxicity after maternal oral exposure to PFOS (potassium salt; purity, > 98%) of 0.1, 1.0, or 5.0 mg/kg per day during GD1–GD17 (Zhong et al., 2016). The results showed alterations in splenic and thymic cellularity, in T- and B-cell proliferation measured ex vivo after 5 mg/kg exposure, in splenic NK-cell activity at age 8 weeks, and in the antibody response at age 4 but not 8 weeks.

Effects in models of infection in mice

Experimental models of infection are often used to address the consequences of immunosuppression on host resistance.

A 21-day exposure to PFOS (potassium salt) at 25 μg/kg per day, corresponding to PFOS at 670 ± 47 ng/mL in the serum, resulted in a significant increase in emaciation and mortality in response to influenza A virus in B6C3F$_1$ mice (Guruge et al., 2009).

In another study in C57BL/6 mice exposed to PFOS (potassium salt; purity, 88.9%) at 1.5 μg/kg per day for 4 weeks or to 3 μg/kg per day for 14 days, no effects were found on influenza virus clearance or antibody or T-cell-specific antiviral responses, indicating that for these doses

and durations, PFOS did not suppress the antigen-specific immune response. The PFOS serum levels were 99.6 ± 4.4 and 116 ng/mL ± 2.8 ng/mL for 1.5 and 3 µg/kg per day, respectively (Torres et al., 2021).

In a mouse model of *Citrobacter rodentium* infection, persistent treatment with PFOS at 2 mg/kg (potassium salt; purity, > 98%) increased the bacterial count, and this was accompanied by increases in inflammatory cytokine concentrations and dysbiosis, suggesting an alteration in intestinal immunity (Suo et al., 2017).

(c) Mechanism of action of PFOA and PFOS on the immune system

Taylor et al. (2023) confirmed in C57BL6 mice treated for 15 consecutive days the significant decrease of IgM antibodies directed against SRBCs. They also observed changes in the numbers of B-cell subsets as well as mitochondrial markers after PFOA (free acid) exposure, indicating that the B-cell developmental trajectory could be altered through effects on B-cell differentiation or proliferation, leading to suppression of the T-cell-dependent antibody response (TDAR).

PFOA and PFOS have been described to have pro-inflammatory effects, mediated through cytokine production (as also reported in Section 4.2.6), which could indirectly modulate the immune response. Son et al. (2009) found that levels of TNF-α, IL-1β, and IL-6 were augmented in the spleen in male mice exposed to PFOA (ammonium salt; purity, 98%) in drinking-water for 21 days. However, most in vitro studies conducted with human or rodent immune cells showed a decrease in pro-inflammatory cytokine production. PFOA and PFOS reduced the number of macrophages (CD11b+ cells) in the bone marrow, but not in the spleen or peritoneal cavity, in C57BL/6 mice receiving 0.02% (w/w) PFOS (tetraethylammonium salt; purity, 98%) or PFOA (free acid; purity, 96%) in the diet for 10 days (Qazi et al., 2009). This high-dose, short-term exposure also augmented the inflammatory responses to LPS. Dietary treatment of male C57BL/6 mice with 0.002% (w/w) PFOA or 0.005% (w/w) PFOS for 10 days attenuated the hepatic levels of TNF-α, IFN-γ, and IL-4 (Qazi et al., 2010).

[The Working Group noted that the mechanisms of action of PFOA and PFOS on the immune system are still a matter of debate.]

A role of PPARα in the PFOA-induced reduction in thymus weight and cellularity has been evoked in mice (Yang et al., 2000, 2001). In PPARα-null mice, reductions in spleen weight and in the number of splenocytes after PFOA treatment were absent, and the decrease in the number of thymocytes was significantly less marked (Yang et al., 2002b). The response of splenocytes isolated from the spleens of PFOA-treated PPARα-null mice to appropriate T- or B-cell activators in vitro was not altered compared with wildtype mice (Yang et al., 2002b). However, De Witt et al. (2016) found that exposure to PFOA at 30 mg/kg suppressed the TDAR in both WT and PPARα KO C57BL/6 mice, suggesting that the suppression of TDAR might be independent of PPARα involvement.

To determine whether the immunotoxicity of PFOA (free acid; purity, > 96%) is associated with lipid metabolism, male BALB/c mice were fed either a regular diet or an HFD, and exposed to PFOA at doses of 0, 5, 10, or 20 mg/kg per day for 14 days (Wang et al., 2014a). The results suggested that an excess of dietary lipids did not prevent the PFOA-induced immune suppression caused by peroxisome proliferators. Moreover, immunomodulation by PFOA was via the PPAR pathway and involved the induction of mitochondrial damage and the lymphocyte apoptosis pathway.

Ten-day treatment of male 129/Sv PPARα-null mice with different dietary doses (0.001%–1% w/w) of PFOS showed that the thymic changes were partially dependent on PPARα (Qazi et al., 2009). It has been suggested that PFOS (free acid;

purity, > 98%) may indirectly affect the immune organs by interfering with lipid metabolism, leading to co-senescence of the thymus and spleen (Wang et al., 2011).

Corsini et al. (2011) conducted in vitro experiments showing that PFOA and PFOS (free acids) suppressed LPS-induced TNF-α production in human primary cultures and THP-1 cells, whereas IL-8 was suppressed only in THP-1 cells, and IL-6 release was reduced only by PFOS. Both PFOA and PFOS reduced PHA-induced IL-4 and IL-10 release from T-cells, whereas IFN-γ release was affected only by PFOS. In all instances, PFOS was more potent than PFOA. Using siRNA, a role for PPARα in PFOA-induced immunotoxicity could be shown, whereas an inhibitory effect on LPS-induced I-κB degradation was identified that could explain the immunomodulatory effect of PFOS, suggesting different mechanisms of action (Corsini et al., 2011).

Maddalon et al. (2023b) showed that PFOS (free acid) reduced RACK-1 expression in a recent report. RACK-1 is a kinase involved in immune function and cytokine expression.

Recently, Zhang et al. (2023e) used a systemic evidence map (SEM) approach and found 1155 studies showing that PFAS were involved in either immune effects or chronic inflammation, of which 321 qualified for inclusion in their data set. The SEM showed decreased B-cell activation and altered levels of T-cell subtypes and immunoglobulins, confirming PFAS-induced immunosuppression (Zhang et al., 2023e).

Cell death is often observed after immunosuppressant treatment, explaining the observations of lymphoid organ atrophy. Adult male C57BL/6 mice treated with PFOS (potassium salt; purity, > 98%) at 0, 1, 5, or 10 mg/kg per day by gavage daily for 7 days had more apoptotic cells than in control mice. The PFOS-induced production of ROS and alteration in mitochondrial membrane potential could lead to the apoptosis of splenocytes and thymocytes (Zhang et al., 2013d).

In adult C57BL/6 mice dosed daily by oral gavage with PFOS (potassium salt; purity, > 98%) at 0, 0.0167, 0.0833, or 0.8333 mg/kg per day, yielding target PFOS TADs of 0, 1, 5, or 50 mg/kg, respectively, for 60 days, PFOS induced p53-dependent apoptosis through Bcl-xl downregulation, without changing Bcl-2 or Bax expression (Dong et al., 2012b). The release of cytochrome c and activation of caspase-3 confirmed the involvement of the mitochondria.

Synopsis

[The Working Group noted that in exposed humans, PFOA and PFOS have been found to be associated with the augmentation of LRTIs and diarrhoea and reduced vaccination efficacy in children in several environmental studies conducted in different countries. An association between PFOA and a reduced response to influenza vaccination has recently been identified in adults, and also for SARS-CoV-2 vaccination in a maternal cohort. The limitations of environmental studies are mainly associated with the use of self-reported data in questionnaires and the existence of confounding factors. Importantly, these observations were supported by evidence that PFOA and PFOS affect the response of human primary immune cells (proliferation and cytokine production). Moreover, evidence of immunosuppression after exposure to PFOA and PFOS, which mainly affected the TDAR (T-cell-dependent antibody response), has also been reported in several animal experiments.]

4.2.8 Modulates receptor-mediated effects

See Tables S4.23–S4.26 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636), and Table 4.27.

With regard to the modulation of receptor-dependent pathways in humans, most epidemiological data have come from studies on the receptor

ligands (e.g. changes in the serum concentrations of ligands that activate receptor-dependent pathways) or hormones that regulate the expression of receptor ligands (e.g. thyroid-stimulating hormone TSH and luteinizing hormone LH). The epidemiological studies considered here can be found in Table S4.23. Human in vitro studies have investigated the activation/antagonism of multiple nuclear receptors, with a focus on the activation of PPARα. However, rodent studies have made it clear that PFOA and PFOS target more than just PPARα (Rosen et al., 2010; Attema et al., 2022; Su et al., 2022a). Table S4.24 summarizes the available studies that investigated the ability of PFOA and PFOS to bind and/or activate nuclear receptors in human systems in vitro. Evidence was gathered from transcriptional and biological assays performed in human primary hepatocytes and human liver cell models, as well as binding and reporter assays.

(a) Humans

(i) Thyroid hormone pathway

Exposed humans

See Table S4.23 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Child–adult exposure

Several studies have investigated the associations of PFOA and PFOS with markers of thyroid function and disease in children and adults. [The Working Group selected the most informative studies, based on a prospective study design, a high level of exposure (including occupational and contaminated community exposure), and large study size.]

Two prospective cohort studies of female and male adults (USA) and children (Republic of Korea) have investigated the associations of serum PFOA and PFOS levels with TSH, free triiodothyronine (FT3), total triiodothyronine (TT3), free thyroxine (FT4), and/or total thyroxine (TT4) (Blake et al., 2018; Kim et al., 2020).

In the study conducted in adults, serum collected at the initial enrolment examination and at subsequent follow-up examinations during the years 1991–2008 was analysed for PFOA, PFOS, TSH, and TT4 levels. An IQR increase in serum PFOS, but not PFOA, was associated with a 9.75% (95% CI, 1.72%–18.4%) increase in TSH, but neither PFOA nor PFOS was associated with TT4 (Blake et al., 2018). [The Working Group noted, however, that due to a change in protocol, fewer repeated TT4 measurements than TSH measurements were available. This was also a highly exposed community.]

In children, TSH levels were measured at age 2, 4, and 6 years. The relation of serum PFOA and PFOS concentrations with TSH levels at the three time points was assessed by repeated-measures analysis using linear mixed models. Serum levels of FT4 and T3 were measured once (at age 6 years). PFOA was associated with a low TSH level only in male participants, and no association was detected for PFOS (Kim et al., 2020). The PFOA level at age 6 years was not associated with T3, but it was associated with high FT4 at age 6 years, primarily in male participants (Kim et al., 2020). The PFOS level at age 6 years was associated with high levels of T3 at the same age, primarily in male participants, but was not associated with FT4 (Kim et al., 2020). Age 6 years was the one time point at which a significantly higher PFOS concentration was reported in male than female participants (Kim et al., 2020).

There have been several cross-sectional studies conducted in highly exposed populations. A cross-sectional study of highly exposed adult male workers in a fluorochemical plant, conducted by the industry, showed that their serum PFOA levels were associated with a decrease in FT4 within the normal reference range, without associations with TSH, T3, or TT4 (Olsen and Zobel, 2007). A cross-sectional study

of highly exposed adult female and male workers exposed to fluorochemicals from two manufacturing facilities showed that the serum PFOS levels in men from all the locations combined were associated with an increase in T3, but no associations were seen with TSH, FT4, or TT4. No associations of the serum PFOS levels with TSH, FT3, FT4, or TT4 were found in women in another industry-conducted study (Olsen et al., 2003b). Large cross-sectional studies ($n > 10\,000$) have reported few associations of serum PFOA or PFOS levels with TSH or thyroid hormones. A cross-sectional study of males and females aged 14–39 years in the Veneto region of Italy found no associations of serum PFOA or PFOS levels with TSH (Gallo et al., 2022). [The Working Group noted that this was an analysis of a highly exposed community.]

In a large study of children and adolescents from a community contaminated by PFOA from a fluorochemical-production plant (the C8 study cohort in the Mid-Ohio Valley, USA), serum PFOS, but not PFOA, was associated with a small (1.1%) IQR increase in TT4 (Lopez-Espinosa et al., 2012). Neither the PFOA nor the PFOS serum level was associated with TSH (Lopez-Espinosa et al., 2012).

Similarly, moderately sized cross-sectional studies (n = 1000–2000) have reported associations between serum PFOA or PFOS levels and TSH and thyroid hormones. In a study of cross-sectional data from male and female adolescents, adults, and older adults in the USA (NHANES 2007–2008), serum PFOA level was associated with increased TSH and TT3 levels, but not with free iodothyronine (FT3), FT4, or TT4 (Jain, 2013). No associations of serum PFOS level with TSH, FT3, TT3, FT4, or TT4 were found (Jain, 2013). However, when low iodine and high thyroid peroxidase antibody (TPOAb) levels were taken into account in a subset of the same study sample, serum PFOA and PFOS levels were associated with increases in TSH, FT3, and TT3, and PFOS was negatively associated with FT4. The authors observed that in the T0I0 group, with normal TPOAb and iodine concentrations, serum PFOA was associated with increased FT3 levels (1.2%; 95% CI, 0.1–2.4%) (Webster et al., 2016).

A study of NHANES data from the 2007–2008 and 2009–2010 cycles stratified by age and sex reported that whereas PFOA was associated with an increase in FT3 in both sexes, it was associated with an increase in TT3 only in women (Wen et al., 2013). PFOA was not found to be associated with TSH or FT4/TT4, and the PFOS level was not found to be associated with TSH or thyroid hormones in this study (Wen et al., 2013).

A similar analysis of NHANES data from the 2011–2012 participants showed that the PFOA level was not associated with TSH, FT3, TT3, FT4, or TT4, and that the PFOS level was only associated with an increase in FT4 (van Gerwen et al., 2020). However, when analysed by sex and age, the PFOA level in the same cohort was associated with reduced TSH (in females aged 12–19 years), increased TT3 and FT3 (in women aged 60–80 years), and increased FT4 (in women aged 20–39 years). PFOA was not associated with TSH or thyroid hormones in male participants in any age group (Lewis et al., 2015). The PFOS levels in the 2011–2012 NHANES cohort were reported to be associated with increased TSH (in male participants aged 12–19 years) and increased FT4 (in women aged 20–39 years). PFOS was not associated with TT3, FT3, or TT4 in male or female participants, nor with FT4 in male participants (Lewis et al., 2015).

Lastly, in a cross-sectional study of older (aged 63.5 ± 13.6 years, mean ± SD) women and men, the PFOA serum level was associated with lower TSH concentrations and higher FT4 concentrations, but was not associated with FT3 (Li et al., 2022d). The PFOS serum level in this study was associated with lower TSH and FT3, but higher FT4 (Li et al., 2022c). In contrast, in a study incorporating cross-sectional data from adolescents, adults, and older adults, no associations of PFOA

or PFOS with TSH or FT4 levels were observed (Ji et al., 2012).

Several smaller studies (*n* = 31–633) have been conducted to investigate associations of the PFOA and PFOS serum levels with TSH and thyroid hormones in children and adults. [The Working Group considered these studies to be moderately informative, because of the smaller number of participants and/or the lower PFOA or PFOS serum levels.]

No association between the PFOA serum level and TSH was found in the majority of the small studies (Bloom et al., 2010; Ji et al., 2012; Raymer et al., 2012; Lin et al., 2013; Shrestha et al., 2015; Crawford et al., 2017; Li et al., 2017c; Kang et al., 2018; Khalil et al., 2018; Caron-Beaudoin et al., 2019; Li et al., 2021b, women only).

Two studies reported that the PFOA level in serum was associated with increased TSH level (Byrne et al., 2018; Liu et al., 2022b). One study reported that the PFOA level in adolescent male participants was associated with decreased TSH level (Li et al., 2021b).

No association between the PFOA serum level and TT3 was found in the majority of the small studies (Raymer et al., 2012; Shrestha et al., 2015; Byrne et al., 2018; Liu et al., 2022b). One study reported that the PFOA serum level in adult women was associated with increased TT3 level (Crawford et al., 2017). In the four small studies of adults that examined the relation between PFOA serum level and FT3 level, no association was found (Li et al., 2017c, 2021b; Byrne et al., 2018; Liu et al., 2022b).

In the six small studies in adults that have examined the relation between the PFOA level and TT4 level, no association was found (Ji et al., 2012; Raymer et al., 2012; Shrestha et al., 2015; Crawford et al., 2017; Byrne et al., 2018; Liu et al., 2022b).

No association between the PFOA serum level and FT4 was found in the majority of the small studies (Bloom et al., 2010; Lin et al., 2013; Shrestha et al., 2015; Crawford et al., 2017; Li et al., 2017c, 2021b; Byrne et al., 2018; Kang et al., 2018; Khalil et al., 2018; Caron-Beaudoin et al., 2019; Liu et al., 2022b).

No association between the PFOS serum level and TSH was found in the majority of the small studies (Bloom et al., 2010; Ji et al., 2012; Raymer et al., 2012; Lin et al., 2013; Shrestha et al., 2015; Crawford et al., 2017; Li et al., 2021b; Byrne et al., 2018; Kang et al., 2018; Khalil et al., 2018; Caron-Beaudoin et al., 2019; Liu et al., 2022b).

One study reported that the PFOS level was associated with increased TSH level (Li et al., 2017c).

Another study reported that the PFOS levels in adult men and women were associated with reduced TSH level (Dallaire et al., 2009). No association between the PFOS serum level and TT3 was found in the majority of the small studies (Shrestha et al., 2015; Crawford et al., 2017; Byrne et al., 2018; Liu et al., 2022b). One study reported that the PFOS levels in adult men and women were associated with reduced TT3 level (Dallaire et al., 2009). One study reported that the PFOS level in adult males was associated with increased TT3 level (Raymer et al., 2012). In the majority of the small studies in adults, the PFOS serum level was not associated with the FT3 level (Byrne et al., 2018; Li et al., 2021b; Liu et al., 2022b). One study reported that the PFOS levels in adult women and men were associated with reduced FT3 (Li et al., 2017c). In the majority of small studies in adults, the PFOS serum level was not associated with the TT4 level (Ji et al., 2012; Crawford et al., 2017; Byrne et al., 2018; Liu et al., 2022b). One study reported that the PFOS level in adult men was not associated with TT4 (Raymer et al., 2012), and another reported that the PFOS levels in adult women and men were associated with increased TT4 (Shrestha et al., 2015). No association between the PFOS serum level and FT4 was found in the majority of small studies (Bloom et al., 2010; Lin et al., 2013; Crawford et al., 2017; Byrne et al., 2018; Kang et al., 2018; Khalil et al., 2018; Caron-Beaudoin et al., 2019;

Li et al., 2021b). Three studies reported that the PFOS levels in adult women and men were associated with increased FT4 (Dallaire et al., 2009; Shrestha et al., 2015; Liu et al., 2022b). One study reported that the PFOS levels in adult men and women were associated with reduced FT4 (Li et al., 2017c).

[The Working Group noted that a recent meta-analysis of 12 epidemiological studies showed a significant association between blood levels of PFOS and increased FT4, although the effect size was small (pooled z value, 0.05; 95% CI, 0.03–0.08) (Kim et al., 2018). No associations were found of PFOS with TT4, TT3, or TSH; no associations were found between PFOA and TSH or thyroid hormones (Kim et al., 2018).]

Maternal exposure/maternal hormone status

A large number of studies have been conducted on serum PFOA and PFOS concentrations during pregnancy and associations with thyroid hormone outcomes in mothers and newborns. No prospective or large (n > 10 000) cross-sectional studies have been conducted; however, moderately sized cross-sectional studies (n = 1000–2000) have been conducted. [The Working Group thus considered these to be the most informative.] A cross-sectional study in Sweden tested the association of the maternal PFOA and PFOS levels (blood collected during the first and second trimester, median, 10 weeks) with TSH, FT3, TT3, FT4, and TT4. Neither PFOA nor PFOS were associated with TSH, and PFOA was only associated with higher FT4. PFOS was associated with a lower TT3 and a non-linear (inverted U) increase in TT4 (Derakhshan et al., 2022).

Similarly, a cross-sectional study conducted in Shanghai, China, which tested the associations of the maternal PFOA and PFOS levels (blood collected during the first and early second trimesters) with TSH, FT3, and FT4, reported that PFOA was associated with an increase in FT4 (Aimuzi et al., 2020).

A cross-sectional study conducted in Odense, Denmark, which examined the associations of the maternal PFOA and PFOS (blood collected during the first and second trimesters) with TSH and FT4, found that both PFOA and PFOS were associated with an increase in FT4 (Jensen et al., 2022).

In contrast, a cross-sectional study conducted in Demark that tested the associations of the maternal PFOA and PFOS levels with TSH and FT4 during the first and second trimesters reported no associations (Inoue et al., 2019). [The Working Group noted that haemodilution occurs during the early third trimester (≥ 30 weeks of gestation) (Assali and Brinkman, 1972). Moreover, because in all these studies blood was collected no later than the late second trimester (< 27 weeks of gestation), the Working Group considered that haemodilution could not be a factor that would have had an impact on the outcomes.]

Several smaller studies (n = 152–919) have been conducted to investigate associations of the maternal PFOA and PFOS serum levels with TSH and thyroid hormones. [The Working Group considered these studies to be moderately informative, because of the smaller number of participants and/or the lower PFOA or PFOS serum levels.]

In seven of the eight small studies of mothers, no association between the PFOA serum level and TSH was found (Berg et al., 2015; Kato et al., 2016; Preston et al., 2018; Itoh et al., 2019; Reardon et al., 2019; Xiao et al., 2020; Sarzo et al., 2021), but one showed an increase in TSH in women with high TPOAb titres (Webster et al., 2014).

No association was found between maternal PFOA level and TT3 in one study (Berg et al., 2015), but in another study a decrease in TT3 was reported (Sarzo et al., 2021).

In the four small studies of mothers, no association between the PFOA serum level and FT3

was found (Berg et al., 2015; Itoh et al., 2019; Reardon et al., 2019; Xiao et al., 2020). In the four small studies of adults that examined the relation between PFOA level and TT4 level, no associations were found (Berg et al., 2015; Preston et al., 2018; Xiao et al., 2020). In the seven small studies that examined the relation between PFOA level and FT4, no associations were found (Webster et al., 2014; Berg et al., 2015; Kato et al., 2016; Itoh et al., 2019; Reardon et al., 2019; Xiao et al., 2020; Sarzo et al., 2021).

Mixed results have been reported on the association between the maternal PFOS serum level and TSH. Four studies have reported no association (Preston et al., 2018; Itoh et al., 2019; Sarzo et al., 2021; Xiao et al., 2020). Three studies have reported that the maternal PFOS level was associated with an increase in TSH (Berg et al., 2015; Reardon et al., 2019; Xiao et al., 2020), although one of these only reported this association in people with high TPOAb titres (Webster et al., 2014). One study reported that the maternal PFOS level was associated with low TSH only in TPOAb-positive participants (Preston et al., 2018). One study reported that the maternal PFOS level was associated with reduced TSH (Kato et al., 2016). The two studies that investigated the relation between the maternal PFOS serum level and TT3 reported no association (Berg et al., 2015; Sarzo et al., 2021). In the four small studies that examined the relation between the maternal PFOS serum level and FT3 level, no associations were found (Berg et al., 2015; Itoh et al., 2019; Reardon et al., 2019; Xiao et al., 2020). No association between the PFOS serum level and TT4 was found in the three studies that examined this relation (Berg et al., 2015; Preston et al., 2018; Xiao et al., 2020). No association between the PFOS serum level and FT4 was found in the seven small studies that tested this relation (Webster et al., 2014; Berg et al., 2015; Kato et al., 2016; Itoh et al., 2019; Reardon et al., 2019; Xiao et al., 2020; Sarzo et al., 2021).

Maternal exposure/neonate hormone status

Studies of the associations of prenatal PFOA or PFOS serum levels (measured in the neonatal cord serum) with neonatal TSH and thyroid hormones have also been conducted.

No prospective, large (n > 10 000) or moderately sized (n > 1000) cross-sectional studies have been conducted; however, several studies with 300–700 participants have been conducted. [The Working Group selected the largest available studies and/or those with the highest PFOA or PFOS levels as being the most informative.] Three cross-sectional studies measured both PFOA or PFOS and hormone levels in neonatal cord blood, two of which were conducted in China and one in the Republic of Korea. Guo et al. (2021c) assessed the associations of PFOA or PFOS with TSH, TT3, FT3, TT4, and FT4 in cord sample samples. [The Working Group noted that the cord blood PFOA or PFOS levels may be a more reliable measure of exposure over the full course of gestation, rather than a single maternal sample that may be affected by haemodilution.] Associations were found only with thyroxine.

The neonatal cord blood PFOA level was associated with increased TT4, and the PFOS level was associated with increased TT4 and FT4 (Guo et al., 2021c). Aimuzi et al. (2019) also assessed the associations of PFOA or PFOS with TSH, FT3, and FT4 in cord blood samples. PFOA was associated with an increase in FT4 and a decrease in FT3; however, this only occurred in male neonates (Aimuzi et al., 2019). PFOS was associated with reduced TSH and increased FT3 across all the neonates and increased FT3 in the male neonates only (Aimuzi et al., 2019). In contrast, no association was reported of the neonatal cord serum PFOA or PFOS level with TSH, TT3, or TT4 in a small study (n = 43) (Kim et al., 2011).

A cross-sectional study conducted in Japan tested the association of the maternal PFOA and PFOS levels during the first trimester of pregnancy

with neonatal cord blood thyroid hormone levels (TSH, FT3, and FT4). Only one association was found: maternal PFOS was directly associated with an increase in TSH in male neonates (Itoh et al., 2019). A second study in Japan, which tested the association of the maternal PFOA and PFOS levels during the second or third trimester with neonatal TSH and FT4 in blood collected by heel puncture, also reported that maternal PFOS was associated with an increase in the cord blood TSH concentration in all the newborns (Kato et al., 2016). A cross-sectional study conducted in China tested the relationship between the maternal PFOA or PFOS levels during the second trimester and the neonatal cord blood thyroid hormone levels (TSH, TT3, FT3, TT4, and FT4). The maternal PFOA and PFOS levels were both associated with increased neonatal cord blood TT3 and FT3, but no associations were found for TSH or thyroxine (Liang et al., 2020). A cross-sectional study conducted in the USA that evaluated the relation between maternal PFOA and PFOS levels during the first and second trimesters with T4 levels in newborn heel-puncture blood found no associations (Preston et al., 2018).

The remaining studies on the relations between maternal PFOA or PFOS levels and neonatal TSH and thyroid hormones were very small ($n < 200$), and therefore the Working Group considered them to be of only moderate importance. In the one study that analysed associations of the maternal PFOA and PFOS levels during the third trimester with neonatal cord blood hormones (TSH, TT3, FT3, TT4, and FT4), TSH was the only hormone that was associated with the maternal PFOA and PFOS levels, with both PFOA and PFOS being associated with an increase in TSH (Xiao et al., 2020). In the one study that analysed maternal PFOA and PFOS levels during the first and second trimesters and neonatal cord blood hormones (TSH, TT3, FT3, TT4, and FT4), the only association that was found was that maternal PFOA and PFOS levels were both associated with lower FT4 levels in neonates, but only when the neonates were born to mothers with high TPOAb levels (Lebeaux et al., 2020). The cord blood PFOA level, but not that of PFOS, was positively associated with newborn heel puncture TT4 in female, but not in male, neonates (de Cock et al., 2014). Lastly, the neonatal dried blood spot PFOA and PFOS concentrations were positively associated with T4 and negatively associated with TSH (Rosen Vollmar et al., 2023).

Human cell lines and reporter assays

Few studies have investigated the thyroid hormone receptor (TR) pathway in human in vitro models. Human thyroid peroxidase activity in FTC-238 human follicular carcinoma cells (48-hour exposure) was consistently inhibited by PFOS (≥ 0.1 nM), but by only a high concentration of PFOA (1 μM) (Song et al., 2012). PFOA (IC_{50} = 1.8–3 μM, in cell-free transthyretin preincubation) and PFOS (IC_{50} = 0.6 μM) displaced T4 from transthyretin (TTR) in a TTR-TRβ CALUX assay performed in U2OS human osteosarcoma cells (Behnisch et al., 2021; Sprengel et al., 2021). PFOS (≥ 0.1 μM) antagonized TRβ activation by T3 in a one-hybrid reporter assay performed in CV-1 cells (Du et al., 2013) (see Table S4.23).

Synopsis

[The Working Group noted that the evidence for associations of PFOA and PFOS with TSH or thyroid hormones in children and adults was generally weak (see summary findings for KC8 in Table 4.27). In the most informative studies (prospective study design, high level of exposure (including occupational and contaminated community exposure), or large study size), the results were mixed, with some pattern to the reported associations of PFOA or PFOS levels with the TSH or thyroid hormone levels being identified. The strongest associations were between the PFOS serum level and increased FT4; however, the collective evidence (for TSH, TT3, FT3, and TT4) did not support an association

with the PFOA or PFOS serum level. Importantly, changes in thyroid hormones were rarely accompanied by clinically relevant changes in TSH level in adults or children. Also, there was little evidence about the effects of PFOA and PFOS on the human thyroid hormone receptor (TR) pathway in human cells in vitro.]

(ii) Steroid receptors – androgen and estrogen pathways

Exposed humans

See Table S4.23 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Child–adult exposure

Several studies have investigated the associations of PFOA and PFOS with LH, testosterone, and estrogens in children and adults. LH is responsible for stimulating testosterone production in the gonads, and testosterone is converted to estrogens through the action of aromatase. Therefore, these hormones are analysed together. [The Working Group selected the most informative studies based on a prospective study design, a high level of exposure (including occupational and contaminated community exposure), and large study size.] A single prospective cohort study of pre-adolescent girls (n = 704) in the USA found no associations between serum PFOA and estradiol pre- or post-thelarche but did show negative associations with estrone and testosterone 6 months before thelarche (Pinney et al., 2023). The same study showed no associations of serum PFOS with estradiol, estrone, or testosterone at any time point pre- or post-thelarche (Pinney et al., 2023). A cross-sectional study (n = 1041) of men aged 18–21 years in Denmark found no associations of serum PFOA or PFOS with LH, testosterone, or estradiol (Petersen et al., 2022). Similarly, a cross-sectional study (n = 920) of men aged 28–25 years in Shanghai, China, found no associations of serum PFOA with LH, testosterone, or estradiol (Luo et al., 2021). PFOS was also found to not be associated with LH or testosterone, but PFOS was reported to be negatively associated with estradiol (Luo et al., 2021). Two cross-sectional studies have been conducted of the C8 cohort, composed of participants who lived in contaminated communities in the Mid-Ohio Valley, USA. In a study of children aged 6–9 years (n = 2292), serum PFOA was positively associated with lower testosterone only in male children, whereas serum PFOS was associated with lower testosterone in both girls and boys (Lopez-Espinosa et al., 2016). In the same study, PFOA was not associated with estradiol in male or female children, but PFOS was associated with lower estradiol in boys (Lopez-Espinosa et al., 2016). In a large cross-sectional analysis of adult women in the C8 cohort (n = 29 957), serum PFOA was not found to be associated with estradiol, but serum PFOS was found to be associated with lower estradiol in women aged > 42 years (Knox et al., 2011).

In a cross-sectional study of young men (age 24–26 years; n = 263) in the Faroe Islands, Denmark, the PFOA level was not found to be associated with LH, free or total testosterone, or estradiol; however, the PFOS level was reported to be associated with an increase in LH (Petersen et al., 2018).

In a cross-sectional analysis of NHANES data (USA) regarding people aged > 12 years (n = 1682) from the 2011–2012 cohort, no association was found in any group between the PFOA or PFOS level and the total serum testosterone (Lewis et al., 2015). However, in an analysis of the NHANES 2015–2016 cohort, including people aged > 12 years (n = 1886), PFOS, but not PFOA, was found to be associated with higher testosterone levels in male participants only (Xie et al., 2021). PFOA was associated with a linear decrease in estradiol only in women aged 20–49 years, whereas PFOS concentrations in the second quartile only were found to be associated

with higher estradiol in Q2 in women aged 20–49 years (Xie et al., 2021).

In another cross-sectional study of women in the USA, in which the participants aged 47–52 years had a median serum PFOS concentration of 25 ng/mL, neither the PFOA nor the PFOS serum level was found to be associated with testosterone or estradiol (Harlow et al., 2021).

In a cross-sectional study of male and female adolescents aged 13–15 years in Taiwan, China, in whom the median serum PFOS concentration was ≥ 28 ng/mL, neither PFOA nor PFOS was associated with testosterone or estradiol in the female participants (Zhou et al., 2016). In the male participants, PFOA was associated with higher estradiol, and PFOS was associated with lower testosterone (Zhou et al., 2016).

In a further analysis of these participants within a larger study, the authors reported that specifically in adolescents with asthma, both PFOA and PFOS were associated with lower testosterone and higher estradiol (Zhou et al., 2017).

Several small cross-sectional studies (n = 59–651) have also been conducted. [The Working Group considered these studies to be moderately informative, because of the smaller number of participants and/or the lower PFOA or PFOS serum levels.] The majority of studies found no association of serum PFOA or PFOS with LH (Joensen et al., 2009; Joensen et al., 2013; Tsai et al., 2015; Cui et al., 2020). One study reported that PFOA was associated with an increase in LH in young men, whereas PFOS showed no association with LH (Raymer et al., 2012). Mixed results have been reported for the relation of serum PFOA with testosterone (free or total), including no association (Joensen et al., 2009, 2013; Tsai et al., 2015), a positive association (Raymer et al., 2012; Heffernan et al., 2018; Wang et al., 2021b), and a negative association (Cui et al., 2020).

Mixed results also have been reported for the relation of serum PFOS with testosterone (free or total), including no association (Joensen et al., 2009; Raymer et al., 2012; Heffernan et al., 2018), a positive association (Wang et al., 2021b), and a negative association (Joensen et al., 2013; Tsai et al., 2015; Cui et al., 2020). None of these studies reported an association between serum PFOA or PFOS with estradiol (Joensen et al., 2009, 2013; Raymer et al., 2012; Barrett et al., 2015; Tsai et al., 2015; Heffernan et al., 2018; Cui et al., 2020; Wang et al., 2021b).

Two cross-sectional studies in Italy investigated the associations of PFOA and PFOS serum levels with the expression of androgen and estrogen receptors in leukocytes.

In a study of fertile and infertile women (age 18–40 years; n = 111), PFOA was not found to be associated with sex hormone receptor mRNA expression, whereas PFOS was reported to be associated with higher androgen receptor (AR) expression (Caserta et al., 2013). In a study of fertile and infertile men (age 27–40 years; n = 153), only PFOA was associated with lower expression of both estrogen receptors and androgen receptors (La Rocca et al., 2015).

[The Working Group noted that a recent meta-analysis of 11 studies examined the associations of PFOA and PFOS with reproductive hormones (estradiol and total testosterone) (Li et al., 2024). PFOS was associated with reduced serum testosterone in men, although the effect size was notably small, and no association was found in women (Li et al., 2024). However, earlier systematic analyses did not report significant associations between PFOS and low testosterone (Bach et al., 2016; Petersen et al., 2020). No associations of PFOA or PFOS with estradiol were reported by Li et al. (2024).]

Prenatal exposure

Several studies of prenatal exposure to PFOA and PFOS have investigated their associations with intermediates in the sex hormone synthesis

pathway, as well as with testosterone and estradiol. No prospective cohort or large (*n* > 10 000) cross-sectional studies were available to the Working Group.

In a cross-sectional study of amniotic fluid in Denmark (*n* = 645; male fetuses only), PFOS was found to be associated with increased progesterone, 17-hydroxyprogesterone, androstenedione, and testosterone (PFOA was not analysed) (Toft et al., 2016).

In a cross-sectional analysis of the association between maternal serum PFOA and PFOS concentrations during the third trimester and the male and female neonatal cord blood hormones (Shandong, China; *n* = 349), neither maternal PFOA nor PFOS were reported to be associated with neonatal testosterone (Yao et al., 2021). Maternal PFOA, but not PFOS, concentration during pregnancy was found to be associated with higher levels of neonatal estradiol (Yao et al., 2021).

A second cross-sectional analysis of the association between maternal serum PFOA and PFOS concentrations during the third trimester and male and female neonatal cord blood hormones (Sapporo, Japan; *n* = 224) similarly reported that neither maternal PFOA nor PFOS concentrations during pregnancy were associated with neonatal testosterone (Kobayashi et al., 2021). In the study by Kobayashi and colleagues, maternal concentration of PFOS, but not PFOA, during pregnancy was reported to be associated with increased neonatal levels of estradiol (Kobayashi et al., 2021).

Two cross-sectional studies in Hubei, China, investigated the associations of neonatal cord blood PFOA and PFOS with neonatal estrogens (estrone, estradiol, and estriol) in male and females. In the larger of the two studies (*n* = 942), both neonatal PFOA and PFOS were positively associated with neonatal cord blood estrone and estradiol (Liu et al., 2021). However, only PFOA was associated with higher estriol (Liu et al., 2021). In the second study (*n* = 424), neonate PFOA was positively associated with only estrone, whereas PFOS was associated with higher estrone and estriol (Wang et al., 2019).

In a cross-sectional study of neonatal cord blood PFOA and PFOS and sex hormones in Shandong Province, China (*n* = 351), PFOA was reported to be positively associated with cord blood estradiol, but not total testosterone, and PFOS was positively associated with cord blood total testosterone, but not estradiol (Yao et al., 2019).

There have been a few other moderately informative, smaller (*n* = 72–373) cross-sectional studies of associations between maternal PFOA and PFOS levels during pregnancy and steroid hormones in neonates, children, and young adults. The maternal PFOA concentration after the second trimester was negatively associated with the testosterone precursor dehydroepiandrosterone (DHEA) in neonatal cord blood, and maternal PFOS was positively associated with neonatal DHEA (Goudarzi et al., 2017b). Neither maternal PFOA nor PFOS concentrations during pregnancy were associated with neonatal androstenedione (Goudarzi et al., 2017b). Two cross-sectional studies have tested the associations of the maternal serum PFOA and PFOS concentrations during pregnancy with anatomical biomarkers of prenatal hormone exposure: the ratio of the lengths of the second and fourth digits (2D:4D; negatively associated with testosterone exposure and positively associated with estrogen exposure) and anogenital distance (positively associated with testosterone exposure). The maternal serum PFOA concentration during the second and third trimesters, but not that of PFOS, was associated with an increased mean 2D:4D digit ratio in male, but not female, neonates with different *ESR1* genotypes (Nishimura et al., 2022).

No associations between the maternal serum PFOA or PFOS concentration during pregnancy and the anogenital distance of male or female neonates were observed (Arbuckle et al., 2020). No associations of the maternal serum PFOA or

PFOS concentration during the first and second trimesters with the infant (age 4 months) serum LH, DHEA, androstenedione, 17-hydroxyprogesterone, or testosterone were found (Jensen et al., 2020). The maternal PFOA and PFOS concentrations during the first and second trimesters were positively associated with testosterone in the adolescent daughters (Maisonet et al., 2015). However, the maternal PFOA and PFOS concentrations during the third trimester were not found to be associated with LH, total testosterone, DHEAS, or estradiol in the young adult daughters (Kristensen et al., 2013). The maternal PFOA and PFOS concentrations during the third trimester were not associated with testosterone or estradiol in the young adult sons, although PFOA was associated with higher LH (Vested et al., 2013).

In vitro effects on estrogen receptors α and β

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636)

Human primary cells

Only three studies have investigated the effects of PFOA on the estrogen receptor (ER) (ERα and ERβ) pathway in human primary cells. In human primary hepatocytes exposed for 24 hours to PFOA (≥ 25 µM), the expression of the ERα gene (*ESR1*) was significantly downregulated (Buhrke et al., 2015).

In HUVECs, PFOS (100 µM, 48 hours) significantly increased the expression of the ERα gene (*ESR1*) (Liao et al., 2012). In human primary placental cytotrophoblasts, PFOS (≥ 0.001 µM, 24 hours) reduced CYP19 protein expression and 17β-estradiol secretion (Zhang et al., 2015b).

Human cell lines

In MCF7 human breast cancer cells, PFOA (100 µM, 24–48 hours) did not affect the mRNA expression of the ER target genes *TFF1*, *EGR3*, *ESR1*, *GREB1*, or *PGR* (Behr et al., 2018; Li et al., 2020e); however, PFOA significantly suppressed the estradiol-dependent expression of *EGR3* and *TFF1* in one of these studies (Li et al., 2020e). In contrast, PFOS (50 µM, 24–48 hours) upregulated *TFF1* but not *EGR3* and suppressed the estradiol-dependent expression of these target genes (Li et al., 2020e). In the other study; however, PFOS (100 µM, 24 hours) had no effect on *TFF1*, *ESR1*, *GREB1*, *PGR*, or *CTSD* expression (Behr et al., 2018). In T47D human breast cancer cells, whereas neither PFOA (0.001 µM, 24 hours) nor PFOS (0.001 µM, 24 hours) increased the expression of known ER target genes (*PR* and *pS2*), they did increase the estradiol-dependent induction of *pS2* (Sonthithai et al., 2016). In addition, PFOS induced the expression of ERβ in HepG2 cells (Xu et al., 2017).

PFOA and PFOS have also been investigated for their ability to modulate estradiol production and secretion. In H295R human adrenocortical carcinoma cells, PFOA (48 hours) was reported to have no effect on 17β-estradiol secretion at ≤ 100 µM (Kraugerud et al., 2011; Wang et al., 2015d; Behr et al., 2018), but to reduce 17β-estradiol secretion at 1.6 µM (Rosenmai et al., 2013), and to increase its secretion at 50 µM (Rosenmai et al., 2013). In H295R cells, Kang et al. also reported increases in estradiol at 10 µM and 100 µM PFOA (Kang et al., 2016). PFOA (600 µM, 48 hours) was shown to increase aromatase activity in H295R cells (Kraugerud et al., 2011) and to reduce aromatase activity (IC_{50} = 80 µM) in JEG-3 human placental carcinoma cells (Gorrochategui et al., 2014). In H295R cells, PFOS was shown to have no effect on 17β-estradiol secretion at ≤ 100 µM (Behr et al., 2018), but to increase this at 0.03, 200, or 600 µM (Kraugerud et al., 2011; Du et al., 2013; van den Dungen et al., 2015; Kang et al., 2016).

Human binding and reporter assays

PFOA and PFOS have been shown to bind human ERα at high concentrations in competitive binding assays using the human ERα ligand-binding domain with similar binding affinities (Qiu et al., 2020). The majority of ERα reporter studies employing endogenous human ERα and estrogen response element-driven reporters performed in breast and ovarian carcinoma cell lines (BG1, ovarian; MCF7 and T47D, breast) showed that PFOA and/or PFOS did not transactivate ERα (Yao et al., 2014; Kang et al., 2016; Sonthithai et al., 2016; Evans et al., 2022). However, other studies using this approach showed that PFOA (EC_{50} = 65 µM) and PFOS (EC_{50} = 29 µM) stimulated ERα transactivation (Kjeldsen and Bonefeld-Jørgensen, 2013) or that PFOS (EC_{20} = 12 µM), but not PFOA, stimulated ERα transactivation (Li et al., 2020e), but with significantly lower efficacy than did estradiol. In addition, PFOA (10 nM to 1 µM) and PFOS (1 nM to 1 µM) increased estradiol-stimulated ERα transactivation in an endogenous ERα-driven reporter assay performed in T47D cells (Sonthithai et al., 2016).

In ER reporter studies employing the forced expression of full-length human ERα in HEK293T human kidney cells, PFOA (≥ 0.1 µM) and PFOS (≥ 0.001 µM) stimulated the transactivation of ERα (Benninghoff et al., 2011; Houck et al., 2021). ERα reporter studies using a one-hybrid approach have also been conducted. In one study of human HepG2 cells, PFOA (half-maximal activity concentration (AC_{50} = 8 µM) and PFOS (AC_{50} = 4.4 µM) stimulated ERα-mediated transactivation (Houck et al., 2021). In one study of human kidney HEK293T cells, neither PFOA (≤ 100 µM) nor PFOS (≤ 100 µM) transactivated ERα or ERβ (Behr et al., 2018). However, in experimental designs in which PFOA and PFOS were applied together with estradiol, PFOA (100 µM) was shown to enhance estradiol-driven ERβ activation, and PFOS (100 µM) was shown to enhance estradiol-driven ERα and ERβ activation in a one-hybrid assay in HEK293T cells (Behr et al., 2018).

In vitro effects on the androgen receptor

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Human primary cells

No studies available to the Working Group investigated the effects of PFOA or PFOS on androgen receptor (AR) pathways in human primary cells.

Human cell lines

In LNCaP human prostate adenocarcinoma cells (24-hour exposure), neither PFOA (≤ 100 µM) nor PFOS (≤ 100 µM) induced the mRNA expression of AR target genes (e.g. *AR* and *PSA*) (Behr et al., 2018). In H295R human adrenocortical carcinoma cells, PFOA has been reported not to have an effect on testosterone secretion (PFOA ≤ 100 µM, 48 hours) (Rosenmai et al., 2013; Wang et al., 2015d; Behr et al., 2018) or to increase testosterone secretion (PFOA ≥ 0.6 µM) (Kraugerud et al., 2011). The effect of PFOS has also been tested using the H295R steroidogenesis assay. PFOS was reported to have no significant effect on testosterone secretion in H295R cells (PFOS ≤ 200 µM) (Behr et al., 2018), to reduce testosterone production (PFOS ≥ 1 µM) (Du et al., 2013), and to increase testosterone secretion (PFOS, 0.6–600 µM) (Kraugerud et al., 2011; van den Dungen et al., 2015).

Human binding and reporter assays

PFOA (≤ 300 µM) and PFOS (≤ 300 µM) did not transactivate endogenous human AR in reporter assays performed in breast (MDA-kB2) or prostate (22Rv1/MMTV) cancer cells (Kang et al., 2016; Behr et al., 2018) or HepG2 hepatoma cells (Houck et al., 2021). PFOS (≤ 3 µM) did not

transactivate endogenous human AR in reporter assays performed in breast (MDA-kB2) cancer cells (Du et al., 2013). PFOA (≤ 100 μM) did not transactivate full-length overexpressed human AR in a reporter assay performed in CHO cells (Kjeldsen and Bonefeld-Jørgensen, 2013; Rosenmai et al., 2013). PFOS (≤ 100 μM) also did not transactivate full-length overexpressed human AR in a reporter assay performed in CHO cells (Kjeldsen and Bonefeld-Jørgensen, 2013). PFOS (> 50 μM), but not PFOA, enhanced testosterone-induced AR reporter transactivation in MDA-kB2 cells (Behr et al., 2018), but PFOA (IC_{50} = 11 μM) and PFOS (IC_{50} = 5 μM) inhibited AR reporter transactivation in CHO cells (Kjeldsen and Bonefeld-Jørgensen, 2013). No studies that used binding assays were identified.

Synopsis

[Overall, the Working Group noted that in the studies in exposed humans reviewed above there was no evidence that PFOA or PFOS were associated with LH in children and adults, and there was little evidence that PFOA was associated with testosterone. Whereas the results of a recent meta-analysis (Li et al., 2024) showed that a small but significant decrease in serum total testosterone was associated with PFOS in men, the most informative and moderately informative studies reviewed here reported no association between PFOS and testosterone.

There was also little evidence provided by either the most informative or the moderately informative studies that PFOA is associated with estradiol level (either higher or lower). There were several studies that supported an association of PFOS with lower estradiol level; however, this association was not recapitulated in the moderately informative studies. There does appear to be the potential for sex-specific differences, with men being more likely to show associations of PFOA and PFOS with testosterone and estradiol than women.

Human studies of in utero exposure to PFOA and PFOS did not show associations with sex hormones or sex hormone-dependent end-points in neonates or later in life. More studies reported no association than reported significant associations. However, most of the associations reported were between PFOA or PFOS and higher concentrations or effects of sex hormones.

In addition, PFOA and PFOS seemed not to modulate testosterone production or act as AR ligands, as measured with AR reporter assays.

Overall, inconsistent findings were reported with regard to PFOA and PFOS modulating the ER pathway-mediated effects in human primary cells, human cell lines, and human reporter assays. In systems in which ERs are expressed endogenously, high (i.e. non-human-relevant) PFOA and PFOS concentrations were required to modulate ER-dependent gene expression and transactivation, as well as estradiol production (see summary findings for KC8 in Table 4.27).]

(iii) Steroid receptors – progesterone pathway

See Table S4.23 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Exposed humans

One cross-sectional study conducted in Norway (*n* = 178) assessed the association between serum PFOA and PFOS and salivary progesterone in women and found that in nulliparous, but not parous, adult women, PFOS, but not PFOA, was negatively associated with salivary progesterone (Barrett et al., 2015).

One cross-sectional study conducted in Denmark (*n* = 545) assessed the association between PFOS and progesterone in amniotic fluid during the second trimester and reported that PFOS was associated with higher progesterone (Toft et al., 2016).

Two cross-sectional studies ($n \leq 224$ and $n = 189$, respectively) have assessed the associations of prenatal exposure to PFOA and PFOS with neonatal progesterone levels. Maternal concentration of PFOS, but not PFOA, during the third trimester was negatively associated with cord blood progesterone in the first of these (Kobayashi et al., 2021). In the second study, the maternal pregnancy (from the first to third trimester) PFOA level was negatively associated with the cord blood progesterone in male and female neonates, whereas the maternal PFOS level was not associated with progesterone in neonates of either sex (Itoh et al., 2016).

A single cross-sectional study conducted in China ($n = 374$, women and men) investigated the relations between PFOA or PFOS and progesterone in neonatal cord blood and reported that neonatal levels of PFOA and PFOS were not associated with neonatal levels of progesterone or 17-hydroxyprogesterone (Liu et al., 2020b).

A single cross-sectional study conducted in Belgium ($n = 170$, women and men) investigated the associations between PFOA or PFOS in neonatal cord blood and the expression of progesterone-receptor target genes in leukocytes and found that the blood levels of PFOA and PFOS were associated with higher levels of progesterone receptor-mediated gene expression (Remy et al., 2016).

Synopsis

[The Working Group noted that, in the few studies described above, little evidence was available on the potential association of in utero exposure to PFOA and PFOS with the progesterone pathway. PFOS appeared to be more strongly associated with progesterone and progesterone-induced pathways than PFOA, but both positive and negative associations were reported (see summary findings for KC8 in Table 4.27).]

(iv) Steroid receptors – glucocorticoid pathway

See Table S4.23 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Exposed humans

A single cross-sectional study conducted in Sweden ($n = 1048$) assessed the association between maternal PFOA or PFOS exposure during the first trimester and the urinary cortisol and cortisone concentrations during the third trimester (Dreyer et al., 2020). The maternal serum PFOA was found not to be associated with urinary cortisol or cortisone. The maternal serum PFOS was associated with lower urinary cortisone but not cortisol (Dreyer et al., 2020). One cross-sectional study conducted in Denmark ($n = 545$) assessed the association between PFOS and cortisol in amniotic fluid and reported that PFOS was associated with higher cortisol (Toft et al., 2016). Two cross-sectional studies ($n < 400$) that assessed the associations of prenatal exposure to PFOA and PFOS with neonatal corticosteroid levels have been conducted. Maternal concentration of PFOS, but not PFOA, after the second trimester was negatively associated with cord blood cortisol, cortisone, and the cortisol/cortisone ratio (Goudarzi et al., 2017b). Neonatal cord blood PFOA and PFOS were found to be positively associated with 11-deoxycortisol, but not cortisol or cortisone (Liu et al., 2020b).

In vitro – human primary cells, cell lines, and reporter assays

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

A single study has investigated the effect of PFOA or PFOS on the glucocorticoid receptor (GR) pathway in human primary cells. PFOS (0.001–1 μM, 24 hours) reduced the upregulation

of 11β-hydroxysteroid dehydrogenase (HSD)1, the enzyme responsible for the generation of cortisol, an endogenous GR ligand, in human primary decidual stromal cells (Yang et al., 2016).

Using microsomes from human and rat kidneys, PFOA and PFOS were demonstrated to inhibit the catalytic activity of 11β-HSD2 (the enzyme that deactivates cortisol), with PFOS having a lower IC_{50} than PFOA (0.05 versus 24.4 µM, respectively) (Zhao et al., 2011b, 2023). In the same studies, rat enzyme activities were measured, and the PFOS IC_{50} was 0.29 µM, whereas that of PFOA was 3.8 µM.

In H295R cells, PFOA (≤ 600 µM, 48 hours) had no effect on cortisol secretion (Kraugerud et al., 2011; Rosenmai et al., 2013; Wang et al., 2015d). In H295R cells, PFOS was shown to have no effect on cortisol secretion (PFOS ≤ 600 µM) (Kraugerud et al., 2011), nor to increase cortisol secretion (200 µM) (van den Dungen et al., 2015). In a full-length GR reporter assay performed in T47D cells, PFOS (~30–60 µM, 24 hours) enhanced cortisol-driven GR reporter activation to a modest extent (Wilson et al., 2016).

Synopsis

[The Working Group noted that there was a small number of available studies in which alteration of glucocorticoids and exposure to PFOA or PFOS during pregnancy were investigated, and few of these studies provided evidence that was supportive of a positive association. PFOS appeared to be more strongly associated than PFOA with corticosteroid levels, and the associations were generally negative (see summary findings for KC8 in Table 4.27).

A modest set of data suggested that PFOS suppressed cortisol production and deactivation but could enhance GR activation by cortisol, whereas PFOA had little effect on the GR pathway.]

(v) Other nuclear receptors and types of receptors

See Table S4.23 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Exposed humans

Several studies have investigated the association of PFOA and PFOS with vitamin D receptor-related biomarkers.

Two cross-sectional studies conducted in the USA, one of which was in adults (n = 7040) and one in children (n = 78), investigated the associations of serum PFOA and PFOS levels with total serum 25-hydroxyvitamin D (Khalil et al., 2018; Etzel et al., 2019). In adults, the PFOS level, but not that of PFOA, was associated with a lower serum vitamin D level (Etzel et al., 2019). In children, neither PFOA nor PFOS was associated with the serum vitamin D level (Khalil et al., 2018). A cross-sectional study of pregnant women in the USA (n = 442) investigated the associations of serum PFOA and PFOS levels during the first trimester with free and total serum 25-hydroxyvitamin D levels during the first and second trimesters and reported that serum PFOA level was not associated with the vitamin D level (Chang et al., 2021). In the same study, the serum PFOS level was associated with higher total serum 25-hydroxyvitamin D during both the first and second trimesters. The serum PFOS level was only associated with higher free serum 25-hydroxyvitamin D during the second trimester, and only in pregnancies with male fetuses (Chang et al., 2021).

In a cross-sectional study in China (n = 992) that investigated the associations of neonatal cord blood PFOA and PFOS levels with the total serum 25-hydroxyvitamin D, it was reported that the serum PFOS level, but not that of PFOA, was associated with higher neonatal levels of vitamin D (Liu et al., 2023c).

[The Working Group noted that PFOS appeared to be more associated with serum vitamin D level than PFOA; however, the direction of the association was negative in one study and positive in two studies.]

Three studies have investigated the associations of serum PFOA and PFOS in adult women and men with the expression of nuclear receptor and *AHR* mRNA expression in leukocytes.

In a cross-sectional study of adult women conducted in Italy (n = 154), serum PFOA was negatively associated with *PXR* and *AHR* expression in peripheral blood cells but was not associated with *PPARG* expression (Caserta et al., 2013). In the same study, the serum PFOS concentration positively correlated with *PXR* expression in peripheral blood cells (Caserta et al., 2013).

In a cross-sectional study of adult men conducted in Italy (n = 153), serum level of PFOA was negatively associated with the expression of *PXR* and *AHR* in peripheral blood cells but not with the expression of *PPARG* (La Rocca et al., 2015). In the same study, PFOS was not associated with the expression of *PXR*, *AHR*, or *PPARG* (La Rocca et al., 2015).

In a cross-sectional study of adult women and men conducted in the USA (n = 290), serum PFOA level was associated with lower expression of *LXRB*, but not *LXRA*, in peripheral blood cells, and the serum PFOS level was associated with lower expression of *LXRA*, but not *LXRB* (Fletcher et al., 2013). In the same study, no associations of PFOA or PFOS with *PPARA*, *PPARD*, or *PPARG* were reported (Fletcher et al., 2013).

[The Working Group noted that it is difficult to interpret the significance of associations between serum PFOA and PFOS levels and the expression of nuclear receptors in leukocytes, because of the small number of studies and disparate results.]

PPARα – in vitro

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

The majority of human in vitro studies have focused on the activation of PPARα. However, rodent studies have made it clear that PFOA and PFOS target more than just PPARα (Rosen et al., 2010; Attema et al., 2022; Su et al., 2022a). Table S4.24 summarizes the available studies that investigated the ability of PFOA and PFOS to bind and/or activate nuclear receptors in human systems in vitro. Evidence was gathered from transcriptional and biological assays performed in human primary hepatocytes and human liver cell models, as well as binding assays and reporter assays.

Human primary cells

Multiple studies in human primary liver cells have tested whether PFOA or PFOS activate human PPARα. In human primary hepatocytes treated with PFOA or PFOS for 24–28 hours, human *PPARA* mRNA expression was induced by PFOA (≥ 5 μM) and PFOS (≥ 25 μM) (Bjork and Wallace, 2009; Bjork et al., 2011; Rosen et al., 2013; Buhrke et al., 2015; Marques et al., 2022). Ingenuity Pathway Analysis of microarray mRNA expression data induced by PFOA identified PPARα as a predicted upstream regulator of effects on the cell cycle (Buhrke et al., 2015).

Similarly, in human primary liver spheroids composed of hepatocytes and Kupffer cells that were treated with PFOA or PFOS for 1–14 days and analysed by targeted RNA sequencing (RNASeq), the fatty acid β-oxidation pathway was significantly upregulated by PFOA (20 μM) at all time points and by PFOS (20 μM) after 1 and 4 days (Rowan-Carroll et al., 2021). PPARα was predicted to be a strongly activated upstream regulator of the gene expression changes induced by PFOA and PFOS (Rowan-Carroll et al., 2021).

When comparing full transcriptomes, the benchmark concentrations for PFOA and PFOS were similar after 14 days of exposure (5–10 µM); however, PFOS was found to regulate the transcription of more genes, in general, than PFOA (Rowan-Carroll et al., 2021).

In studies that tested the effects of PFOA or PFOS in both rodent and human primary hepatocytes, PPARα was less potently activated in human versus mouse primary hepatocytes: 5.1 times less potently for PFOA and 1.9 times less potently for PFOS (Rosen et al., 2013). Differences in efficacy have also been noted, with PFOA and PFOS more efficaciously activating PPARα in rat than human primary hepatocytes (although the authors noted that there were differences in the baseline expression of genes in rat and human hepatocytes that affected the apparent induction of expression) (Bjork et al., 2011).

Human cell lines

Similarly, studies of human liver cell lines have shown that PFOA and PFOS increase the mRNA expression of human PPARα target genes. In HepaRG human liver cells, 24-hour exposure to PFOA (100 µM) or PFOS (100 µM) upregulated the mRNA expression of known targets of PPARα, including those associated with fatty acid β-oxidation (Louisse et al., 2020, 2023; Murase et al., 2023). In addition, PFOS, but not PFOA, except at the highest concentration of 200 µM, was shown to increase lipid accumulation in HepRG cells (Louisse et al., 2020). In HepG2 human liver cancer cells, PFOA (≥ 25 µM) had similar effects on the mRNA expression of PPARα target genes to the PPARα agonists WY14 643 and GW7647, although with lower potency (Behr et al., 2020b). In contrast, in HepG2/C3a human hepatoma cells, PFOA (≤ 200 µM) and PFOS (25 µM) did not induce the mRNA expression of the PPARα target genes *ACOX1*, *ACOT*, and *CYP4A1* (Bjork and Wallace, 2009).

Human binding and reporter assays

The results obtained from human primary hepatocytes and other human cell models have been corroborated by ample evidence that PFOA and PFOS activate human PPARα derived from binding and reporter assays. In competitive binding assays performed using the human PPARα ligand-binding domain, PFOA and PFOS were shown to displace well-known PPARα ligands (Li et al., 2018b; Ishibashi et al., 2019). PFOA has been shown to activate PPARα-mediated transcription in reporter assays using full-length human PPARα (Maloney and Waxman, 1999; Nielsen et al., 2022; Sakai et al., 2022) or chimaeras of the human PPARα ligand-binding domain with the GAL4 DNA-binding domain (Vanden Heuvel et al., 2006; Takacs and Abbott, 2007; Wolf et al., 2008a, 2012; Corsini et al., 2012; Buhrke et al., 2013; Rosenmai et al., 2016, 2018; Behr et al., 2020b; Houck et al., 2021; Evans et al., 2022). PFOS also has been shown to activate PPARα reporter assays (full-length: Shipley et al., 2004; Nielsen et al., 2022; Gal-4: Wolf et al., 2008a; Behr et al., 2020b; Houck et al., 2021; Evans et al., 2022). However, some studies also showed that PFOS failed to activate PPARα reporter assays (Gal-4: Takacs and Abbott, 2007; Corsini et al., 2012; Rosenmai et al., 2018). In the reporter assays using full-length hPPARα, Cos1, Cos7, or MDA-MB-231 cells were transfected with an expression vector for the full-length hPPARα protein and a reporter construct in which reporter gene expression was driven by PPARα binding to PPAR response elements and treated with multiple concentrations of PFOA or PFOS, with reporter activity being assessed after 24 hours of exposure. In the reporter assays that used a one-hybrid approach, 3T3-L1, COS-1, THP1, HEK293, HEK293T, or HepG2 cells were transfected with an expression vector for a chimaera of the human PPARα ligand-binding domain and the GAL4 DNA-binding domain and a reporter construct in which reporter gene

expression was driven by GAL4 binding to upstream activating sequences (also known as a one-hybrid approach), and treated with multiple concentrations of PFOA or PFOS, with reporter activity being assessed after 6–24 hours of exposure. It is important to note that whereas PFOA is a full agonist of human PPARα, PFOS is a partial agonist in human PPARα reporter assays (Vanden Heuvel et al., 2006; Rosenmai et al., 2016; Behr et al., 2020b; Nielsen et al., 2022). Human PPARα is also less activated than mouse PPARα in reporter assays, with effective concentrations being 1–2.7 times higher for PFOA to activate human than mouse PPARα and 2.1–2.8 times higher for PFOS to activate human than mouse PPARα (Maloney and Waxman, 1999; Shipley et al., 2004; Vanden Heuvel et al., 2006; Takacs and Abbott, 2007; Wolf et al., 2008a).

Synopsis

[The Working Group noted that, on the basis of the studies reviewed above that were conducted in human primary cells, and various human cell lines, using binding and reporter assays, there was evidence that PFOA and PFOS transcriptionally activated PPARα. However, human PPARα was less effectively activated by PFOA or PFOS than was rodent PPARα. Also, PFOA activated human PPARα more effectively than did PFOS (see summary findings for KC8 in Table 4.27).]

CAR/PXR – in vitro

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Human primary cells

Several studies in human primary hepatocytes have tested whether PFOA or PFOS activate human constitutive androstane receptor (CAR)- and pregnane X receptor (PXR)-mediated gene transcription. These receptors are discussed together because they regulate overlapping transcriptional programmes, which can make it challenging to definitively identify receptor-specific target genes (Tojima et al., 2012). Here, we have deferred to the study authors with respect to the identification of receptor–target gene pairs.

In human primary hepatocytes exposed for 24–48 hours, PFOS (25 μM) induced the expression of the CAR target genes *CYP2B6* and *CYP2C19*, but PFOA (25 μM) only induced CAR target gene expression after 48 hours (Bjork et al., 2011; Marques et al., 2022).

In transcriptomic analyses of human primary hepatocytes, PFOA (25 μM) was shown to partially activate the PXR-dependent pathway, but not the CAR pathway (Buhrke et al., 2015). The PXR target gene *CYP3A4* has also been shown to be upregulated by PFOA and PFOS (100 μM, 48 hours) in human primary hepatocytes (Rosen et al., 2013); however, when tested at 25 μM for 24 hours, only PFOS significantly increased *CYP3A4* mRNA expression (Bjork et al., 2011).

Human cell lines

In human liver cell models, PFOA activated CAR-dependent gene expression (*CYP2B6*) in HepaRG human liver cells (30 μM, 48 hours; Abe et al., 2017) and in HepG2 cells (250 μM, 24 hours; Behr et al., 2020b). In contrast, very low-level exposure to PFOA (0.001 μM) or PFOS (0.001 μM) for 24–48 hours was shown to reduce CAR-mediated gene expression (*CYP2C19*) (Franco et al., 2020). In HepaRG human liver cells exposed for 48 hours, PFOA (≥ 50 μM) and PFOS (≥ 1 μM) induced mRNA expression of the PXR target gene *CYP3A4* (Behr et al., 2020a). Very low-level exposure to PFOA (0.001 μM) or PFOS (0.001 μM) for 24–48 hours also reduced PXR-mediated gene expression (*CYP3A4*) (Franco et al., 2020).

Table 4.27 Modulation of receptor-mediated effects: pathway characterization for PFOA and PFOS

Hormone pathway	Consistent and coherent evidence		Suggestive evidence		Paucity of data	
	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS
Exposed humans						
Thyroid pathway			X	X		
Estrogen pathway				X	X	
Androgen pathway		X	X			
Progesterone pathway			X	X		
Glucocorticoid pathway				X	X	
AHR pathway					X	X
Primary human cells						
Thyroid pathway					X	X
Estrogen pathway			X			X
Androgen pathway					X	X
Progesterone pathway					X	X
Glucocorticoid pathway					X	X
AHR					X	X
PPARα	X	X				
CAR/PXR	X	X				
PPARγ			X	X		
HNF4α					X	X
Human cell lines, binding assays and reporter assays						
Thyroid pathway					X	X
Estrogen pathway			X	X		
Androgen pathway					X	X
Progesterone pathway					X	X
Glucocorticoid pathway					X	X
AHR					X	X
PPARα	X	X				
CAR/PXR	X	X				
PPARγ			X	X		
HNF4α					X	X
Experimental systems (rodents)						
Thyroid pathway		X			X	
Estrogen pathway		X	X			
Androgen pathway	X	X				
Progesterone pathway					X	X
Glucocorticoid pathway						X
AHR					X	X
PPARα	X	X				
CAR/PXR	X	X				
PPARγ	X	X				
HNF4α					X	X

AHR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; HNF4α, hepatocyte nuclear factor 4 alpha, PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor.

Human binding and reporter assays

Reporter assays also have been employed to investigate the abilities of PFOA and PFOS to stimulate the transcriptional activities of CAR and PXR.

It has been shown that PFOS activates CAR indirectly, by modulating its phosphorylation, rather than binding to the ligand-binding domain (Abe et al., 2017); therefore, traditional reporter assays have not detected PFOA- or PFOS-induced transactivation of CAR (e.g. Behr et al., 2020b; Houck et al., 2021; Murase et al., 2023).

In reporter studies employing full-length human PXR, PFOA (0.1–300 μM, 24 hours) was shown to stimulate PXR transactivation when the host cell was a human hepatocyte model (Zhang et al., 2017b; Houck et al., 2021). In reporter assays using a one-hybrid approach, PFOA (0.1–300 μM, 24 hours) stimulated PXR-mediated transcription in only one of the two available studies (Behr et al., 2020a; Houck et al., 2021). In reporter studies employing full-length human PXR, PFOS (0.14–300 μM, 24 hours) was shown to stimulate PXR transactivation when the host cell was a human hepatocyte model (Zhang et al., 2017b; Houck et al., 2021). In reporter assays using a one-hybrid approach, PFOS (0.14–300 μM, 24 hours) stimulated PXR-mediated transcription in only one of the two available studies (Behr et al., 2020a; Houck et al., 2021).

Synopsis

[Overall, the Working Group noted that there was evidence in human primary cells and other human cell in vitro models that PFOA and PFOS stimulated CAR and PXR transcriptional activity. Reporter assays were not able to detect effects of PFOA or PFOS on CAR activity, because activation probably occurred through the modulation of phosphorylation, rather than through ligand binding. The results of PXR reporter assays suggested the transactivation of PXR by PFOA and PFOS, but the effects of PFOA and PFOS appeared to be dependent upon the type of reporter assay and host cell type (see summary findings for KC8 in Table 4.27).]

PPARγ – in vitro

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Human primary cells

Only four studies in human primary cells have examined whether PFOA and PFOS activate human PPARγ-related pathways. In HUVECs, PFOS (100 μM) exposure for either 24 or 48 hours increased the mRNA expression of *PPARG* (Liao et al., 2012). In human primary hepatocytes exposed for 48 hours, PFOA (25 μM) and PFOS (25 μM) induced the mRNA expression of *CD36* and *PPARG*, PFOA alone induced the expression of *SCD*, and neither induced the expression of *FASN* or *GPAM* (Marques et al., 2022). In transcriptomic analyses of human primary hepatocytes, PFOA (≥ 1 μM) strongly upregulated the PPARγ pathway (Buhrke et al., 2015). In primary hMSCs stimulated to undergo adipogenic differentiation (7–14 days), PFOA (≥ 0.1 μM) and PFOS (≥ 0.1 μM) stimulated the expression of *FABP4* and *PPARG* and lipid accumulation (an indicator of adipocyte differentiation, which requires PPARγ; Qin et al., 2022c). The authors reported similar findings in mammalian cells (Qin et al., 2022b). In another similar study (7–21 days of differentiation), PFOS (≥ 0.1 μM) induced *FABP4* and *PPARG* expression Gao et al. (2020). In human primary subcutaneous preadipocytes stimulated to undergo differentiation (10 days), PFOA (≥ 6 μM) induced the expression of *FABP4*, *PLIN1*, and *PPARG* and increased lipid accumulation (Li et al., 2019b). In human primary bone marrow mesenchymal stromal cells differentiating for 21 days, PFOA (≥ 0.1 μM) and PFOS (1 μM) increased lipid accumulation (Bérubé

et al., 2023). Similarly, in human primary visceral preadipocytes differentiated for 11 days, PFOS (≥ 5 µM) increased lipid accumulation (Xu et al., 2016). [The Working Group noted that none of the differentiation studies tested the ability of PFOA or PFOS to stimulate adipocyte differentiation in the absence of a hormone cocktail, which would be a stronger indicator of PPARγ agonism.]

Human cell lines

In the human liver cell HepaRG model, exposure to PFOA (100 µM) for 24 hours induced the expression of the classic PPARγ target gene *FABP4* (Attema et al., 2022).

Human binding and reporter assays

PFOA and PFOS have been shown to bind to human PPARγ at high concentrations in competitive binding assays using the human PPARγ ligand-binding domain, with PFOS having higher binding affinity than PFOA (Zhang et al., 2014b; Li et al., 2018b, 2019b). In a human PPARγ reporter assay in which full-length human PPARγ was expressed in HepG2 cells, PFOA and PFOS had similar potencies (~10 µM) for the stimulation of PPARγ transactivation (Zhang et al., 2014b). Less consistent results have been obtained using human PPARγ one-hybrid reporter assays. PFOA (at concentrations of ≥ 25 µM) and PFOS have been reported not to activate human PPARγ hosted in human kidney HEK293T cells (Behr et al., 2020b); or to activate human PPARγ in HEK293 cells with low efficacy (Li et al., 2019b); or to activate human PPARγ in INDIGO cells with very low potency (Evans et al., 2022); or to activate human PPARγ in HEK293 cells (Buhrke et al., 2013), human epithelial HeLa cells (Garoche et al., 2021), and HepG2 cells (Houck et al., 2021).

Synopsis

[The Working Group noted that there was evidence in human primary cells and other human cell in vitro models that PFOA and PFOS stimulated PPARγ transcriptional activity. Caveats remain, however, in that it is unclear if increases in PPARγ-mediated gene expression result from the direct activation of PPARγ or from PFOA and PFOS increasing the expression of *PPARG*. Additionally, the results of reporter assay studies were inconsistent with regard to the stimulation of PPARγ-transactivation by PFOA and PFOS, even when the host cell line was the same (see summary findings for KC8 in Table 4.27).]

HNF4α – in vitro

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Human primary cells, cell lines, and reporter assays

Hepatocyte nuclear factor (HNF)4α is a critical receptor for liver development and function (Hayhurst et al., 2001) and is an orphan nuclear receptor (Bogan et al., 2000). [Fatty acids are thought to become stably associated with the ligand-binding domain (Wisely et al., 2002); therefore, PFOA and PFOS are unlikely to interact with the HNF4α ligand-binding domain and are more likely to have indirect effects on HNF4α activity.] Importantly, HNF4α is essential for the activation of CAR and PXR, by acting as a coactivator (Tirona et al., 2003).

PFOA (≥ 25 µM, 24 hours) has been shown to downregulate HNF4α, as evidenced by a large decrease in *HNF1A* mRNA expression in human primary hepatocytes (Buhrke et al., 2015). Similarly, in HepG2 cells, PFOA (25 µM, 48 hours) reduced the protein expression of HNF4α and a transcriptional target (HNF1α)

(Scharmach et al., 2012). Lastly, PFOA (≥ 1 µM, 24 hours) reduced endogenous HNF4α transcriptional activity in HepG2 cells and the activity of overexpressed full-length HNF4α in HEK293 cells in reporter assays (Scharmach et al., 2012).

[The Working Group noted that PFOA was shown to suppress the activity of a critical liver transcription factor, HNF4α. However, all the evidence came from a single research group. The possibility that PFOS may interfere with the HNF4α pathway has not been investigated.]

Synopsis

[The Working Group noted that, overall, in exposed humans, the data showed some association of PFOA with modulation of the thyroid, androgen, and progesterone pathways. However, the data did not support an association between PFOA and the estrogen pathway, because the results were largely negative. There was a paucity of data regarding an association of PFOA with the glucocorticoid pathway.

In exposed humans, there was some evidence for PFOS being associated with modulation of the androgen pathway, with PFOS potentially being associated with lower testosterone levels in men, although the effect size was small. For the thyroid hormone, estrogen, progesterone, and glucocorticoid pathways, the data were inconsistent, and there was insufficient explanation for the differences, meaning that the data were only indicative of an association with PFOS.

There was a paucity of data to permit the assessment of any association of either PFOA or PFOS with the aryl hydrocarbon receptor (AHR) pathway.

In human primary cells, there was evidence that both PFOA and PFOS modulated PPARα activity (receptor activation), which was corroborated by data from human cell lines, binding, and reporter assays. There was evidence that both PFOA and PFOS modulated CAR/PXR activity (receptor activation), which was corroborated by data from human cell lines.

There was some evidence that PFOA and PFOS modulated the estrogen pathway (estradiol secretion and estrogen-receptor activation) in human primary cells, but the results were inconsistent across studies in other human cell in vitro models, with more evidence of estrogen-receptor activation coming from reporter assays in which estrogen receptor was overexpressed. There was some evidence that PFOA and PFOS modulated the PPARγ pathway (adipocyte differentiation, lipid accumulation, and PPARγ activation) in human primary cells, but the results were inconsistent across studies in other human cell in vitro models.

There was scarce evidence of associations of PFOA or PFOS with androgen-receptor activation in human primary cells, with results in other human cells in vitro (testosterone secretion and androgen-receptor activation) having largely obtained negative results. There was a paucity of data regarding any association of PFOA or PFOS with the thyroid pathway (thyroid peroxidase activity, TTR binding/T4 displacement). There was a paucity of data with which to assess the modulation of HNF4α activity (receptor activation) by PFOA and no studies examined the modulation of HNF4α by PFOS. There was a paucity of data (no studies in human primary cells) from studies examining the modulation of the progesterone receptor, the glucocorticoid receptor (and negative results regarding cortisol secretion and receptor activation in experimental human models), or the AHR by PFOA or PFOS (see summary findings for KC8 in Table 4.27).]

(b) Experimental systems

See Table S4.26 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636) and Table 4.27.

Both PFOA and PFOS have been shown to modulate receptor-mediated effects in experimental systems. Because the data were robust for many endocrine receptor outcomes, this review

primarily focuses on in vivo studies in experimental systems. The identified studies were peer-reviewed, used well-developed methods, and the quality of the study design was deemed to be adequate. Some studies used transgenic models in which the expression of one or more endocrine receptor classes was experimentally altered.

(i) Modulation of PPARs

Non-human mammalian systems in vivo

See Table S4.25 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

One of the most extensively studied effects relates to the ability of these chemicals to activate PPARs. Numerous in vivo mammalian studies that evaluated effects on PPARs were available (Table S4.24). This class of nuclear receptors has three main subtypes: PPARα, PPARβ, and PPARγ (Escher and Wahli, 2000). PPARα is highly expressed in the liver and influences the expression of genes involved in lipid metabolism, including peroxisomal β-oxidation, fatty acid uptake, and triglyceride synthesis (Rakhshandehroo et al., 2007). PFOA and PFOS are structural analogues of fatty acids, the endogenous ligands for PPARα.

In rodents, peroxisome proliferation and other metabolic effects occur in the liver after PPARα activation by either PFOA or PFOS. PFOA or PFOS exposure results in hepatomegaly in rodents (Costello et al., 2022). Studies of the exposure of PPARα-KO 129/Sv mice to PFOA or PFOS have shown that hepatomegaly was independent of PPARα activation (Filgo et al., 2015; Su et al., 2022a). PFOA or PFOS exposure in rodents also alters the activity of certain members of the cytochrome P450 (CYP) system. PPARα regulates the expression of acyl-CoA oxidase (ACOX) and CYP4A. CYP4A is involved in the oxidation of fatty acids and lipid metabolism.

The increased expression of certain CYP genes in rodents exposed to either PFOA or PFOS has been used as an indicator of the activation of the receptor subclasses. PPARα target genes include acyl-CoA oxidase (*Acox1 or Pco*), cytochrome P450 4a10 (*Cyp4a10*), and acetyl-CoA acetyltransferase 1 (*Acat1)* (Mandard et al., 2004). It has been estimated that PPARα mediates more than 75% of the changes in hepatic gene expression induced by PFOA and PFOS in mice (Rosen et al., 2008a, 2017).

Hepatic palmitoyl-CoA oxidase (PCO; alias Acox1) activity has been used as a marker of PPARα activation and peroxisome proliferation (Klaunig et al., 2003). [The Working Group noted that in the literature, acyl-CoA oxidase (*Acox1*) is also termed PCO (NCBI, 2023).]

One study in male cynomolgus monkeys (*S. cynomolgus* or *M. fascicularis*) examined the effects of 6 months of oral exposure to PFOA on PPARα (Butenhoff et al., 2002). [The Working Group noted that the increase in PPARα activation caused by PFOA, assessed by PCO activity in this study, is qualitatively similar to that seen in male rats after PFOA exposure.] Male and female rats exposed to PFOS at 20 ppm in the diet for 14 weeks developed hepatocellular hypertrophy and vacuolation, as well as increased relative liver weight, without a significant increase in hepatic PCO activity (Seacat et al., 2003). Increased relative liver weight, hepatocellular hypertrophy, and a modest (0.2-fold) increase in hepatic PCO activity occurred in rats after 28 days of exposure to PFOS at 20 ppm (Elcombe et al., 2012b).

PPARα-null mice have been studied after exposure to PFOA or PFOS. Yang et al. (2002a) reported that liver weight increased in both wildtype and PPARα-null mice treated with PFOA (0.02% in the diet) for 7 days, whereas the PCO activity was only increased in the wildtype mice. Wolf et al. (2008b) also reported that the oral PFOA exposure of wildtype and PPARα-null mice increased liver weight and hepatic cell proliferation and caused hepatocyte

hypertrophy. Increased numbers of peroxisomes were present in the livers of treated wildtype mice, whereas the livers of PPARα-null mice had numerous vacuoles without peroxisomes (Wolf et al., 2008b). [The Working Group noted that, collectively, these findings suggest that the effects of PFOS on the rat liver do not appear to primarily occur through a PPARα-dependent mode of action.]

Loveless et al. (2006) evaluated the effects of linear, branched, and a mixture of linear/branched isomers of PFOA on the relative liver weight and hepatic PCO activity of male CD rats and CD-1 mice. The liver weight of the mice increased with increasing PFOA dose and serum level, but the PCO activity was lower at the highest dose and serum level than at lower doses and serum levels. In the rats, the branched isomers of PFOA were more potent at increasing relative liver weight than the linear isomer but were less potent at increasing PCO activity. Perkins et al. (2004) reported that the subchronic dietary exposure of rats to PFOA was associated with a reversible increase in liver weight, increased hepatic PCO activity, and minimal-to-mild hepatocyte hypertrophy.

Short-term exposure to PFOS was associated with increased liver weights in wildtype and PPARα-null mice (Qazi et al., 2009). In addition, altered expression of genes related to lipid metabolism, inflammation, and xenobiotic metabolism were observed in PPARα-knockout mice exposed to PFOS, suggesting that additional pathways are activated by this chemical (Rosen et al., 2010).

Differences have been reported in the function of mouse and human PPARα (Gonzalez and Shah, 2008). The activation of mouse PPARα results in hepatocyte proliferation and dysregulation of cell-cycle genes, which does not occur in humans (Morimura et al., 2006). Transgenic mouse models that express the human PPARα gene (hPPARα) have also been used to explore the role of PPARα in PFOA and PFOS hepatotoxicity. For example, female and male hPPARα mice exposed to PFOA showed increased liver mass and histologically evident lipid accumulation (Schlezinger et al., 2020, 2021). Other studies using humanized transgenic mice have shown that hPPARα may be less responsive to either PFOA or PFOS when compared with PPARα in wildtype mice (Nakamura et al., 2009; Albrecht et al., 2013; Su et al., 2022a). [The Working Group noted that differences in the response in humanized mice are not necessarily indicative of a different response of human PPARα to PFOA, because species differences in the binding of PFOA to recognition sites on mouse DNA may exist. The Working Group also noted that the relevance to humans of the activation of PPARα with the altered hepatocyte growth and survival and clonal expansion of preneoplastic foci cells, leading to PPARα-dependent rodent liver tumour responses, has been questioned (Corton et al., 2018).]

The effects of PFOA and PFOS on PPARγ have also been examined in rodents. This pathway regulates adipocyte differentiation and lipid metabolism (Casals-Casas and Desvergne, 2011). Four studies in mice (Abbott et al., 2012; Nakagawa et al., 2012; Yan et al., 2015b; Schlezinger et al., 2020) have shown increased liver *Pparg* mRNA expression after PFOA exposure. One study (Wan Ibrahim et al., 2013) showed increased brain *Pparg* mRNA expression in neonatal mice after the PFOS exposure of pregnant dams.

Two studies in mice (Yan et al., 2015b; Li et al., 2019c) yielded mixed results regarding the liver expression of PPARβ/δ after PFOA exposure.

Non-human mammalian systems in vitro

See Table S4.26 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Cell-based in vitro studies involving either reporter assays or the expression of PPAR target genes in hepatic cells have shown that these

chemicals activated mouse, rat, and human PPARα, as well as PPARγ (Vanden Heuvel et al., 2006; Takacs and Abbott, 2007; Wolf et al., 2008b; Bjork and Wallace, 2009; Bjork et al., 2011; Houck et al., 2021). Both PFOA and PFOS exposure resulted in the transactivation of PPARγ constructs derived from humans, mice, zebrafish, and *Xenopus* in reporter assays (Garoche et al., 2021). Both PFOA and PFOS have been shown to modulate PPARγ signalling in various in vitro cell systems (Watkins et al., 2015; Liu et al., 2019; Li et al., 2021a; Modaresi et al., 2022; Qin et al., 2022c). Studies performed by Takacs and Abbott (2007) using COS-1 cells transfected with mouse or human PPARα, PPARβ/δ, or PPARγ reporter plasmids and exposed to either PFOA or PFOS showed that PFOA causes significant increases in both mouse and human PPARα reporter activity, whereas PFOS induced activation of the mouse PPARα reporter alone. PFOA and PFOS exposure also increased the activity of the mouse PPARβ/δ, but not the human PPARβ/δ reporter construct. Neither PFOA nor PFOS activated the mouse or human PPARγ reporter (Takacs and Abbott, 2007).

(ii) Modulation of CAR/PXR

Non-human mammalian systems in vivo

See Table S4.25 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

In vivo studies using CAR-null mice (Abe et al., 2017) or mice expressing hPPARα (Schlezinger et al., 2020) have shown that PFOA exposure modulates the CAR and PXR pathways. These studies reported effects on the expression of CAR target genes, which include *Cyp2b10*, *Cyp2c29*, *Cyp2c55*, *Akr1b7*, and *Ugt2b34* (Dusek et al., 2019). The PXR target genes examined in some studies included *Oatp1a4*, *CYP3A4*, *CYP2B6*, *MDR1*, and *MRP2* (Smutny et al., 2022). Two studies performed in male C57BL/6 mice showed increased hepatic expression of CAR after PFOA exposure (Cheng and Klaassen, 2008; Li et al., 2019c).

Increased liver expression of the CAR target gene *Cyp2b1* has been reported to occur in male Sprague-Dawley rats after either a 7- or 28-day (Elcombe et al., 2012a, b) exposure to PFOS at 100 ppm. Increased liver activity of the PXR target gene *Cyp3a1was* also identified after 28 days (Elcombe et al., 2012b). Three other rodent studies (Bijland et al., 2011; Dong et al., 2016; Lai et al., 2017a) also reported PFOS effects on the PXR pathway.

Non-human mammalian systems in vitro

In vitro studies using transcriptome profiling in rodent and human hepatocytes showed that PFOA and PFOS may regulate PXR target gene expression and, to lesser extent, that of CAR (Bjork et al., 2011; Buhrke et al., 2015; Abe et al., 2017; Houck et al., 2021).

(iii) Modulation of thyroid-receptor function

Non-human mammalian systems in vivo

See Table S4.25 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

One of the most extensively studied endocrine responses seen after PFOA or PFOS exposure relates to the effects of these chemicals on thyroid hormone function. In rats, PFOS exposure reduced serum T4 and T3 levels without increasing TSH secretion (Chang et al., 2008; Davidsen et al., 2022). In mice, PFOS had less consistent effects on thyroid hormone levels (Table S4.25). One study in cynomolgus monkeys reported no effects of PFOS on the serum TSH, FT4, or TT3 concentrations (Chang et al., 2017). Monkeys exposed to PFOS had low serum TT4 concentrations, which was not deemed to be clinically significant. [However, the Working Group noted that the exposure level in this study

was limited and that long delays between exposure and the measurement of thyroid hormone concentrations occurred.]

The exposure of rodents to PFOS during pregnancy was often not associated with altered maternal or pup thyroid hormone levels (Fuentes et al., 2006; Chang et al., 2009), although one study reported reduced maternal serum TT3 and TT4 concentrations after exposure during gestation (Conley et al., 2022), and this finding was replicated in pups (Lau et al., 2003).

Adult exposure to PFOS was generally associated with reduced serum thyroid hormone concentrations, whereas the TSH level was often unaffected (see Table S4.26, Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Putative mechanisms for the PFOS-induced disruption of thyroid hormone function include inhibition of the sodium/iodide symporter, altered synthesis of thyroglobulin, effects on thyroperoxidase, and the displacement of thyroid hormones from TTR (Weiss et al., 2009; Yu et al., 2009; Dong et al., 2016; Ren et al., 2016; Coperchini et al., 2021a; Davidsen et al., 2022).

In vivo studies examining the effects of PFOA on thyroid hormone function have been more limited in number (Table S4.26, Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636), with mixed effects reported in exposed non-human primates, mice, and rats (Butenhoff et al., 2002, 2012a; Blake et al., 2020).

Non-human mammalian systems in vitro

Several studies have shown that PFOA and PFOS affect the binding of thyroid hormones to the thyroid hormone transport protein TTR (Weiss et al., 2009; Ren et al., 2016; Behnisch et al., 2021). Both PFOA and PFOS have been shown to modulate thyroid hormone signalling in rat cell systems (Croce et al., 2019; Selano et al., 2019; De Toni et al., 2022).

(iv) Modulation of estrogen-receptor function

Non-human mammalian systems in vivo

Data concerning the direct effects of PFOA or PFOS on the estrogen receptor are limited to those obtained in single studies with negative or mixed findings (Yao et al., 2014; Xu et al., 2017). The estradiol concentrations in mice and rats after either PFOA or PFOS exposure can be variable, with some studies reporting elevated levels, and others showing a reduction in serum estrogen concentration (Table S4.26, Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636). Male mice exposed to PFOS did not demonstrate altered serum estradiol concentrations (Wang et al., 2014b; Qu et al., 2016; Qiu et al., 2021; Huang et al., 2022a), whereas female ICR mice exposed to PFOS had reduced estradiol concentrations (Wang et al., 2018b). Reductions in serum estradiol concentrations occurred at exposure levels that did not affect male mice (Qiu et al., 2021; Huang et al., 2022a). However, male rats exposed to PFOS had reduced serum estradiol concentrations in two studies (López-Doval et al., 2015; Salgado et al., 2015).

One study conducted in male cynomolgus monkeys (*S. cynomolgus* or *M. fascicularis*) showed no effect on serum estradiol concentration after a 26-week exposure to PFOA (Butenhoff et al., 2002). Several rodent studies also reported no effect of PFOA on serum estradiol concentration (Perkins et al., 2004; Zhao et al., 2010; Tucker et al., 2015; Su et al., 2022b; Yang et al., 2022). Other studies reported increased serum estradiol concentrations in male rats upon PFOA exposure (Biegel et al., 2001; Han et al., 2022) and in female rats upon PFOS exposure (Qiu et al., 2020).

Zhang et al. (2020b) reported lower serum estradiol concentrations in female ICR mice exposed to PFOA.

Short-term studies using zebrafish have shown that PFOS acts as an estrogen-receptor agonist (Du et al., 2013).

Non-human mammalian systems in vitro

In vitro studies using ER reporter assays performed in trout or human HEK293T cells and transcription factor activation bioassays performed in human HepG2 cells have shown that PFOA and PFOS regulate ERα target gene expression (Benninghoff et al., 2011; Buhrke et al., 2015; Qiu et al., 2020; Houck et al., 2021).

(v) Modulation of androgen-receptor function

Non-human mammalian systems in vivo

Several studies performed in mice have shown that PFOS exposure results in reduced serum or testicular testosterone concentrations (Table S4.25, Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636). However, other studies in mice showed no effects of PFOS exposure (Wang et al., 2014b; Li et al., 2022e). Most studies performed in rats have also shown reduced serum testosterone concentration after PFOS exposure, with fewer demonstrating no effect or increased testosterone concentration (Table S4.25). One study conducted in cynomolgus monkeys showed no effect of a 26-week exposure to PFOA (see also above) (Butenhoff et al., 2002). PFOS exposure in rodents has also been shown to reduce epididymal sperm count and alter the expression levels of several key steroidogenic enzymes in the testes (Wan et al., 2011). Effects of PFOS on testosterone synthesis in Leydig cells have been linked to the CREB/transcription coactivator 2 (CRTC2)/steroidogenic acute regulatory protein (StAR) signalling pathway (Qiu et al., 2021).

Two studies performed in Sprague-Dawley rats showed increased liver testosterone 6β-hydroxylase activity after PFOS administration (Elcombe et al., 2012a, b).

PFOA also showed effects on the androgen receptor pathway. Reduced serum or plasma testosterone concentrations were reported in male rats and mice after PFOA exposure (Li et al., 2011; Owumi et al., 2021b). Reduced serum testosterone concentrations were identified in the male offspring of Kunming mice exposed during gestation (Song et al., 2018). Increased serum testosterone concentrations were identified in female CD-1 mice (Yang et al., 2022) and male CD rats (Biegel et al., 2001) after PFOA administration. However, one study reported no effect of PFOA on the circulating testosterone concentration of exposed male CD rats (Perkins et al., 2004).

(vi) Other receptor systems

Non-human mammalian systems in vivo

Effects of PFOA and PFOS on prolactin and insulin function have been reported in studies that evaluated the function of multiple hormones (Table S4.25, Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636). Hines et al. (2009) reported that exposure to PFOA at 0.01 mg/kg bw significantly increased serum insulin in mice. Wan et al. (2014) reported that perinatal exposure to PFOS resulted in elevated levels of glucose and insulin in F_1 pups and adult CD-1 mice. Combined gestational and lactational exposure to PFOS elevated fasting serum insulin concentrations and impaired glucose tolerance in rat offspring (Lv et al., 2013).

Neither PFOS nor PFOA altered AHR transcriptional activity in transfected mouse Hepa1.1 2cR cells (Long et al., 2013). [The Working Group noted that a positive control, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, was included with this

AHR–luciferase reporter gene bioassay.] Other studies examined whether PFOA or PFOS altered the expression of *Cyp1a1*, which encodes an enzyme and is regulated by AHR. One study performed in juvenile Atlantic salmon (*Salmo salar*) fed either PFOA or PFOS at 0.2 mg/kg for < 1 week showed no relation between PFOA or PFOS treatment and the hepatic expression of CYP1A1 and AHR isoforms; however, a temporary but significant increase in *Cyp1a1* expression was identified in PFOA-fed salmon after 5 days and PFOS-fed salmon after 2 days (Mortensen et al., 2011). The exposure of rare minnow (*Gobiocypris rarus*) to PFOA increased the *Ahr* mRNA level in the gills and was associated with the downregulation of *Cyp1a* mRNA (Liu et al., 2008). One study examined the effects of PFOS on intestinal immunity and infection in mice with *Citrobacter rodentium* infection (Suo et al., 2017). PFOS prevented the replication of *C. rodentium* by promoting IL-22 production by group 3 innate lymphoid cells through the activation of AHR. The large intestinal lamina propria lymphocyte mRNA expression of *Cyp1a1* was also elevated after PFOS treatment (Suo et al., 2017).

In the liver, HNF4α regulates multiple genes and lipid metabolism and is involved in hepatocellular differentiation (Yeh et al., 2019). In mice, the conditional hepatocyte-specific deletion of HNF4α results in hepatomegaly and hepatic steatosis. This phenotype is similar to that induced in rodents exposed to PFOA or PFOS (Bonzo et al., 2012). However, only one study has examined the role of HNF4α after PFOA or PFOS exposure in experimental systems (Yan et al., 2015b). This showed reduced hepatic mRNA expression of *Hnf4a* in mice exposed to PFOA at 1.25 or 5 mg/kg per day. The high PFOA dose used in this study was not associated with altered *Hnf4a* expression. Finally, an in vitro study using proteomic profiling in human hepatocytes showed that PFOA alters the expression of genes regulated by HNF4α (Scharmach et al., 2012).

Synopsis

[The Working Group noted that, overall, the studies described above in experimental systems in vivo reported evidence that both PFOA and PFOS could modulate PPARα activity. In addition, there was evidence that PFOA modulated PPARγ activity. In one rodent study PFOS was shown to modulate PPARγ activity.

There was a paucity of available data for the effects of either PFOA or PFOS on PPARβ/δ. There was evidence that both PFOA and PFOS modulated CAR/PXR function.

PFOS modulated thyroid function in exposed animals. However, there were limited data suggesting that thyroid effects were induced by PFOA exposure. There was some evidence that PFOA and PFOS altered serum estradiol concentrations in rodents; however, both non-significant and significant findings were reported.

Evidence that PFOS altered the testosterone concentrations of rodents was available from multiple studies, but there was more limited evidence for the effects of PFOA on androgen-receptor function and testosterone concentration. Finally, there was a paucity of information on the effects of both PFOA and PFOS on other receptor systems in experimental systems (see summary findings for KC8 in Table 4.27).]

4.2.9 Causes immortalization

(a) Humans

(i) Exposed humans

See Table 4.28.

The shortening or lengthening of telomeres has been associated with some types of cancers and may contribute to carcinogenesis. Telomere shortening may increase genetic instability, whereas telomere lengthening may promote deleterious cell survival and proliferation and serve as a marker of immortalization.

Table 4.28 End-points relevant to immortalization in humans exposed to PFOA or PFOS

End-point, assay	Biosample type	Location, setting, study design	Exposure level[a] No. of study participants	Response (significance)	Covariates adjusted for	Comments	Reference
Telomere length, by Sigma TeloTAGGG Telomere Length assay	Peripheral blood leukocytes and serum	Firefighters from San Francisco Fire Department and office workers, City and County of San Francisco, USA	163 participants (84 firefighters and 79 office workers) Women PFOA, 1.16 ± 1.76 ng/mL PFOS, 4.18 ± 2.08 ng/mL	Linear regression models (minimally adjusted model, Model 1, and a fully adjusted model, Model 2, used to assess the associations between continuous log-transformed PFAS and TL In Model 1, a doubling of PFOA concentration was associated with a 273 (95% CI, 54–493) bp increase in TL; in Model 2, a doubling in PFOA was associated with a 240 (95% CI, 25–455) bp increase in TL A doubling in PFOS concentration was associated with a 183 (95% CI, 15–352) bp increase in TL in Model 1, and a 172 (95% CI, 5–340) bp increase in TL in Model 2	Age, occupation, the number of times dairy products were eaten per week, and the number of times eggs were eaten per week. Covariates assessed include demographic variables such as race/ethnicity and education; health variables such as BMI, stress, and sleep metrics; and food frequency variables.	Potential confounders were selected a priori, on the basis of results from previous literature and prior analyses performed on these data. No tables provided for the above statistics.	Clarity et al. (2021)

Table 4.28 (continued)

End-point, assay	Biosample type	Location, setting, study design	Exposure level[a] No. of study participants	Response (significance)	Covariates adjusted for	Comments	Reference
Telomere length, by qPCR	Maternal whole blood and delivery cord blood	Chemicals in Our Bodies cohort	Mothers (*n* = 125) and newborns (*n* = 326) PFOA, 0.8 ± 2.0 ng/mL PFOS, 2.0 ± 2.1 ng/mL	Quantile g-computation PFAS mixture alone was associated with a modest increase in maternal TL (mean change in maternal TL per quartile increase, 0.04; 95% CI, −0.01 to 0.09) In the analysis restricted to maternal–fetal paired samples, an increase in the PFAS mixture of one quartile was positively associated with umbilical cord blood TL (mean change in umbilical cord blood TL per quartile increase, 0.11 (95% CI, 0.01–0.22) Individual PFOA (β = 0.01; 95% CI, −0.03–0.06), and PFOS (β = −0.01; 95% CI, −0.05 to 0.03)	Maternal education, race/ethnicity, maternal age, gestational age at delivery, pre-pregnancy BMI, parity, and infant sex		Eick et al. (2021)
Telomere length, by qPCR	Leukocytes, serum	US NHANES 1999–2000 Cross-sectional data set	773 participants; 389 men and 384 women PFOA mean (10th–90th percentile), 5.63 (2.24–8.76) ng/mL PFOS, 33.97 (13.54–57.68) ng/mL	Fully adjusted multiple variable linear regression Each increment of one SD in the $\log_{10}$ PFOS level was associated with a 21-bp increase in the TL (*P* = 0.033), after adjustment for potential confounders No associations of the TL with the concentrations of PFOA or other PFAS	Age, sex, race, BMI, educational level, leukocyte count, C-reactive protein, and PIR status		Huang et al. (2019b)

Table 4.28 (continued)

End-point, assay	Biosample type	Location, setting, study design	Exposure level[a] No. of study participants	Response (significance)	Covariates adjusted for	Comments	Reference
Telomere length, by qPCR	Cord blood leukocytes, cord serum	Wuhan Maternal and Child Health Hospital in Wuhan, China	916 cord blood samples PFOA, 1.81 ± 2.33 ng/mL PFOS, 5.77 ± 10.32 ng/mL	Weighted quantiles of exposure and linear regression Negative association in the adjusted model: % change in neonatal TL, −5.19% (95% CI, −9.44 to −0.73%; $P = 0.040$) for each unit increase in WQS index of PFAS mixture The weights of PFOS and PFOA were 13.16% and 10.75%, respectively	Maternal sociodemographics, age, education, alcohol drinking habits, smoking status, pre-pregnancy BMI, parity, birth weight, gestational age, neonatal sex, and neonatal birth season Alcohol consumption and active smoking were not adjusted for, because no participants drank or smoked during pregnancy		Li et al. (2023a)
Telomere length, by qPCR	Buffy coat from cord blood, cord plasma	Shanghai Allergy Cohort, Shanghai, China; 2012–2013	581 participants PFOA, 7.65 ± 3.88 ng/mL PFOS, 2.93 ± 3.28 ng/mL	Generalized linear models adjusted for covariates LTL was significantly shorter in the female newborns whose PFOS, but not PFOA, concentrations were in the highest quartile, compared with those in the lowest quartile, after adjusting for potential confounders (0.926 ± 0.053 vs 0.945 ± 0.054, respectively (mean ± SD); $P = 0.023$)	Maternal and paternal ages, maternal education, maternal pre-pregnancy BMI, mode of delivery, gestational age at birth, infant sex, birth weight, and antepartum obstetric risk		Liu et al. (2018b)

Table 4.28 (continued)

End-point, assay	Biosample type	Location, setting, study design	Exposure level[a] No. of study participants	Response (significance)	Covariates adjusted for	Comments	Reference
Telomere length, by qPCR	Buffy coat containing leukocytes, serum	Flemish Environment and Health Study, Flanders, Belgium	175 participants PFOA geometric mean (25th–75th percentile): 2.78 (2.13–3.65) µg/L PFOS: 7.52 (5.31–10.9) µg/L	sPLS regression model followed by OLS regression Serum PFOA level was inversely associated with LTL; sPLS model (raw coefficient, −0.017; 95% CI, −0.032 to −0.002; $P = 0.03$; and OLS model (relative change, −3.64%; 95% CI, −6.60 to −0.60%; $P = 0.02$	In linear regression models the a priori covariates selected from among socioeconomic status, lifestyle, smoking habits, and ethnicity were age, sex, BMI, smoking habits, household education, and ethnicity		Vriens et al. (2019)
Telomere length, by qPCR	Leukocytes from cord blood, maternal serum	Birth cohort study in Guangxi, China, 2015–2018	PFOA, geometric mean, 2.379; 25th–95th percentile, 1.716–5.599 ng/mL PFOS geometric mean, 0.983; 25th–95th percentile, 0.662–3.736 ng/mL 499 mother–umbilical cord blood pairs	Multivariable linear regression: each ln-transformed unit concentration increase in PFOA was associated with 20.41% (95% CI, −30.44 to −8.93%) shorter LTL in spring-born infants but not in those born in other seasons	Cigarette smoking, passive exposure to tobacco, alcohol consumption, pre-pregnancy BMI, maternal age, occupation, parity, pregnancy complications, gestational age, date of birth (birth season), infant sex, birth weight		Pan et al. (2022)

BMI, body mass index; bp, base pair; CI, confidence interval; LTL, leukocyte telomere length; NHANES, National Health and Nutrition Examination Survey; OLS, ordinary least-squares; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid: PIR, poverty income ratio; qPCR, quantitative polymerase chain reaction; SD, standard deviation; sPLS, sparse partial least-squares; TeloTAGGG, commercial telomere length assay; TL, telomere length; vs, versus; WQS, weighted quantile sum.

[a] PFOS and PFOA concentrations are expressed as mean ± SD or geometric mean ± geometric SD, unless otherwise stated in the table.

Several cross-sectional studies examined potential links of PFOA and PFOS exposure in humans with changes in telomere length (TL).

Clarity et al. (2021) conducted a study on blood collected from female firefighters from the San Francisco Fire Department and office workers in the City and County of San Francisco, USA. The minimally adjusted model (Model 1) and fully adjusted model (Model 2) assessed the association between the continuous log-transformed PFOA and PFOS serum concentrations and leukocyte telomere length (LTL). Statistically significant positive associations of LTL with PFOA (β = 0.57; 95% CI, 0.12–1.02) and PFOS (β = 0.44; 95% CI, 0.05–0.83) were observed in both cohorts, and especially in firefighters in Model 1, after adjustment for age. In Model 2, after adjustment for age, dairy consumption, and egg consumption, only PFOA was found to be significantly associated with LTL in the firefighters. [The Working Group noted that firefighters were occupationally exposed to many different chemicals, including benzene, PAHs, formaldehyde, dioxins, and polybrominated diphenyl ethers, but the effect estimate differences did not account for unmeasured chemical co-exposure in the firefighters that may have affected TL.]

A strong positive association between the blood PFOS concentration and TL was shown mainly in the female participants from among 773 adults in the NHANES cycle of 1999–2000 (Huang et al., 2019b).

In another study, prenatal exposure to PFAS mixtures that included PFOA and PFOS showed a modest association with LTL in umbilical cord blood (mean change in TL per quartile increase, 0.11; 95% CI, 0.01–0.22), and only when maternal–fetal unit pairs were considered (Eick et al., 2021). No significant association was observed of individual PFOA and PFOS blood levels with TL in maternal whole blood (Spearman correlations of 0.09 and 0.05, respectively) or umbilical cord blood (Spearman correlations of 0.22 and −0.06, respectively) (Eick et al., 2021).

In contrast to these findings, Li et al. (2023a) showed a modest negative association between PFOS concentration and umbilical cord blood TL in male newborns only, and Liu et al. (2018b) identified shorter TL in the umbilical cord blood of female newborns exposed to higher levels of PFOS. In both studies there was no significant association between the level of PFOA and TL in umbilical cord blood.

No significant associations of umbilical cord blood TL with the maternal serum levels of PFOA or PFOS were observed in a birth cohort study of 499 mother–newborn pairs conducted in Guangxi, China, between June 2015 and May 2018 (Pan et al., 2022).

In addition, serum PFOA level was inversely associated with TL in 175 adults aged 50–65 years in the cross-sectional Flemish Environment and Health study (Vriens et al., 2019). [The Working Group noted that TL displays large inter-individual variation at birth and throughout the human lifespan and may depend on the differentiation and activation status of leukocytes. All the available studies were cross-sectional, and the lack of prospective studies investigating the effects of PFOA and PFOS exposure on TL in humans was a notable research gap.]

(ii) Human cells in vitro

The inhibition of gap junctional intercellular communication (GJIC) may lead to a loss of intercellular communication and play a role in mitogenic activation, promoting tumour formation and cellular proliferation, migration, and invasion. Some carcinogens may inhibit GJIC and promote cancer development.

Saejia et al. (2019) showed that treatment with PFOA did not affect cell migration, but enhanced cell invasion, adhesion, and the activity of matrix metalloproteinase-2 through the activation of the NF-κB signalling pathway in human follicular thyroid carcinoma (FTC133) cells. Treatment

of non-tumorigenic human breast epithelial MCF-10A cells with 10 μM PFOS for 72 hours induced cell-cycle progression, cell migration, and invasion, which may lead to breast cancer initiation and development (Pierozan and Karlsson, 2018).

(b) Experimental systems

Non-human mammalian cells in vitro

It has been shown that PFOA and PFOS inhibit GJIC in rat liver epithelial cells (Upham et al., 1998). The activation of extracellular receptor kinase and phosphatidylcholine-specific phospholipase C in an oxidation-dependent manner was suggested to be a mechanism of GJIC dysregulation (Upham et al., 2009).

The morphological transformation of Syrian hamster embryo cells in vitro assay is recommended by the Organisation for Economic Co-operation and Development for the detection of genotoxic and non-genotoxic carcinogens. Seven days of exposure to non-cytotoxic concentrations of PFOS alone or PFOA in combination with benzo[*a*]pyrene (B[*a*]P, 0.4 μM) pre-treatment induced cell transformation (measured as colony forming unit frequency) in a non-genotoxic manner (Jacquet et al., 2012a, b). PFOS and its acid form induced cell transformation only at 0.37 and 3.7 μM concentrations, in a non-dose-dependent manner without an initiator, whereas PFOA at concentrations from 3.7×10^{-4} to 37.2 μM induced cell transformation in B[*a*]P-sensitized cells (Jacquet et al., 2012a, b). [The Working Group noted that immortalization-specific studies of primary or cultured human cells were not available.]

Synopsis

[The Working Group noted that the evidence that PFOA or PFOS induces cell immortalization was sparse for all test systems.]

4.2.10 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

(i) Cell proliferation

Exposed humans

Xie et al. (2023) identified positive correlations of PFOA and PFOS concentrations with the expression of molecular markers of glioma, specifically Ki-67 and p53. A total of 137 glioma tissue and 40 non-glioma tissue samples were collected. The study showed that PFOA and PFOS is commonly present in cancerous and noncancerous brain tissue, but higher concentrations of PFOA and PFOS were present in glioma samples from the brain than in non-glioma samples, albeit without statistical significance. The data revealed a positive correlation between PFOA and PFOS concentrations and tumour grade. Positive correlations of PFOA and PFOS with the expression of the glioma molecular markers Ki-67 or p53 were also observed. Significant correlations were observed of tumour grade with Ki-67 and p53 expression ($r^2 = 0.33$ and $r^2 = 0.10$, respectively). Ki-67 expression significantly correlated with the level of PFOA (univariate linear regression; $n = 97$; $r^2 = 0.24$; $P < 0.05$). A multiple regression model indicated that approximately 30% of Ki-67 expression could be explained by variation in the PFOS and PFOS concentrations, and predictor importance analysis suggested that Ki-67 expression was mainly driven by the PFOA concentration. No significant univariate regression associations were observed between the concentrations of individual PFAS and p53 (Xie et al., 2023). [The Working Group noted several limitations to this study. PFOA and PFOS were measured in the glioma tissue; however, it was not specified which part(s) of the brain was sampled and compared. The PFAS concentrations varied substantially in different brain areas (Di Nisio et al., 2022). Paired glioma and non-glioma

samples were available for 18 patients, and no statistically significant differences in the concentrations of PFAS were observed between the 18 pairs of glioma and non-glioma tissue samples, possibly because of the limited sample numbers. The 137 glioma and 40 non-glioma brain tissue samples included in the study were from patients from whom only one of the two tissue types was collected. It was not informative to compare PFAS levels in tumour and non-tumour brain tissue from different participants, because PFOA and PFOS exposure varied substantially among the participants. In addition, it was noted that the expression of Ki-67 was absent in 40 out of 137 glioma samples and that the relevance of cell proliferation measurements in tumour tissues as an end-point for this KC is questionable.]

Bassler et al. (2019) explored the mechanisms of the associations of PFOA and PFOS with the development of NAFLD. Two hundred adult samples from a cross-sectional study of participants from districts with PFOA contamination of drinking-water were analysed. The serum concentrations of biomarkers of hepatocyte death/apoptosis were altered. A univariate analysis showed that serum cytokeratin 18 M30 (a marker of hepatocyte apoptosis) and cytokeratin C18 M65 (a marker of hepatocyte total cell death, necrosis and apoptosis) were both positively associated with serum PFOA and PFOS. In a multivariate analysis, M30 was positively associated with PFOA, and there was a similar trend for PFOS [The Working Group noted that although caspase-cleaved cytokeratin 18 fragments (CK-18 forms both M30 and M65 antigens) are markers of hepatocyte death, they have also been considered to be candidate markers for the detection of non-alcoholic steatohepatitis and fibrosis (Feldstein et al., 2009).] [The Working Group also noted that this study observed associations of PFOA and PFOS with markers of inflammation. See Section 4.2.6.]

Human primary cells in vitro

Human primary normal prostate epithelial cells were cultivated to form a 3D spheroid model and exposed to PFOA or PFOS at 10 nM for 3–4 weeks. PFOA and PFOS significantly increased the total number of spheroids and their size, indicating elevated stem cell self-renewal and progenitor cell proliferation. Transcriptome analyses showed an upregulation of genes encoding signalling pathway intermediates involved in cell proliferation (G2-M checkpoint, mitotic spindle, E2F targets) and oncogenesis (kRAS and MYC signalling, TNF-α via NF-κB, IL-6/JAK/STAT3, TGFβ, and inflammatory signalling). Metabolomic analysis of PFOA and PFOS-exposed prostaspheres (spheroids) revealed upregulation of glycolytic pathways, including those involved in the Warburg effect, in response to PFOA or PFOS exposure (Hu et al., 2022).

In addition, transcriptomic analysis of human primary hepatocytes treated with non-cytotoxic doses (10 nM–10 μM PFOA or PFOS, potassium salt) for 48 or 96 hours revealed that PFOA predominantly changed the expression of genes involved in lipid metabolism and hepatic steatosis, whereas PFOS predominantly induced changes in the expression of genes involved in carcinogenesis and cell death signalling. PFOA and PFOS caused a decrease in *CLDN1* mRNA. Claudin-1 protein is involved in maintaining cellular adhesion and the formation of cell junctions. PFOA and PFOS also caused an induction of the *AKR1B10* gene, which is associated with the progression of hepatocellular carcinoma (Matkowskyj et al., 2014).

The effect of PFOS on human primary oesophageal epithelial cell proliferation was assayed using a cell counting kit (CCK)-8. PFOS at 10 nM had no significant effect on proliferation but increased the migration and invasion of the oesophageal squamous cell carcinoma cell lines KYSE150, KYSE140, and KYSE70 by regulating

the transcription and protein stability of ZEB1 (Liu et al., 2022c).

Human cell lines in vitro

Exposure to PFOS (10 μM) or PFOA (100 μM) for 72 hours induced breast epithelial cell (MCF-10A cell line, expected to be ERα- and β-negative) proliferation and the alteration of regulatory cell-cycle proteins (cyclin D1, CDK6, p21, p53, p27, ERK 1/2, and p38), and this persisted after multiple cell divisions. Interestingly, PFOA and PFOS increased proliferation and caused a persistent increase in cyclin D1 levels, but through two different mechanisms. PFOS activated the ERK pathway and PFOA acted by inactivating p38 and reducing cyclin D1 degradation. Both compounds promoted cell migration and invasion (Pierozan et al., 2020). [The Working Group noted that this study also observed associations of PFOA and PFOS with alterations in epigenetic end-points (see Section 4.2.4).]

Gimenez-Batista et al. investigated the effects of several concentrations of PFOA (100, 72.5, 7.25, 3.6, and 0.36 μM) or PFOS (100, 60, 6, and 0.6 μM) on the growth of a human colon myofibroblast (CCD-18Co) cell line using an MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay, in the presence or absence of the pro-inflammatory cytokine IL-1b. After 96 hours of treatment, CCD-18Co myofibroblast proliferation was significantly induced in the presence or absence of IL-1b (Giménez-Bastida et al., 2015).

Exposure of a human non-small cell lung carcinoma cell line (A549) to one of several concentrations of PFOA or PFOS (10, 200, or 400 μM) for 48 hours caused alterations to the cell cycle and apoptosis. Lower doses of these compounds caused the cell balance to shift towards cell proliferation, whereas exposure to higher concentrations shifted the balance towards apoptosis, as evaluated by MTT assay and confirmed by measurement of the mRNA expression of genes involved in the cell cycle and proliferation. Increases in cellular proliferation were noted at 100 and 200 μM PFOA and PFOS exposure levels; however, significant reductions in cell viability at ≥ 600 μM PFOA and 400 μM PFOS were also observed (Jabeen et al., 2020).

Spheroids produced from COV434 and KGN human ovary granulosa tumour cell lines were exposed to PFOA (0.02, 0.2, 2, 20, or 200 ng/mL, or 2 mg/mL) or PFOS (0.08, 0.8, 8, 80, or 800 ng/mL, or 8 mg/mL). The proliferation of the spheroid cultures was estimated by the measurement of ATP using the CellTiter-Glo 3D cell viability assay. PFOA and PFOS increased COV434 and KGN cell proliferation in a dose-dependent manner, compared with untreated control cells (Gogola et al., 2019).

In other studies, lower viability or induction of apoptosis was observed after PFOA or PFOS exposure in primary cultures of normal human thyroid cells (NHT) (Coperchini et al., 2021b), in a human hepatoma cell line (HepG2) (Hu and Hu, 2009), and in monolayers (2D) and spheroids (3D) of neuronal cells (N2a) (Choi et al., 2013).

Two cancer cell lines, prostate (DU145) and breast (MCF7), were treated with very low doses of PFOA (10^{-6} to 10^{-12} M), for 48 hours. PFOA increased the proliferation of DU145 and MCF7 cells, as monitored by real-time imaging. PFOA acted through distinct signalling pathways in these two cell lines (AKT/mTORC1 and plexin D1-dependent pathways in MCF7 and DU145 cells, respectively) (Charazac et al., 2022).

PFOA at 50 or 100 μM after 72 hours significantly promoted viability, migration, and invasion of RD cells (a human embryonal rhabdomyosarcoma cell line), and significantly inhibited apoptosis. Higher concentrations (500 and 1000 μM) inhibited cell viability. Treatment with a PI3K inhibitor antagonized the effects of PFOA on migration, invasion, and apoptosis. The authors concluded that PFOA promoted RD cell migration and invasion and inhibited apoptosis through the PI3K/AKT signalling pathway (Zhang et al., 2019).

Trophoblast cells HTR-8/SVneo were exposed to 100–1000 µM PFOA for 72 hours. Low doses increased trophoblast viability, whereas concentrations of > 400 µM reduced cell viability. The activation of ROS-dependent ERK signalling at low doses triggered trophoblast proliferation, whereas the activation of UPR signalling triggered trophoblast apoptosis under conditions of severe ERS (Du et al., 2022).

Immortalized human granulosa HGrC1cells (a cell type of ovarian follicles) were exposed to PFOA at 1–100 µM for 24–96 hours. Low doses (up to 10 µM) caused increased viability and proliferation, whereas the highest dose (100 µM) caused reductions in these parameters. YAP1-mediated proliferation was confirmed by adding a YAP1 inhibitor, which prevented the effects of PFOA. Thus, PFOA was shown to induce proliferation, migration, and invasion of HGrC1 cells, potentially via upregulation of the Hippo pathway effector YAP1 and of cell-cycle regulators such as cyclin D1 (Clark et al., 2022).

PFOA (≥ 100 nM) treatment also stimulated A2780 ovarian cancer cell invasion and migration, and increased the expression of the matrix metalloproteinases MMP-2 and 9 (Li et al., 2018c).

Human normal HL-7702 hepatocyte line was exposed to PFOA at 0–800 µM for 48 or 96 hours. Low doses (50–100 µM) increased cell proliferation by promoting a shift from the G1 to S phase, whereas at higher doses (200–400 µM), the cell numbers were comparable with those of the control, mainly because of cell-cycle arrest in the G0/G1 phase. Notably, no apoptosis was detected, even at 400 µM. Corroborating these data, proteomic analysis detected 111 significantly expressed proteins, of which 46 were related to cell proliferation and apoptosis. The induction of cyclin D1, CDK6, cyclin E2, cyclin A2, and CDK2 in the low-dose PFOA groups was associated with larger numbers of cells in S phase, as well as fewer cells in the G0/G1 phase, indicating that PFOA shifted cells from G1 to S phase (Zhang et al., 2016b).

The viability of L-02 cells increased slightly after 24 hours of treatment with PFOA at 8 µM, but decreased at higher PFOA concentrations (16–512 µM) after 24 hours (Wang et al., 2022a).

In an experiment performed in HepG2 cells, which were treated with 200–500 µM PFOA for 12–48 hours, it was observed that 50 µM PFOA increased the number of cells in the G2/M phase, while reducing the number in S phase; 100 and 150 µM PFOA increased the G0/G1 cell percentages and reduced the S/G2/M cell percentages; and ≥ 400 µM reduced the proportion of G0/G1 cells (Shabalina et al., 1999).

In contrast, cell proliferation was not altered in other cell types, for example in human choriocarcinoma (Jeg-3) and in endometrial adenocarcinoma (RL95-2) cells treated with 0.01–100 µM PFOA for 24 hours (Tsang et al., 2013), in ovarian cancer cells (A2780 cells) treated with 0–200 nM PFOA for different periods of time, and in human ovarian GC cells (KGN) treated with 0.03–300 µM PFOA for 12–48 hours (Zhou et al., 2020).

To examine the effects of PFOA during human endometrial carcinogenesis, Ishikawa cells were treated with 50 nM PFOA for 48 hours. This treatment did not affect proliferation, but it promoted both the migration and invasion of these endometrial cancer cells (Ma et al., 2016).

Pierozan and Karlsson (2018) showed that PFOS-induced cell proliferation and cell death are dependent on the exposure time and concentration. The breast cell line MCF-10A was treated with 0–1 mM PFOS for 24, 48, or 72 hours. Significantly lower cell viability was observed at ≥ 250 µM PFOS at all time points. In contrast, exposure to 10 µM PFOS for 48 hours, or 1 or 10 µM PFOS for 72 hours, increased MTT production and the number of cells, reduced the percentage of cells in the G0/G1 phase, and increased the percentage of cells in S phase at all time points. There were also decreases in the mean fluorescence intensities associated with p27, p21, and p53 staining and an increase in the mean

fluorescence intensity associated with CKD4 staining in PFOS-treated cells, compared with the controls. In addition, treatment with 10 µM PFOS for 72 hours also stimulated MCF-10A cell migration and invasion.

However, PFOS at concentrations ranging from approximately 1 nM to 100 µM did not induce significant effects on cell viability and/or cell death in hMSCs (Gao et al., 2020), human primary placental cytotrophoblasts isolated from placenta at full-term pregnancy (Zhang et al., 2015b), human embryo liver cells (L-02) (Zeng et al., 2021; Dong et al., 2022), human choriocarcinoma cell lines HTR-8/SVneo and JEG-3, embryonic stem cell-derived cardiomyocytes (Cheng et al., 2013; Li et al., 2021a), and renal proximal tubular epithelial cells NRK-52E (Wen et al., 2016).

Notably, ferroptosis, a process of regulatory cell death that is induced by excessive lipid peroxidation, was investigated in two studies. Cui et al. (2022) investigated the role of ferroptosis in HUVECs exposed to 180 µM PFOS for 12–48 hours. The results showed that the viability of the HUVECs was significantly reduced by the PFOS treatment after 12 hours. PFOS increased the expression of the ferroptosis-related protein ACSL4 and reduced the expression of GPX4, HO-1, and FTH1. The results were confirmed using a ferroptosis inhibitor (Cui et al., 2022).

In the human proximal tubular epithelial cell line HK-2, treatment with PFOS at 50–250 µM reduced cell viability after 12 hours in a dose-dependent manner and induced ferroptosis and apoptosis (Wang et al., 2022b).

(ii) Cell death inhibition

Human hepatocarcinoma HepG2 cells exposed to 200 µM PFOA for ≤ 72 hours showed autophagosome accumulation. [The Working Group noted that the autophagosome accumulation may have been the result of autophagy activation or have been caused by the inhibition of autophagy at the degradation step.] The proteomic analysis performed in this study suggested the inhibition of autophagy. The same study observed autophagosome accumulation in mouse livers (Yan et al., 2017). [The Working Group noted that these results are helpful in understanding the potential mechanisms of the hepatotoxicity induced by PFOA. In addition, this study showed that the PFOA-dependent reduction in HepG2 cell viability may not be directly attributable to a dysfunction of autophagy.]

(iii) Angiogenesis

In HTR8/SVneo cells, an embryonic trophoblast cell type that is able to form vessel-like vascular networks in 3D matrices, exposure to PFOA at 100 µg/mL affected morphological parameters of the pseudo-vascular network in a dose-dependent manner, including the number of pseudo-vascular junctions and the total lengths of non-branching segments. In contrast to PFOA, PFOS did not have significant effects on angiogenesis (Poteser et al., 2020).

(b) Experimental systems

(i) Non-human mammalian systems in vivo

In the chronic carcinogenicity study by Butenhoff et al. (2012a) (see Section 3.1.2), a significantly increased incidence of tubular hyperplasia of the ovaries of rats was observed in groups treated with PFOA, compared with controls – 0/48 (0%), 7/50 (14%), and 15/47 (33%) at 0 (control), 30, and 300 ppm, respectively. There were also increases in the incidence of hepatocellular hypertrophy in male and female rats. [The Working Group noted that although the differences were not statistically significant, the incidence of pancreas acinar hyperplasia in male rats was 0/46 (0%), 2/46 (4%), and 2/49 (4%) for the groups at 0 (control), 30, and 300 ppm, respectively).]

Dietary administration of PFOA at 300 ppm for 2 years to CD rats resulted in increased Leydig cell proliferation (46% versus 14% for the control

group) and a higher incidence of adenoma (11% versus 0% for the control groups) in the testes. Also, PFOA showed a tendency to increase pancreatic acinar cell proliferation at 15, 18, and 21 months, when compared with the control groups. The incidence of acinar cell hyperplasia was 39% versus 18% or 10% for the ad libitum or pair-fed control groups, respectively; and that of adenoma was 9% versus 0% or 1% for the ad libitum or pair-fed control groups, respectively. No cell proliferation was observed in the livers (Biegel et al., 2001).

In a review of the pancreatic lesions in male rats in the studies by both Biegel et al. (2001) and Butenhoff et al. (2012a), using the same diagnostic criteria as those applied in the study by Biegel et al. (2001), a significant positive trend ($P < 0.05$, Cochran–Armitage trend test) in the incidence of pancreatic acinar cell hyperplasia was observed, with the incidence being significantly increased [$P = 0.0382$, Fisher exact test] at the highest dose (3/46 (7%), 1/46 (2%), and 10/47 (21%) for 0, 30, and 300 ppm PFOA, respectively) (Caverly-Rae et al., 2014). In addition, histopathological examination of the pancreas revealed focal ductal hyperplasia in C57Bl/6 mice treated with PFOA at 2.5 or 5.0 mg/kg orally for 7 days (Kamendulis et al., 2014).

In a 2-year carcinogenicity study with and without perinatal exposure (NTP, 2020) (see Section 3.1.2), exposure to PFOA resulted in increases in the incidence of hepatocellular hypertrophy in male and female rats and increases in the incidence of papillary urothelium hyperplasia at the 16-week interim time point of the feeding study. In this 2-year feeding study of PFOA, increases in the incidence of hepatocyte hypertrophy, bile duct hyperplasia, hyperplasia of the renal papillary epithelium, and epithelial hyperplasia of the forestomach were observed in female rats at 300 or 1000 ppm. In the male rats, increases in the incidence of hepatocyte hypertrophy and bile duct hyperplasia were also observed. In addition, acinus hyperplasia was also significantly increased in all the postweaning-only exposure groups in the chronic study and in the groups at 300 ppm in the perinatal and postweaning study, and this lesion was considered to be potentially preneoplastic.

Filgo et al. (2015) (see Section 3), described non-neoplastic lesions in four strains of mice (age, 18 months) after exposure to PFOA during gestation. Significant increases in the incidence of non-neoplastic liver lesions were observed in CD-1 mice after PFOA exposure, including oval cell hyperplasia, Ito cell hypertrophy, and centrilobular hepatocyte hypertrophy. Several non-neoplastic changes were observed after PFOA exposure in PPARα-KO mice, but not in 129/Sv WT mice, including significant dose-related increases in the incidence of both bile duct hyperplasia and bile duct inclusion bodies (hyaline droplets). In addition, the incidence of centrilobular hepatocyte hypertrophy and of haematopoietic cell proliferation was significantly increased by PFOA exposure in PPARα-KO mice, but not in 129/Sv WT mice. The incidence of Ito cell hypertrophy was reduced by PFOA treatment in PPARα-KO mice (Filgo et al., 2015). [The Working Group noted that this study demonstrated that PPARα is not essential for the liver effects of PFOA in mice, because these were observed in PPARα-KO mice.]

Male Sprague-Dawley rats were exposed orally to PFOA (300 ppm) in the diet for 1, 7, or 28 days. The results showed that the hepatic bromodeoxyuridine (BrdU) labelling index was increased by approximately three-fold 1 day after the start of treatment. The increase in the hepatic BrdU labelling index versus the control group was largest after 8 days (approximately five-fold compared with controls). The labelling index values were near the background levels for male rats after 2 and 29 days, even though they were statistically significantly higher than those for the respective controls. The administration of PFOA to rats led to hepatomegaly, characterized by hypertrophy and hyperplasia, as a result

of early increases in cell proliferation, which would ultimately lead to liver tumour formation. (Elcombe et al., 2010). [The Working Group noted that technical problems occurred with fixation and immunostaining for BrdU, so a second study was conducted to evaluate cell proliferation. The results were those of both studies together.]

Male Sprague-Dawley rats were treated intravenously via a tail vein with methylpalmitate, a Kupffer cell inhibitor, 24 hours before a single oral dose of PFOA (100 mg/kg) and were killed 24 hours later. PFOA significantly increased the BrdU labelling index in the liver, and pre-treatment with methylpalmitate reduced PFOA-induced labelling by 57% (Alsarra et al., 2006).

Thottassery et al. (1992) investigated the role of adrenal hormones in the hepatomegaly induced by PFOA. Male Sprague-Dawley rats underwent adrenalectomy and received a single dose of PFOA at 150 mg/kg by oral gavage. In intact rats and in rats that had undergone adrenalectomy, PFOA caused increases in ornithine decarboxylase activity and significantly reduced hepatic DNA levels (by 30%; $n = 4–10$; $P < 0.05$) (Thottassery et al., 1992). [The Working Group noted that the reduced hepatic DNA concentration was inversely proportional to the hepatomegaly observed. In rats that had undergone adrenalectomy, however, hepatomegaly was mostly caused by hyperplasia.]

Thirty-six adult Sprague-Dawley rats received ethane dimethyl sulfonate to eliminate Leydig cells, then were treated with PFOA at 0, 25 or 50 mg/kg per day by oral gavage for 9 consecutive days. The number of proliferating cell nuclear antigen (PCNA)-positive Leydig cells in testes sections was reduced after 21 days, indicating that PFOA exposure may reduce the proliferation of stem Leydig cells (Lu et al., 2019).

Male C57BL/6 mice were fed a low-fat control diet or a high-fat diet (HFD) for 16 weeks to model normal and steatotic livers, respectively. After 16 weeks on these diets, the mice were treated with PFOA (1 mg/kg bw per day) for 2, 8, or 16 weeks. PFOA induced hepatocyte hypertrophy, regardless of the diet, as indicated by histological examination. In rats consuming the low-fat control diet, PFOA induced an increase of 3.30-fold in hepatocyte DNA synthesis compared with vehicle-treated control diet-fed mice by week 2. HFD and PFOA had a synergistic effect on the BrdU labelling index by week 2. By week 8, HFD significantly increased DNA synthesis in hepatocytes compared with the vehicle-treated control diet-fed group. The control diet + PFOA and the HFD + PFOA groups also showed elevated DNA synthesis levels of 11.23-fold and 15.72-fold, respectively, compared with the vehicle-treated control diet-fed group (Li et al., 2019c). [The Working Group noted that, taken together, these results suggest that pre-existing NAFLD enhanced PFOA-stimulated hepatocyte hyperplasia only at the early time point studied. The Working Group also noted that this study shows that PFOA activates PPARα, CAR, and PXR and that PFOA reverses HFD-induced steatosis and reduces the size of adipose tissue depots.]

Gestational intrauterine exposure to PFOA at 5 mg/kg in female Kunming mice was used to characterize the potential effects of prenatal PFOA exposure on the cerebral cortex cells of offspring at PND21. PFOA-treated PND21 mice demonstrated increased levels of nerve growth factor (NGF) in serum and cortex cells. In addition, PFOA-exposed cerebral cortex cells showed higher NGF and PCNA expression, and exposure to PFOA and an NGF-specific inhibitor downregulated the expression (Qin et al., 2018). In contrast, in other studies in PFOA-exposed Kunming mice, increases in apoptotic markers were observed in the liver (Liu et al., 2015b) and uterus (Li et al., 2018c; Zhang et al., 2021b).

Adult male and female cynomolgus monkeys (*S. cynomolgus* or *M. fascicularis*) were treated with PFOS (potassium salt) at 0, 0.03, 0.15, or 0.75 mg/kg per day for 182 days and were monitored for 1 year after the treatment. Hepatocellular hypertrophy and lipid vacuolation were present

at term in the group at 0.75 mg/kg per day. The treatment had no significant effect on cell proliferation in the liver, pancreas, or testes after 182 days of treatment, as determined using the PCNA immunohistochemistry cell labelling index (Seacat et al., 2002).

Male and female Sprague-Dawley rats were exposed to PFOS (potassium salt) at a dietary concentration of 0.5, 2, 5, or 20 ppm for up to 104 weeks (Butenhoff et al., 2012b). A 20-ppm dose recovery group received 20 ppm PFOS in the diet for up to 53 weeks, after which it was fed control diet. The male rats at 20 ppm that were killed during week 53 had an increased incidence and severity of centrilobular hepatocytic hypertrophy and vacuolation, whereas the female rats had only centrilobular hypertrophy and the changes were less severe. These alterations were also observed in rats killed at the scheduled end of the study. There were no statistically significant increases in hepatocellular S-phase labelling index (cell proliferation index), as measured using BrdU immunohistochemistry, in any of the experimental groups.

Hepatocellular hypertrophy, increased cell proliferation, and reduced liver apoptotic index were observed in Sprague-Dawley rats fed PFOS (potassium salt; 20 or 100 ppm) for 28 days, and this was reported to be mediated by the nuclear receptors PPARα, CAR, and PXR (see also Section 4.8) (Elcombe et al., 2012c). A subsequent study showed that after a recovery period of 84 days after treatment, increased liver proliferative index, reduced liver apoptotic index, and a lower number of hepatocellular glycogen vacuoles were observed (Elcombe et al., 2012a).

Han et al. (2018b) treated male Sprague-Dawley rats daily by gavage with PFOS (1 or 10 mg/kg) for 28 days. PFOS exposure triggered Kupffer cell activation and significantly upregulated the expression of PCNA, c-Jun, c-MYC, and cyclin D1 in the liver (Han et al., 2018b). [The Working Group noted that this study showed inflammatory cell infiltration; see Section 4.2.6.]

Groups of 25 pregnant Sprague-Dawley rats were given daily oral doses of PFOS (potassium salt) at 0.1, 0.3, or 1.0 mg/kg per day from GD0 to PND20. The mean number of Ki-67^{+} thyroid follicular epithelial cells in female fetal thyroids from the 1.0 mg/kg per day group was higher by 2.1-fold than for the control group ($P < 0.05$) (Chang et al., 2009).

The effects of PFOS on GJIC in vivo were studied using Sprague-Dawley rats (n = 4–6) exposed to PFOS (5 mg/kg per day) orally for 3 days or 3 weeks. GJIC was significantly reduced in the livers of PFOS-treated rats, with an EC_{50} of 30 µM (15 mg/L) after 3 days of exposure, and the magnitude of inhibition was the same for the longer exposure period of 21 days (no statistical significance was shown) (Hu et al., 2002).

Male Sprague-Dawley rats were treated with PFOS (0, 5, or 10 mg/kg per day) by gavage for 7 days and injected intraperitoneally with ethane dimethyl sulfonate the next day to eliminate Leydig cells, so that the Leydig cell regeneration process could be investigated. PFOS pre-treatment significantly lowered the serum testosterone level and reduced the number of regenerated Leydig cells (Mao et al., 2021). [The Working Group noted that transverse sections of the testes immunohistochemically stained for CYP11A1 and 11β-HSD1 were used to analyse cell number. In addition, in a 3D seminiferous tubule culture system, PFOS inhibited stem Leydig cell proliferation and differentiation, as well as the hedgehog signalling pathway.]

Qu et al. (2016) showed alterations in testicular ER expression, together with reduced proliferation and increased apoptosis of germ cells, which might be involved in the PFOS-induced testicular toxicity. In male ICR mice, oral administration of PFOS (0–10 mg/kg bw) for 4 weeks caused reduced sperm count, testosterone level, and CRTC2/StAR expression, and damage to the testicular interstitium, paralleled by increases in phosphorylated PKA, CREB, and p38 in the testes (Qiu et al., 2021). PFOS impaired normal

placental angiogenesis in female CD-1 mice by disrupting the lncRNA Xist/miR-429/VEGF-A pathway, adversely affecting fetal development (Chen et al., 2018).

(ii) Non-human mammalian systems in vitro

The 3T3-L1 preadipocyte culture system has been used to test numerous compounds that influence adipocyte differentiation or function. Cells were treated with PFOA (5–100 μM) or PFOS (50–300 μM), the PPARα agonist WY-14 643, or the PPARγ agonist rosiglitazone. The cells were assessed morphometrically and biochemically for number, size, and lipid content. There was a significant concentration-related increase in cell number and reduced cell size after exposure to PFOA or PFOS (Watkins et al., 2015). However, Fischer rat thyroid line-5 (FRTL-5) cells exposed to 10^5 nM PFOA or PFOS showed significant inhibition of cell proliferation. In particular, the percentage of proliferating FRTL-5 cells was 14.2% of the total number of cells in the medium, and this was reduced to 7.5% by PFOA and to 3.9% by PFOS (Coperchini et al., 2015).

PND4 neonatal ovaries from CD-1 mice were cultured in control medium (dimethyl sulfoxide < 0.01% final concentration) or PFOA (50 μM or 100 μM). The results showed that exposure to PFOA at 50 μM for 96 hours increased the number of secondary follicles, the expression of Ki-67 and the protein and gene expression of *Ccna2*, *Ccnb2*, *Ccne1*, *Ccnd1*, *Ccnd2*, *and Ccnd3*. PFOA also induced the expression of the Hippo pathway components *Mst1/2*, *Lats1*, *Mob1b*, *Yap1*, *and Taz*, as well as the downstream Hippo pathway targets *Areg*, *Amotl2*, *and Cyr61*, although it reduced the expression of the anti-apoptotic gene *Birc5*. Inhibition of the Hippo pathway effector YAP1 with verteporfin resulted in the attenuation of PFOA-induced follicular growth and proliferation (Clark et al., 2022). [The Working Group noted that PFOA can disrupt the Hippo pathway, leading to changes in the cell cycle, increased cell growth, and enhanced follicle development.]

Rat liver epithelial cells were cultured with PFOA at 10 μM, 50 μM, or 100 μM for 38 weeks and compared with passage-matched control cells. PFOA-treated cells showed increases in MMP-9 secretion and cell migration, and they developed more and larger colonies in soft agar. Microarray data showed Myc pathway activation at 50 μM and 100 μM, associated with Myc upregulation and PFOA-induced morphological transformation. Western blotting confirmed that PFOA caused significant increases in c-MYC protein expression in a time- and concentration-related manner. The tumour invasion indicators MMP-2 and MMP-9, the cell-cycle regulator cyclin D1, and the oxidative stress protein GST were all significantly upregulated at 100 μM (Qu et al., 2023).

The role of Wnt/β-catenin signalling in PFOS-induced neurotoxicity has also been investigated. C17.2 neural stem cells (mouse-derived multipotent neural stem cells isolated from the cerebellum) were treated with PFOS at 12.5, 25, 50, 100, or 200 nM for ≤ 48 hours (Dong et al., 2016). The CCK-8 assay was used to count the cells and indicated that PFOS exposure impaired the proliferation of the cells in a dose-dependent and time-dependent (at 50 nM) manner. Furthermore, flow cytometry analyses of the cell-cycle distribution revealed a decrease in the number of cells in S phase. The protein levels of cyclin D1 and PCNA were significantly reduced after PFOS exposure for 12 hours, compared with the control group. Gene expression of *Myc* and *Cox2* and survival were significantly impaired in a dose- and time-dependent manner after PFOS exposure, suggesting the involvement of β-catenin signalling (Dong et al., 2016).

In ovine primary theca cells, PFOS at 50 ng/mL was not cytotoxic after 24 hours and had no effect on GJIC (Gingrich et al., 2021).

Synopsis

[The Working Group noted that the above studies reported some evidence that PFOA increased cell proliferation in human primary cells. Transcriptomic analyses in human primary cells suggested that PFOA modulated gene signalling pathways involved in cell proliferation. PFOA induced cell proliferation, migration, or invasion in human cell lines and cell proliferation or hyperplasia in multiple tissues in rodents, including in PPARα-null mice.

Similarly, PFOS seemed to increase cell proliferation, migration, or invasion in human cell lines and cell proliferation or hyperplasia in the liver of rats.]

4.2.11 Multiple key characteristics of carcinogens

See Tables 4.29 to 4.31.

Data from transcriptomic and metabolomic studies were assessed for quality with respect to study design parameters, quality control of the raw data, data processing criteria, and differential analysis and information content regarding the 10 KCs of carcinogens. The results of the studies were mapped/associated to the KCs. To establish the association, the Working Group mapped genes, metabolites, or enriched pathways to each KC, based on the known association of the transcript or metabolite with a biological process underlying the KC.

(a) Humans

(i) Exposed humans

Chang et al. (2022) used a meet-in-the-middle approach to investigate the interrelationships between serum PFOA and PFOS concentrations, maternal metabolomic perturbations, and fetal growth. The authors showed an association of maternal serum PFOA level with reduced fetal growth in a population of 313 African-American women. Changes in amino acid, lipid and fatty acid, and bile acid metabolism were associated with PFOA, and to a lesser extent PFOS, exposure. Uric acid was suggested to be a potential intermediate biomarker of the early response to PFOA exposure and to predict reduced fetal growth (see Table 4.30). This study was in good agreement with a previous cross-sectional study performed in the US adult population (NHANES 2009–2014; $n = 4917$), which showed associations of PFOA and PFOS exposure with serum uric acid and gout (Scinicariello et al., 2020).

Rhee et al. (2023) identified several metabolites that were significantly associated with both *n*-PFOA and *n*-PFOS in eight nested case–control serum metabolomic profiling studies as part of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. The strongest associations were observed for sphingolipids, fatty acid metabolites, and androgenic steroids. [The Working Group noted that sphingolipids, derivatives of the amino alcohol sphingosine, are biologically active components of cell membranes that play a significant role in intracellular signal transduction, regulate cellular processes (e.g. proliferation, maturation, and apoptosis), and are involved in cellular stress responses.] The associations of specific metabolites with *n*-PFOS remained significant after the model was adjusted for serum *n*-PFOA, but the *n*-PFOA–metabolite associations were substantially attenuated after adjustment for *n*-PFOS. [The Working Group noted that differences in the associations of PFOA or PFOS levels with metabolites in patients with cancer and controls were not discussed in this study.]

A metabolome-wide association study using non-targeted ultra-high-resolution mass spectrometry identified metabolites associated with serum PFOA and PFOS levels in 115 children aged 8 years (Kingsley et al., 2019). In this cross-sectional study, serum PFOA and PFOS concentrations correlated with lipid metabolism and with arginine, proline, aspartate, asparagine, and butanoate metabolism. In addition,

Table 4.29 Omics data relevant to multiple key characteristics of carcinogens in humans exposed to PFOA or PFOS

End-point	Location, setting, study design	Sample, tissue, or cell type No. of study participants	Methods	Type of exposure	Results	Covariates adjusted for	Potentially related KCs	Comments	Reference
MWAS with pathway enrichment analysis	Emory University African American Vaginal, Oral, and Gut Microbiome in Pregnancy Study, Atlanta, Georgia, USA Prospective birth cohort study	Serum 313 pregnant African-American women at 8–14 wk of gestation	Isotope dilution calibration Untargeted, high-resolution metabolomic profiling using hydrophilic interaction LC with positive ESI and reverse-phase (C18) chromatography with negative ESI Multiple linear regression models	General population PFOA, Q1: < LOD–0.45; Q4: 1.07–4.42 ng/mL PFOS, Q1: < LOD–1.44; Q4: 3.24–12.4 ng/mL	Significant association was found between serum PFOA (OR, 1.20; 95% CI, 0.94–1.49) and small-for-gestational age birth; and correlations with level 1 biomarkers (uric acid and ferulic acid) and level 2 biomarkers (unsaturated fatty acid C18:1, parent bile acid, and bile acid–glycine conjugate) PFOS correlated with 2-hexyl-3-phenyl-2-propenal and parental bile acid	Maternal age, education, BMI, parity, tobacco use, marijuana use, and infant sex	KC5; lipid metabolism		Chang et al. (2022)

Table 4.29 (continued)

End-point	Location, setting, study design	Sample, tissue, or cell type No. of study participants	Methods	Type of exposure	Results	Covariates adjusted for	Potentially related KCs	Comments	Reference
MWAS	One of 8 nested case–control studies conducted within the PLCO cohort, 1993–2001, collected at 10 screening centres across the USA Cross-sectional	Serum 3647 participants (1818 patients in the cancer study and 1829 controls)	UHPLC-MS/MS, multiple linear regression modelling and meta-analysis using DerSimonian and Laird random effects models were used to combine findings from the various studies. Bonferroni-corrected significance threshold applied	General population. PFOA: < 2.9–> 6.5 µg/L PFOA: < 19.1–> 47.12 µg/L	38 *n*-PFOA-associated metabolites, including 17 lipids, two nucleotides (uric acid, 3-methylcytidine), one carbohydrate (1,5-anhydrosorbitol), and one cofactor/vitamin 51 *n*-PFOS-associated metabolites, including 20 lipids (sphingolipids, fatty acid metabolites, and bile acid metabolites), five cofactors/vitamins, three amino acids, two nucleotides (guanosine, 3-methylcytidine), and one carbohydrate (D-glucose)	Estimated glomerular filtration rate, educational attainment, BMI, sex	KC4, KC10, lipid metabolism	Study used semi-targeted metabolomic analyses, measuring relative metabolite levels, which made direct comparison between populations difficult. Use of non-fasted serum samples may lead to short-term changes in metabolite levels and introduce bias. The majority of participants were postmenopausal women.	Rhee et al. (2023)
MWAS	Cincinnati, Ohio, USA HOME Study, a prospective pregnancy and birth cohort Cross-sectional	Serum 115 children aged 8 yr	Non-targeted, high-resolution metabolomic profiling using LC and Fourier transform high-resolution MS	General population PFOA, mean ± SD, 2.6 ± 1.0 ng/mL PFOS, 4.4 ± 3.2 ng/mL	Serum PFOA and PFOS concentrations correlated with TCA cycle, pyrimidine and purine metabolism, changes in de novo fatty acid biosynthesis	Age, sex, race/ethnicity	KC10, lipid metabolism	Results based on enriched pathways in network-based metabolome-wide correlation analysis.	Kingsley et al. (2019)

Table 4.29 (continued)

End-point	Location, setting, study design	Sample, tissue, or cell type No. of study participants	Methods	Type of exposure	Results	Covariates adjusted for	Potentially related KCs	Comments	Reference
MWAS	CHDS (1959–1967), Oakland, California, USA Cross-sectional	Maternal perinatal serum 397 participants	C18 chromatography coupled with quadruple orbitrap MS Metabolic features were annotated with xMSannotator using the Human Metabolome Database	General population PFOA median (25th–75th percentile), 0.4; 0.25–0.6 ng/mL PFOS, 33.9; 16.05–61 ng/mL	301 metabolites were significantly associated with serum PFOS concentration. Pathway enrichment analyses: glycine, threonine, alanine, and serine metabolism, and urea cycle/amino group metabolism, carnitine shuttle, lysine metabolism, and branched-chain amino acid metabolism	Total cholesterol (continuous variable), age (continuous variable), and *p,p′*-DDE level (continuous variable)	KC10	Study included 50 women whose daughters developed breast cancer, but the association with metabolomic changes was not discussed. Linear regression model used.	Hu et al. (2019)
Transcriptomics	C8 Health Project (2005–2006), Mid-Ohio Valley and Parkersburg, West Virginia, USA Cross-sectional	Serum and whole blood 290 participants	TaqMan Low-density array (quantitative RT-PCR), solid-phase extraction, followed by reverse-phase HPLC-MS/MS	General population PFOA GM (95% CI): 40.9 (33.7–49.5) ng/mL in men; 25.5 (20.7–31.2) ng/mL in women PFOS: 8.3 (7.3–9.45) ng/mL in men; 5.5 (20.7–31.2) ng/mL in women	In men, inverse associations of PFOA level were identified with *ABCG1*, *NPC1*, and *PPARA* transcripts; no associations with PFOS In women, an inverse association between the *NR1H2* (LXRB) transcript with PFOA and a positive association of PFOA with *NCEH1* expression were identified; *NCEH1* and *PPARA* expression were positively correlated with the level of PFOS	Age, sex, BMI, household family income, smoking status	KC8	Adjusted linear regression model.	Fletcher et al. (2013)

Table 4.29 (continued)

End-point	Location, setting, study design	Sample, tissue, or cell type No. of study participants	Methods	Type of exposure	Results	Covariates adjusted for	Potentially related KCs	Comments	Reference
Transcriptomics	Postmenopausal Norwegian women (age 48–62 years), part of Norwegian Women And Cancer Study Cross-sectional	Serum and whole blood RNA 270 healthy participants	HPLC-QTOF-MS and AB Human Genome Survey Microarray V2.0	General population PFOA median (range), 4.4 (0.79–0.21) ng/mL PFOS median range, 19 (5.7–84) ng/mL	Higher levels of PFOS were associated with the TCA cycle pathway ↓ *NNT*, *PDHB*, *SDHD*, *SDHC*, *SUCLA2*, *IDH3A*, *MDH1*, and *SUCLG2* expression ↑ *ACO2*	None	KC10	No covariates adjusted for	Rylander et al. (2011)

BMI, body mass index; CHDS, Child Health and Development Studies; CI, confidence interval; *p,p′*-DDE, dichlorodiphenyldichloroethylene; ESI, electrospray ionization; GM, geometric mean; HOME, Health Outcomes and Measures of the Environment; HPLC-MS/MS, high-performance liquid chromatography-tandem mass spectrometry; HPLC-QTOF-MS, high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry; KC, key characteristic of carcinogens; LC, liquid chromatography; LOD, limit of detection; MS, mass spectrometry; MWAS, metabolome-wide association study; OR, odds ratio; PFOA, perfluorooctanoic acid; *n*-PFOA, linear isomer of perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; Q, quartile; RT-PCR, reverse transcription-polymerase chain reaction; SD, standard deviation; TCA, tricarboxylic acid; UHPLC-MS/MS, ultra-high performance liquid chromatography-tandem mass spectrometry; USA, United States of America; wk, weeks(s); yr, year(s).

in reversed-phase chromatography with ESI operated in negative mode (C18-negative mode), tyrosine, galactose, vitamin A (retinol), and lysine metabolism pathways, de novo fatty acid biosynthesis, tricarboxylic acid (TCA) cycle, pyrimidine, and purine metabolism pathways were enriched for both PFOA and PFOS. Thus, this study indicated that the serum levels of PFOA and PFOS were associated with a common set of biological pathways related to energy production and catabolism (Kingsley et al., 2019).

Hu et al. (2019) investigated the metabolite profiles associated with the serum PFOS levels of 397 participants in the Child Health and Development Studies (CHDS) cohort. Using a linear regression model, the authors identified 301 metabolites that were significantly associated with the serum PFOS concentration. Pathway enrichment analysis showed that these metabolites were associated with glycine, threonine, alanine, and serine metabolism; the urea cycle/amino group metabolism; the carnitine shuttle; lysine metabolism, which generates carnitine; and branched-chain amino acid metabolism (i.e. valine, leucine, and isoleucine degradation). This cluster of pathways suggested a mechanistic link of PFOS with energy production and lipid regulation. Metabolites involved in the urea cycle and amino group metabolism, such as β-alanine, creatinine, pipecolate, lysine, arginine, creatine, and adrenochrome, were increased in the samples with high PFOS levels; and homocysteine and betaine negatively correlated with the PFOS concentration in serum. [The Working Group noted that there were associations between PFOA or PFOS levels and several common metabolites across multiple human studies, identified using a metabolomic approach.]

Guo et al. (2022b) summarized the application of non-targeted metabolomics in epidemiological studies that assessed metabolite and metabolic pathway alterations associated with exposure to PFOA (11 studies) and PFOS (10 studies) in a scoping review that included human studies involving children and adolescents (three studies), non-pregnant adults (five studies), and pregnant women (three studies). Alterations in tryptophan metabolism and the urea cycle were associated with PFOA or PFOS exposure in multiple studies. Lipid metabolites involved in glycerophospholipid metabolism, which is critical for biological membrane function, and fatty acids and carnitines, which are relevant to the energy supply pathway of fatty acid oxidation, were also associated with PFOA and PFOS exposure. Secondary significant metabolome changes associated with PFOA and PFOS exposure included the components of the TCA cycle, which is involved in energy generation, and purine and pyrimidine metabolism, which are cellular energy pathways. [The Working Group noted that there were commonalities in the associations of PFOA or PFOS level with non-targeted metabolomic alterations. Because of the cross-sectional nature of the studies, the authors were unable to rule out the temporality of the observed associations and minimize confounding bias and measurement errors. The studies had limitations in power, with sample sizes of < 1000 participants and relatively low PFOA and PFOS exposure levels.]

Fletcher et al. (2013) conducted transcriptional profiling of 13 genes in whole-blood samples from 290 out of 69 000 participants in the C8 Health Project that was carried out during 2005–2006 to examine the potential health effects of PFAS on residents of the Mid-Ohio Valley, USA, who lived in six contaminated water districts surrounding a chemical plant. The data showed inverse associations of serum PFOA concentration with the *NR1H2* (*LXRB*), *NPC1*, and *ABCG1* genes, which are involved in cholesterol transport; a positive association between serum PFOS level and the cholesterol mobilization-related *NCEH1* gene, and a negative association with the *NR1H3* gene, which is involved in cholesterol transport, were also identified. Moreover, the authors noted sex-specific differences in the expression of genes related to

cholesterol mobilization and transport and serum PFOA and PFOS levels. Inverse associations of PFOA level with *ABCG1*, *NPC1*, and *PPARA* transcripts was shown in male participants, but no associations of PFOS with any transcript. In women, the authors showed an inverse association of *NR1H2* (*LXRB*), but a positive association of *NCEH1* gene expression, with the PFOA level. The levels of the *NCEH1* and *PPARA* transcripts also positively correlated with the level of PFOS in women.

In another study, the applicability of peripheral blood transcriptomics for exploration of the effects of PFOA and PFOS exposure on 270 healthy postmenopausal Norwegian women (age 48–62 years) was investigated (Rylander et al., 2011). The authors identified two significantly dysregulated gene sets related to the TCA cycle in the "PFOS high" group (> 30 ng/mL; $n = 42$), compared with the "PFOS low" group (< 30 ng/mL; $n = 228$), but no significantly enriched genes in the tested sets were detected in the groups with different PFOA levels. Eight key genes (*NNT*, *PDHB*, *SDHD*, *SDHC*, *SUCLA2*, *IDH3A*, *MDH1*, and *SUCLG2*) were downregulated, and one, *ACO2*, was upregulated, in the group with higher level of PFOS. [The Working Group noted that the presence of PFOA and PFOS in the blood of all the participants may have reduced the chance of detecting differentially expressed single genes or metabolites.]

(ii) Human cells in vitro

See Table 4.30.

Buhrke et al. (2015) conducted a study to investigate the potential PFOA-mediated alterations in the transcriptome of human primary hepatocytes. PFOA exposure affected the PPARα pathway, influenced by substantial gene expression alterations, including upregulation of *PPARA*, *JUN*, and *FOS*, and downregulation of *ER1* and *HNF4a*, which is an important factor for liver development and embryogenesis. Activation of the PPARα network and the inhibition of copper-transporting ATPase 2 (ATP7B), sterol regulatory element-binding transcription factors 1 and 2 (SREBF 1 and SREBF 2), sterol regulatory element-binding protein cleavage-activating protein (SCAP), and insulin receptor (INSR) networks of differentially expressed genes was shown in 3D human primary hepatocyte spheroids treated with PFOA or PFOS for 14 days (Rowan-Carroll et al., 2021).

In addition, Rosen et al. (2013) treated human primary hepatocytes with 12 different perfluoroalkyl acids, including PFOA and PFOS, and showed that *CYP2B6*, *CYP3A4*, *PLIN2*, and *FABP1* were among the most upregulated genes.

Proteomic studies showed that the inhibition of GRP78, HSP27, CTSD, HNRNPC, HUWE1, UBQLN1, and hnRNPC, and the activation of PAF1, may be involved in the activation of p53, which triggered the apoptotic process in human hepatic L-02 cells treated with PFOA (Huang et al., 2013, 2014).

Li et al. (2023b) combined metabolomic and proteomic analyses to investigate the altered profiles in metabolite and protein levels in human primary hepatocytes exposed to PFOS at human exposure-relevant concentrations. The authors showed that an alteration in glycerophospholipid metabolism was the most significant lipid metabolism dysregulation induced by PFOS in hepatocytes and was associated with the intracellular transport process.

A transcriptomic analysis performed in HepaRG cells exposed to PFOS showed dose-dependent dysregulation of genes involved in a PPARα-regulated network, cholesterol biosynthesis, ATF4-activated genes in response to ERS, cytosolic tRNA aminoacylation, and amino acid transport across the plasma membrane (Louisse et al., 2023) (see also Section 4.2.8).

PFOA induced changes in the levels of lipid metabolites, arachidonic acid, myristic acid, and oleic acid in L-02 cells; precursors associated with nucleic acid synthesis (e.g. adenine and guanosine diphosphate) in DLD-1 cells; and

Table 4.30 Omics data relevant to multiple key characteristics of carcinogens in human cells in vitro exposed to PFOA or PFOS

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Transcriptomics	Primary human hepatocytes	1, 25, or 100 µM PFOA, 24 h	Human genome GeneChips HG-U133 plus 2.0 (Affymetrix) Statistically significant dysregulated genes were identified by one-way ANOVA ($P < 0.05$)	43 genes were significantly dysregulated (FC, > 2; $P < 0.05$) after 1, 25–109, or 100–215 µM PFOA exposure The PPARα-dependent signalling pathway was identified by IPA to be the most significantly dysregulated pathway Gene expression: ↑ *PPARA*, ↓ *ESR1*, ↓ *HNF4A*, ↑ *JUN*, and *FOS*	KC8, KC10		Buhrke et al. (2015)
Gene expression	3D human primary hepatocytes	0.02–100 µM PFOA or 0.02–100 µM PFOS, 14 days	RNA sequencing human TempO-Seq S1500 panel DESeq2 v1.30, FDR-adjusted $P < 0.05$ and FC > 1.5	PFOA and PFOS-induced changes in gene expression related to cholesterol biosynthesis and lipid metabolism, and PPARα activation network	KC8	Small number of genes analysed.	Rowan-Carroll et al. (2021)
Gene expression	Human primary hepatocytes	0–200 µM PFOA or 0–225 µM PFOS, 48 h	Custom 48-gene TaqMan low-density RT-PCR arrays; the $2^{-\Delta\Delta Ct}$ method was used; dose–response data were evaluated using SAS jmp	*CPT1A*, *ANGPTL4*, *PLIN2*, and *APOA2* were the most dose–dependently responsive genes to PFOA and PFOS exposure Upregulation of *CYP2B6*, *CYP3A4*, and *FABP1* after PFOA or PFOS exposure	Lipid metabolism	Small number of genes were analysed; analysis performed in context of other PFAA.	Rosen et al. (2013)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Proteomics	Human non-tumour hepatocytes (L-02)	0, 25, or 50 mg/L, 72 h PFOA	2D fluorescence DIGE coupled with ultrafleXtreme MALDI-TOF/TOF MS Results with $P < 0.05$, which is equal to a Mascot score > 33 were considered to be a positive identification	~1500 protein spots were detected in the DIGE gels at pH 4–7, and 28 protein spots were statistically significantly changed (1.5-fold increase or decrease, $P < 0.05$) 24 spots were downregulated and 4 were upregulated by PFOA The identified proteins were associated with cancer, cell death and survival, and cellular development networks; TP53 (p53), ERK1/2, and STAT3 were the key regulators of these networks	KC10	Small number of proteins detected.	Huang et al. (2013)
Proteomics	Human non-tumour hepatocytes (L-02)	0, 25, or 50 mg/L, 72 h PFOS	iTRAQ labelling and 2D nanoLC-MS/MS analysis. The acquired peak lists for all the MS/MS spectra were searched using the Mascot search engine (Matrix Science, 2023); IPA network analysis of proteins	~1300 proteins were identified and quantified at more than a 95% CI with an FDR < 0.99% 18 proteins were significantly differentially expressed (11 upregulated and 7 downregulated) in a dose-dependent manner by PFOS exposure Differentially expressed *HNRNPC*, *HUWE1*, *UBQLN1*, *RPL21*, and *PAF1* shown to be involved in p53 and c-myc networks, which are associated with DNA replication, recombination, and repair, RNA post-transcriptional modification, and the cell cycle	KC10	Small number of proteins detected.	Huang et al. (2014)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Metabolomics and proteomics	Primary human hepatocytes	10 µM PFOS, 48 h	UHPLC-MS/MS coupled with quadrupole-Orbitrap high-resolution MS for lipidomics and metabolomics; U3000nano RSLC nanoLC interfaced with a high-resolution MS system and XCalibur 4.3 software for proteomics; DEPs were defined using $\log_2$ FC, ≥ 1.0; $P < 0.05$	82 metabolites present at different concentrations were placed into 13 classes after PFOS treatment; these were involved in glycerophospholipid metabolism and phosphatidylethanolamine biosynthesis 55 proteins (27 upregulated and 28 downregulated) were significantly changed by PFOS treatment; intracellular transport, nuclear lumen, and ribonucleoprotein complex pathway were significantly upregulated; and amide transport and establishment of protein localization to organelle pathway were significantly downregulated after PFOS treatment	KC5, KC10		Li et al. (2023b)
Transcriptomics	HepaRG cells	6.25, 12.5, 25, 50, 100, 200, or 400 µM PFOS, 24 h	Whole-genome gene expression microarray and BMDExpress as the software tool; ANOVA was used, $P < 0.05$; Benjamini–Hochberg applied; FC filter, 1.0	18 Reactome gene sets were upregulated and 90 downregulated. 10 genes were selected that showed clear concentration–response curves for PFOS and were involved in diverse biological processes (*ATF4*, *SLC7A11*, *YARS1*, *PDK4*, *ANGPTL4*, *LSS*, *HMGCR*, *OAT5*, *THRSP*, and *CXCL10*	KC8, KC10		Louisse et al. (2023)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Metabolomics	A549 (lung), DLD-1 (intestine) and L-02 (liver) cells	0, 100, or 300 µM PFOA, 48 h	UHPLC with MS system and quadruple orbitrap MS with switching positive and negative mode electrospray ionization; Compound Discoverer 2.1 software and Optimal Scaling analysis using SPSS 20.0 software were used for metabolomic analysis; one-way ANOVA was conducted to compare the relative abundances of metabolites between PFOA-treated groups and the controls	Number of different metabolites (DM) L-02: 10 DLD-1: 12 A549: 67 Most DMs were changed in dose-dependent manner L-02: lipid metabolites: arachidonic acid, myristic acid, and oleic acid DLD-1: precursors associated with nucleic acid synthesis (e.g. adenine and GDP) A549: lipids, amino acids, and carbohydrates In A549 and L-02, PFOA induced the production of pro-inflammatory interleukins (IL-1β, IL-6, IL-8, and IL-13)	KC6, KC10	Small number of metabolites detected.	Zhang et al. (2021c)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Transcriptomics and lipidomics	Primary human lymphocytes	50 µM PFOS, 72 h	mRNA sequencing, edgeR was used to identify DEGs and log_2 FC, ≥ 1.0, $P < 0.05$ applied; HPLC-triple quadrupole time-of-flight MS and XCMS software were used for lipidomic analysis	530 DEGs (247 upregulated and 283 downregulated) were found in lymphocytes after PFOS treatment PFOS exposure dysregulated genes (*BHLHE41*, *DCSTAMP*, *FCRLA*, *MYO7B*, *NOTCH3*, *NTRK2*, *RARRES2*, *SDC2*, *SORT1*, *SPIB*, and *SPP1*) and lipids that play important roles in immune functions, such as lymphocyte differentiation, inflammatory response, and immune response PFOS induced changes in 96 metabolites, including 37 lipids associated with glycerophospholipid, sphingolipid, glycerolipid metabolism; adipocytokine signalling pathway; regulation of autophagy, and arachidonic acid metabolism	KC7, KC10	Single concentration tested.	Li et al. (2020c)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Transcriptomics and metabolomics	PrECs	10 nM PFOA or PFOS, 3–4 weeks	Single-cell RNA sequencing using 10X Cellranger pipeline and Seurat package (Satija Lab, 2023). Agilent GC/MS system was used for metabolite detection. AMDIS 2.71 (NIST, 2023) database and MetaboAnalyst software were used for metabolite analysis. Principal components analysis and uniform manifold approximation and projection were used for cluster visualization. GSEA was performed on normalized gene expression data using Molecular Signatures Database (Broad Institute, 2023).	UMAP plots revealed cell 5 clusters in the PFOS-, and 8 clusters in the PFOA-, exposed prostaspheres. Both chemicals induced changes in expression of the luminal keratin genes *KRT8*/*KRT18* The cells lacked stemness and showed basal keratin gene expression Significant enrichment of pathways involved in cell replication including, E2F targets, G2/M checkpoint, and mitotic spindle; increased TNFα via NFκB pathway and k-RAS signalling; IL-2, IL-6, and TGFβ inflammatory response; and metabolic pathways (glycolysis, oxidative phosphorylation) were shown in PFOA and PFOS-treated spheroids; the top enriched metabolites were involved in glycine and serine metabolism, with an enhancement of glucose metabolism through the Warburg effect; the top individual metabolites significantly induced by PFOA and PFOS exposure included glycerol, glutamic acid, citric acid, urea, serine, alanine, and glucose	KC8, KC9, KC10	Single concentration tested.	Hu et al. (2022)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Transcriptomics	Human Burkitt lymphoma cell line (Namalwa)	100 µM PFOA, 48 h	RNA sequencing, DEGs identified using generalized linear models that incorporated empirical Bayesian methods; canonical pathways identified using IPA	The four most significantly downregulated genes were *RAG1*, *RAG2*, *TCL1A*, and *TFRC*. PFOA affected two cellular processes related to immune function, B-cell development and primary immunodeficiency signalling RT-PCR confirmed that PFOA and PFOS caused a time-dependent reduction in *RAG1* and *RAG2*	KC7	Single concentration tested.	Janssen et al. (2022)
Metabolomics and proteomics	MLTC-1 Leydig cells	0.1, 1, or 10 µM PFOA, 48 h	Proteomic data acquisition using NanoLC-MS/MS and the MaxQuant software (Max Planck Institute of Biochemistry, 2023) were used for protein identification and quantification. UPLC system coupled to a quadruple orbitrap MS and SIMCA-P software (v14.0) were used for multivariate analysis of metabolites; MetaboAnalyst software and IPA software were used for metabolic and molecular network analysis	The expression levels of 67 proteins were significantly changed in PFOA-treated cells ($P < 0.05$; FC, ≥ 1.5 in 10 µM PFOA) involved in lipid and fatty acid metabolic processes, catabolic processes, and steroid hormone regulation Metabolic pathway analysis of 17 DMs showed that these metabolites were involved in lipid and fatty acid, amino acid, and carbohydrate metabolism and steroidogenesis IPA showed that ERK1/2, p38 MAPK, and cAMP were key regulators of 18 proteins and 7 metabolites related to steroid hormone regulation, and that fatty acid and lipid metabolism were affected by PFOA exposure	KC10		Huang et al. (2022b)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Transcriptomics and proteomics	Human breast cancer cells (SKBr3)	10 or 50 µM PFOA, 48 h	RNA sequencing LC-MS/MS analysis using Flex Binary UHPLC System connected to a Hybrid quadrupole–Orbitrap mass spectrometer; the FC thresholds (> 1.05 or < 0.95) and $P < 0.05$ were used to identify DEGs or DEPs	PFOA induced 1390 DEGs and 136 of the DEGs were associated with DEPs; 14 genes/proteins were associated with calcium metabolism cAMP signalling pathway was identified as a key network dysregulated by PFOA exposure; ADORA1 was suggested to be a target for PFOA binding that may have induced the Gi-cAMP-PKA pathway and reduced the concentration of cAMP Low concentrations of PFOA inhibited ADORA2A expression, whereas a high concentration (50 µM) induced its expression, causing opposite cellular effects	KC8, KC10	The suggestion that PFOA can bind to ADORA1 or ADORA2A receptors was not experimentally confirmed.	Li et al. (2022g)

ADORA1, adenosine A1 receptor; ANOVA, analysis of variance; cAMP, cyclic adenosine monophosphate; CI, confidence interval; DEGs, differentially expressed genes; DEPs, differentially expressed proteins; 2D DIGE, 2D fluorescence difference gel electrophoresis; 2D nanoLC-MS/MS, two dimensional nanoliquid chromatography-tandem mass spectrometry; DMs, differential metabolites; ESI, electrospray ionization; FC, fold change; FDR, false discovery rate; GC-MS, gas chromatography-mass spectrometry; GDP, guanosine diphosphate; GSEA, gene set enrichment analysis; IL, interleukin; IPA, Ingenuity Pathway Analysis; IQR, interquartile range; iTRAQ, isobaric tags for relative and absolute quantitation; KC, key characteristic of carcinogens; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOD, limit of detection; MALDI-TOF/TOF MS, matrix-assisted laser desorption-ionization-time of flight/time-of-flight mass spectrometry; MS, mass spectrometry; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PCA, principal components analysis; PFAA, perfluoroalkyl acids; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PKA, protein kinase A; PPAR, peroxisome proliferator-activated receptor; PrECs, primary normal human prostate epithelial cells; RT-PCR, reverse transcription-polymerase chain reaction; STAT, signal transducer and activator of transcription; UMAP, uniform manifold approximation and projection; UPLC, ultra-high performance liquid chromatography.

[a] ↑, increase; ↓, decrease.

lipids, amino acids, and carbohydrates in A549 cells. Regardless of the cell type, A549 or L-02, PFOA induced the production of pro-inflammatory IL-1β, IL-6, IL-8, and IL-13, as determined using a non-targeted metabolomic approach (Zhang et al., 2021c).

Li et al. (2020c), using a transcriptomic analysis in human primary lymphocytes, showed that PFOS exposure induced changes in the expression of genes and lipids that play important roles in immune function, such as in lymphocyte differentiation, the inflammatory response, and immune responses (see Sections 4.2.6 and 4.2.7).

To investigate carcinogenic mechanisms in the prostate after chronic exposure to PFOA and PFOS, Hu et al. (2022) conducted a study in primary normal human prostate epithelial cells in the form of a serial passage of prostasphere cultures that were treated with 10 nM PFOA or PFOS for 3–4 weeks. Exposure to either PFAS caused a significant increase in the total number of spheres, indicating augmentation of stem cell symmetric self-renewal. Transcriptome analysis using single-cell RNA sequencing showed that both chemicals induced changes in the expression of the luminal keratin genes KRT8/18, and the cells lacked stemness and basal keratin gene expression. Significant enrichment of pathways involved in cell replication, including E2F targets, G2M checkpoint and mitotic spindle, increased TNF-α via the NF-κBpathway, kRAS signalling, IL-2, IL-6, TGFβ, the inflammatory response, and metabolic pathways (glycolysis and oxidative phosphorylation), were shown in PFOA and PFOS-treated spheroids. Metabolomic gas chromatography-MS analysis of PFOA- and PFOS-exposed prostaspheres showed an enrichment in metabolites involved in glycine and serine metabolism, with enhancement of anaerobic glucose utilization through the Warburg effect. The top individual metabolites that were significantly induced by PFOA and PFOS exposure included glycerol, glutamic acid, citric acid, urea, serine, alanine, and glucose (Hu et al., 2022).

A transcriptomic-based approach was also used to investigate the carcinogenic potential of PFOA and PFOS in several other in vitro systems, including the bladder (Ye et al., 2022) and breast cancer (Li et al., 2022f), and their immunosuppressive properties in human B lymphoma cells (Janssen et al., 2022).

(b) Experimental systems

Non-human mammalian in vivo

See Table 4.31.

A recent study investigated the effects of PFOA on the hepatic transcriptome of PPARα$^{-/-}$ and wildtype mice fed an HFD and treated with PFOA at 0.05 mg/kg or 0.3 mg/kg bw per day for 20 weeks (Attema et al., 2022). In the wildtype mice, the largest effects were observed in the high-dose PFOA group, with a total of 788 genes being significantly changed. Although the overall effects of high-dose PFOA were substantially reduced in PPARα$^{-/-}$ mice, 294 genes were still significantly altered by high-dose PFOA in the absence of PPARα. Of the genes induced by high-dose PFOA in the wildtype mice, 88% were dependent on PPARα. This result was confirmed by gene set enrichment analysis. Significant positive enrichment was observed for pathways related to xenobiotic metabolism, steroid hormone biosynthesis, and omega-6 fatty acid metabolism in wildtype and PPARα$^{-/-}$ mice. Also, in PPARα$^{-/-}$ mice, many of the genes that were significantly upregulated were regulated by the rodent-specific PXR agonist pregnenolone 16a-carbonitrile, as well as by the CAR agonist 1,4-bis (2-(3,5-dichloropyridyloxy)) benzene, suggesting that they are PXR and CAR target genes.

Rosen et al. (2008a) compared the transcript profiles of the livers of wildtype and PPARα-null mice exposed to PFOA and concluded that the majority of the genes were dependent on PPARα. The independent genes were involved in lipid homeostasis and xenobiotic metabolism. The expression of many of the identified xenobiotic

Table 4.31 Omics data relevant to multiple key characteristics of carcinogens in non-human mammalian systems in vivo exposed to PFOA and PFOS

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Mouse, C57BL/6J, male (wildtype and PPARα$^{-/-}$)	Liver	PFOA in drinking-water, 0.05 or 0.3 mg/kg bw per day, 20 wk	RNA-Seq, Limma analysis with cut-off $P \leq 0.001$; FC, > 1.5	Effects of PFOA were mostly PPARα-dependent. PXR and CAR could be involved in the absence of PPARα.	KC8, lipid metabolism		Attema et al. (2022)
Transcriptomics	Mouse, wildtype and PPARα-null 129S1/SvlmJ, male	Liver	PFOA by gavage, 0, 1, or 3 mg/kg bw per day, 7 days	Applied Biosystems Mouse Genome Survey Microarrays, a two-way ANOVA across dose ($P \leq 0.03$); post-hoc *t*-test of the least-square means was used to evaluate individual treatment effects ($P \leq 0.0025$)	In PFOA-treated mice, the changes in transcripts related to fatty acid metabolism, inflammation, xenobiotic metabolism, and cell cycle regulation were PPARα-independent. Involvement of other PPAR isoforms in fatty acid metabolism and inflammation suggested.	KC6, KC8, KC10, and lipid metabolism	In PPARα-null mice, the number of DEGs was ~5 times as low as that in wildtype mice, which may have led to misinterpretation of the data.	Rosen et al. (2008a)
Proteomics	Mouse, BALB/c, male and female	Liver	PFOA by gavage, 0, 0.05, 2.5, or 5 mg/kg bw per day, 28 days	iTRAQ labelling, FC, ≥ 1.5	Dose-dependent proteomic changes: mitochondrial dysfunction, oxidoreductase activity, peroxisome proliferator activity, ion 236 binding, and transferase activity.	KC5, KC8, KC10	Livers of 10 mice pooled per group (not biological replicates).	Li et al. (2017b)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	ICR mice	Liver	PFOA by gavage, 0 or 10 mg/kg bw per day, 7 days	RNA sequencing, edgeR 3.0.8. $\log_2$ (FC) > 1 and $P < 0.05$	PFOA induced 2426 DEGs associated with fatty acid and lipid metabolism, oxidative stress, alterations of liver cell proliferation and apoptosis of hepatocytes, liver inflammation, necrosis, hepatic steatosis, and steatohepatitis.	KC5, KC6, KC8, KC10, and lipid metabolism	Single dose.	Li et al. (2022g)
Transcriptomics and lipidomics	Mouse, CD-1, female	Neonatal testes (PND1) and male offspring at PND63	PFOS by gavage, 0, 0.3, or 3 mg/kg bw per day, throughout gestation	RNA sequencing, edgeR package. $\log_2$ (FC) > 0.3 and FDR < 0.05; LC-MS/MS using Kinetex C18 column	56 (low dose) and 319 (high dose) DEGs were associated with lipid metabolism, oxidative stress, and cell junction signalling in testes. Levels of adrenic acid, docosahexaenoic acid, and eicosapentaenoic acid were reduced in testes at PND1 by PFOS treatment; and LOX-mediated 5-HETE and 15-HETE derived from arachidonic acid were increased. Male offspring at PND63 showed reductions in serum testosterone and epididymal sperm count.	KC5, KC8, KC10, lipid metabolism		Lai et al. (2017b)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Rat, Sprague-Dawley	Liver	PFOA by gavage, 0, 1, 3, 5, 10, or 15 mg/kg bw per day, 21 days	Affymetrix rat genome 230 2.0 GeneChip For upregulated genes change P-value < 0.0025; downregulated genes change P-value < 0.9975	> 500 genes significantly altered (P < 0.0025, FC > 2) after exposure to PFOA at any dose, with the largest number being at 10 mg/kg (813 genes) and 15 mg/kg (667 genes) PFOA. Dysregulated genes were associated with fatty acid synthesis and degradation, mitochondrial fatty acid β-oxidation (7 genes), apoptosis, cell communication and adhesion, growth and cell cycle, signal transduction and regulation of hormones.	KC10, KC6, KC5, lipid metabolism		Guruge et al. (2006)
Transcriptomics	Rat, Sprague-Dawley (Crl:CD(SD) IGS BR), male	Liver	PFOA or PFOS by gavage (10 mL/kg) for 1, 3, or 5 consecutive days The PFOA and PFOS groups received 20 and 10 mg/kg bw per day, respectively	Microarray, differentially expressed genes filtered at P < 0.05; pathway perturbations were visualized by DrugMatrix	PFOA and PFOS exhibited PPARα agonist-like effects on genes associated with fatty acid homeostasis. PFOA and PFOS exposure also resulted in the downregulation of cholesterol biosynthesis genes.	KC8, lipid metabolism		Martin et al. (2007)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Mouse, C57BL/6, male	Liver	PFOA HFD for 24 wk + 8 wk PFOA, gavage, 1 mg/kg bw per day	RNA sequencing, DESeq2; $\log_2$ (fFC) > 1; and a Benjamini–Hochberg-corrected $P < 0.1$	1233 and 835 DEGs were detected in PFOA-treated chow and HFD groups, respectively. 11 lipid metabolism related pathways were increased by PFOA and most were correlated with the “clearance” (oxidation, hydrolysis, catabolism) of lipids, causing significant downregulation of the hepatic steatosis pathway in HFD-fed mice. KEGG pathway analysis showed pathways including “PPAR signalling pathway,” “Fatty acid degradation,” “Biosynthesis of unsaturated fatty acids,” “Peroxisome,” and “Chemical carcinogenesis” were enriched by PFOA exposure, regardless of diet, leading to activation of the PPARα target genes *Cyp4a10*, *Lpl*, and *Cd36*, and the CAR target genes *Cyp2b10* and *Cyp3a11*, but inhibited Pparγ and Pparδ signalling.	KC8, KC10, lipid metabolism		Li et al. (2019c)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Mouse, athymic nude, male	Prostate tumour xenografts	PFOS and an HFD; gavage of 0 or 10 mg/kg bw per day, 40 days	RNA sequencing of RWPE-kRAS xenografts	Synergistic effect on xenograft growth of PFOS and HFD was observed. Genes involved in pyruvate metabolism, glycolysis pathways, PPARα network, and chromatin organization were significantly upregulated by PFOS in tumours from mice fed an HFD.	KC8, KC4	Single dose; histone modification analysis was not coherent.	Imir et al. (2021)
Transcriptomics	Mouse, CD-1, female	Fetal liver, lung	PFOA Gavage, 0, 1, 3, 5, or 10 mg/kg bw per day on GD1–GD17	Affymetrix mouse 430 2.0 expression GeneChips, two-way ANOVA across dose ($P \leq 0.05$); *t*-test of the least square means was used as a post-hoc test to evaluate individual treatment effects ($P \leq 0.0025$)	Clear dose–response effects in both the fetal liver and lung, with more extensive gene expression changes in liver. In the fetal liver, the DEGs were associated with lipid transport, ketogenesis, glucose metabolism, lipoprotein metabolism, cholesterol biosynthesis, steroid metabolism, bile acid biosynthesis, phospholipid metabolism, retinol metabolism, proteosome activation, and inflammation. These were associated with PPARα (except bile acid and glucose metabolism). Genes related to fatty acid catabolism were changed in both the fetal liver and lung	KC5, KC6, KC10, lipid metabolism		Rosen et al. (2007)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Mouse, CD-1 female	Fetal liver, lung	PFOS Gavage, 0, 5, or 10 mg/kg bw per day, GD1–GD17	Affymetrix mouse 430 2.0 expression GeneChips, two-way ANOVA across dose ($P \leq 0.05$); *t*-test of the least square means was used as a post-hoc test to evaluate individual treatment effects ($P \leq 0.0025$)	PFOS induced similar gene expression changes in liver and lung to PFOA (Rosen et al., 2007), which were primarily related to PPARα activation. In fetal lung: *Cyp4a14*, enoyl-coenzyme A hydratase (*Ehhadh*), and fatty acid binding protein 1 (*Fabp1*).	KC5, KC6, KC10, lipid metabolism		Rosen et al. (2009)
Transcriptomics	Mouse, CD-1	Fetal liver	PFOS Corn oil gavage, from mating to GD18.5, 0.3mg/kgbwper day (equivalent to human tolerable daily intake of 150 ng/kg bw per day)	RNA sequencing, edgeR 3.0.8. $\log_2$ (FC) > 1 and a Benjamini–Hochberg-corrected $P < 0.05$	PFOS activated the synthesis and metabolism of fatty acids and lipids, caused liver damage, and affected liver development in the fetus. Wnt/b-catenin, Rac, and TGF-b pathways activated.	KC8, KC10, lipid metabolism	1 pool (3 samples) per group.	Lai et al. (2017a)
Transcriptomics	Rat, Sprague-Dawley	Fetal liver	PFOS Gavage, 0 or 0.3 mg/kg bw per day, GD2–GD20	Affymetrix RAE 230A microarray, $P \leq 0.05$	225 upregulated and 220 downregulated genes. Peroxisomal proliferation pathway was dysregulated, but no change in *Ppara* gene. *Cyp7a1* reduced.	KC8, lipid and bile acid metabolism	Single dose.	Bjork et al. (2008)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Rat, Sprague-Dawley	Blood	PFOS Gavage, 0, 2.5, or 5 mg/kg bw per day, 28 days	RNA sequencing, DEGseq R package. $\log_2$ (FC) $> 1/ < -1$ and Q-value < 0.001	DEGs in blood of treated rats were associated with spliceosome, B-cell receptor signalling pathway, acute myeloid leukaemia, protein processing in the endoplasmic reticulum, NF-κB signalling pathway, and Fc gamma R-mediated phagocytosis.	KC6, KC8, KC10	No transcriptomic data for the liver or kidney.	Wang et al. (2023b)
Transcriptomics and metabolomics	Rat, Sprague-Dawley	Livers of rat mothers	PFOS Gavage, 0, 0.03, or 0.3 mg/kg bw per day, during pregnancy (GD1–GD18)	RNA sequencing, DESeq and Q-values < 0.05; UPLC/MS Progenesis QI software, OPLS-DA model, FC > 1.5 or ≤ 0.66, and VIP ≥ 1	DEGs were related to several metabolic pathways, such as PPAR signalling, ovarian steroid synthesis, arachidonic acid metabolism, insulin resistance, cholesterol metabolism, unsaturated fatty acid synthesis, and bile acid secretion. Untargeted metabolomics identified 164 and 158 DMs These were enriched with respect to α-linolenic acid metabolism, glycolysis/gluconeogenesis, glycerolipid metabolism, glucagon signalling pathway, and glycine, serine, and threonine metabolism.	KC8, lipid metabolism		Yu et al. (2023)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Mouse, BALB/c, male	Spleen	PFOA Gavage, 0, 0.4, 2, or 10 mg/kg bw per day, 28 days	RNA sequencing, weighted gene co-expression network analysis	7043 DEGs, with enrichment in cell cycle, autoimmunity, and anaemia in the spleen after PFOA.	KC6, KC10	Only one dose (10 mg/kg) was investigated.	Guo et al. (2021b)
Transcriptomics	Mouse, wildtype and PPARα-null 129S1/SvlmJ, male	Liver	PFOS Gavage, PFOS 0, 3, or 10 mg/kg bw per day, 7 days	Applied Biosystems Mouse Genome Survey Affymetrix 430 2.0 GeneChips, two-way ANOVA across doses ($P \leq 0.03$); *t*-test of the least square means was used as a post-hoc test to evaluate individual treatment effects ($P \leq 0.0025$)	The PPARα-dependent DEGs were associated with lipid metabolism, peroxisome biogenesis, proteasome activation, and inflammation; PPARα-independent DEGs were related to lipid metabolism and xenobiotic metabolism. Modest activation of CAR, and possibly PPARγ and/or PPARβ/δ was noted.	KC6, KC8, lipid metabolism		Rosen et al. (2010)

ANOVA, analysis of variance; CAR, constitutive androstane receptor; DEG, differentially expressed gene; DMs, differential metabolites; FC, fold change; FDR, false discovery rate; GD, gestational day; HETE, hydroxyeicosatetraenoic acid; iTRAQ, isobaric tags for relative and absolute quantitation; HFD, high-fat diet; KC, key characteristic of carcinogens; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOX, LOX, lipoxygenase; OPLS-DA, orthogonal partial least squares discriminant analysis; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PND, postnatal day; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; UPLC-MS, ultra-high performance liquid chromatography-mass spectrometry; VIP, variable importance in the projection; wk, week(s).

metabolism genes is known to be under the control of the nuclear receptor CAR and the transcription factor NRF2 (Rosen et al., 2008a).

Exposure of male and female Balb/c mice to PFOA at 0.05, 0.5, or 2.5 mg/kg per day for 28 days induced dose-dependent changes in proteins associated with mitochondrial dysfunction, oxidoreductase activity, peroxisome proliferator activity, ion binding, and transferase activity (Li et al., 2017a). In another study, ICR mice treated with PFOA for 7 days showed transcriptomic changes associated with the metabolism of many fatty acids and lipids, and particularly fatty acid β-oxidation, long-chain fatty acid transport, and the acyl-CoA metabolic process (Li et al., 2022g).

Lai et al. (2017b) conducted transcriptomic and targeted lipidomic analyses using neonatal testes in an effort to identify molecular targets and lipid markers associated with in utero PFOS exposure at doses of 0.3 or 3 μg/g bw, corresponding to the general population and occupational exposure levels, respectively. Analysis of male offspring at PND63 showed significant reductions in serum testosterone and epididymal sperm count. After PFOS exposure, the levels of adrenic acid and docosahexaenoic acid in the testes were significantly reduced by the low and high PFOS concentrations, respectively. Exposure to PFOS significantly induced the generation of 5-HETE and 15-HETE from arachidonic acid by LOX in the testes. Pathway analysis of the transcriptomic data highlighted that PFOS exposure induced changes in redox responses and oxidation–reduction processes in neonatal testes (Lai et al., 2017b).

Guruge et al. (2006) showed that male Sprague-Dawley rats exposed to PFOA at 1, 3, 5, 10, or 15 mg/kg bw per day for 21 days were characterized by a moderately dose-dependent number of significantly dysregulated genes. The upregulated genes are involved in the metabolism of lipids, cell communication, adhesion, growth, apoptosis, hormone regulatory pathways, proteolysis and peptidolysis, and signal transduction. The downregulated genes are related to the transport of lipids, inflammation, cell adhesion, apoptosis, the regulation of hormones, metabolism, and G-protein-coupled receptor protein signalling pathways.

Martin et al. (2007), using transcriptomic analysis, showed that PFOA and PFOS exposure resulted in the downregulation of cholesterol biosynthesis genes and alterations to thyroid hormone metabolism genes. These effects were associated with a decrease in serum cholesterol and serum thyroid hormone depletion, respectively, in the livers of male rats after 1, 3 and 5 days of exposure.

NAFLD could be considered to be a risk factor and potentiate the toxic carcinogenic effects of chemicals. Based on these considerations, Li et al. (2019c) investigated the hepatic effects of PFOA in mice in which NAFLD had been induced. PFOA activated xenobiotic nuclear receptors, inflammation, and cell proliferation in the livers of mice fed an HFD. Transcriptomic analysis showed that PFOA activated PPARα, CAR, and PXR in the livers of mice fed a control diet or an HFD, but reduced the severity of hepatic steatosis and hepatic triglyceride levels, enhanced lipid oxidation pathways, and attenuated HFD-induced hepatic fibrosis.

Imir et al. (2021) investigated the impact of metabolic alterations induced by an HFD combined with PFOS exposure on prostate tumour progression by analysing prostate RWPE–kRAS xenograft tumour growth in vivo. PFOS exposure of athymic nude male that were fed an HFD-induced RWPE–kRAS xenograft tumour growth and caused alterations in metabolites associated with glucose metabolism via the Warburg effect, the transfer of acetyl groups into mitochondria, and the TCA cycle, and in particular pyruvate and acetyl-CoA. Gene set enrichment analysis identified genes involved in pyruvate metabolism and glycolysis pathways to be significantly upregulated by PFOS exposure in tumours in mice fed an HFD. These data

indicate that metabolic alterations induced by HFD combined with PFOS exposure may play a significant role in prostate tumour growth and progression.

Rosen et al. (2007, 2009) conducted a transcriptomic microarray analysis of the lungs and livers of fetuses from pregnant CD-1 mice exposed to PFOA and PFOS to investigate the mechanism whereby they induce developmental toxicity. The expression of genes related to fatty acid catabolism was altered in both the fetal liver and lung. In the fetal liver, exposure to PFOA or PFOS caused significant alterations in the expression of genes associated with lipid transport, ketogenesis, glucose metabolism, lipoprotein metabolism, cholesterol biosynthesis, steroid metabolism, bile acid biosynthesis, phospholipid metabolism, oxidative phosphorylation, retinol metabolism, proteosome activation, and inflammation. Interestingly, PFOA and PFOS altered the expression of genes related to lipid metabolism, inflammation, and xenobiotic metabolism in both wildtype and PPARα-null CD-1 mice, which was consistent with modest activation of CAR, and possibly PPARγ and/or PPARβ/δ (Rosen et al., 2008b, 2010).

Similarly, prenatal PFOS exposure induced transcriptomic changes that may activate the synthesis and metabolism of fatty acids and lipids, leading to liver damage and interference with liver development in the fetuses of CD-1 mice (Lai et al., 2017a) and Sprague-Dawley rats (Bjork et al., 2008).

A transcriptomic analysis of blood samples from rats exposed to PFOS identified differentially expressed genes that were associated with the spliceosome, the B-cell receptor signalling pathway, acute myeloid leukaemia, protein processing in the ER pathway, NF-κB signalling pathway, and Fc gamma R-mediated phagocytosis (Wang et al., 2023b).

Using transcriptome sequencing combined with non-targeted metabolomic assays, Yu et al. (2023) identified differentially expressed genes in the livers of Sprague-Dawley rats given PFOS at 0.03 or 0.3 mg/kg bw per day that were related to several metabolic pathways, such as PPAR signalling, ovarian steroid synthesis, arachidonic acid metabolism, insulin resistance, cholesterol metabolism, unsaturated fatty acid synthesis, and bile acid secretion. Non-targeted metabolomics identified 164 and 158 metabolites present at different concentrations in 0.03 and 0.3 mg/kg bw per day exposure groups, respectively, which could be associated with α-linolenic acid metabolism, glycolysis/gluconeogenesis, glycerolipid metabolism, glucagon signalling pathway, and glycine, serine, and threonine metabolism.

Yu et al. (2016) conducted a high-throughput targeted metabolomic study of 278 metabolites to investigate the effects of PFOA exposure for 28 days on the brains and livers of male Balb/c mice. This study aimed to link the metabolic profiles of the livers and brains of mice exposed to PFOA with alterations in the transcriptome and proteome, and PFOA-induced hepatomegaly and neurobehavioural effects. PFOA treatment induced metabolic changes in the brain and liver that were associated with the metabolism of amino acids, lipids, and carbohydrates. The energy and lipid metabolism pathways were more susceptible to PFOA exposure. Lipidomic data in mice exposed to PFOA suggested that the β-oxidation and biosynthesis of fatty acids and inflammation are involved in PFOA-induced hepatomegaly. An iTRAQ labelling quantitative proteomic technology was used for the global characterization of the liver proteome in mice exposed to PFOS at 1.0, 2.5, or 5.0 mg/kg bw for 24 hours (Tan et al., 2012). Seventy-one of 1038 unique detectable proteins were significantly dysregulated in the mouse livers after PFOS exposure, and these were involved in lipid metabolism, transport, biosynthetic processes, and the response to a stimulus. Long-chain acyl-CoA synthetase, acyl-CoA oxidase 1, bifunctional enzyme, 3-ketoacyl-CoA thiolase A, CYPs, and GSTs were identified as key enzymes that regulate

peroxisomal β-oxidation and the metabolism of xenobiotic compounds that were affected by PFOS exposure.

In the study by Guo et al. (2021b), male mice were fed diet containing PFOA at a dose of 0, 0.4, 2, or 10 mg/kg per day for 28 days to investigate the splenic atrophy induced by PFOA. The authors demonstrated that mice exposed to PFOA reduced spleen weight and relative spleen weight and lower iron levels in the spleen and serum. Weighted gene co-expression network analysis of 7043 genes showed enrichment in those involved in cell cycle, autoimmunity, and anaemia in the spleen of PFOA-treated mice. PFOA exposure resulted in an increase in the ratio of the total number of macrophages to M1 macrophages in the spleen, the phagocytic ability of macrophages, and the levels of cytokines such as TNF-α, IL-1β, and IL-6. These findings suggest that overactivation of macrophages may play an important role in the splenic atrophy induced by PFOA exposure.

[The Working Group noted that these transcriptomic alterations provide an insight into PFOA and PFOS exposure in relation to the KCs of carcinogens. In addition, the Working Group also noted that transcriptomic, metabolomic, and lipidomic data collected after PFOA or PFOS exposure provide information associated with KCs, such as inflammation, cell proliferation, stress responses, and lipid metabolism.]

Synopsis

[The Working Group noted that the above studies showed some evidence that human exposure to PFOA and PFOS alters pathways related to nutrient and energy supply. Metabolomic analyses in exposed humans have suggested that PFOA and PFOS increase the activities of glycolytic pathways. Transcriptomic analyses in human primary cells have shown alterations in cell proliferation pathways, and transcriptomic data in experimental systems have shown alterations in lipid metabolism pathways.]

4.3 Evaluation of high-throughput in vitro screening data

See Tables 4.32 to 4.35.

An analysis of the in vitro bioactivities of PFOA and PFOS was informed by data from high-throughput screening assays generated by the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). PFOA, its ammonium salt APFO, PFOS, and its salt potassium perfluorooctanesulfonate, were among the thousands of chemicals tested in the large assay battery of the Tox21 and ToxCast research programmes of the US EPA and the US NIH. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2021). A supplementary table (Annex 6, Supplementary material for Section 4.3, Evaluation of high-throughput in vitro toxicity screening data, online only, available from: https://publications.iarc.who.int/636) provides a summary of the findings, including the assay names, the corresponding KCs, the resulting "hit calls", both positive and negative, and any reported caution flags for PFOA. The results were generated with the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) (Reisfeld et al., 2022; available from: https://gitlab.com/i1650/kc-hits); the evaluations discussed in the present monograph were performed using the US EPA ToxCast and Tox21 assay data and the curated mapping of KCs to assays available at the time. The findings and interpretation of these high-throughput assays for PFOA and PFOS are discussed below. [The Working Group noted that for PFOA, its ammonium salt, PFOS, and its potassium salt, the chemical purity quality control rating was "Unknown/Inconclusive", so the results should be interpreted with caution.]

Table 4.32 Number of assays available for the evaluation of high-throughput in vitro screening data for PFOA

		PFOA	
		Active (no flag)	Active with flag, or inactive
APFO	Active (no flag)	13	1
	Active with flag or inactive	2	270

APFO, ammonium perfluorooctanoate; PFOA, perfluorooctanoic acid.

4.3.1 PFOA

After mapping against the KCs of carcinogens, the ToxCast/Tox21 database contained 289 assay end-points in which PFOA was tested, and 288 assay end-points in which APFO was tested (US EPA, 2023a). Of these, PFOA and APFO were found to be active and without caution flags in 15 and 14 assay end-points, respectively, relevant to the KCs of carcinogens, with 13 assay end-points active without flags for either (see Tables 4.32 and 4.33).

PFOA and APFO were active in two assay end-points mapped to KC5, “induces oxidative stress”. Both of these assays were performed in the HepG2 cell line, and both were related to transcription factor activity mapping to endogenous human NRF2, which regulates antioxidant response elements.

In addition, PFOA and APFO were both active in nine assay end-points mapped to KC8, “modulates receptor-mediated effects”, with a tenth assay end-point for which PFOA was active with < 50% efficacy, and APFO was active without flags. Three assay end-points were for ER, one for antagonist activity in a human embryonic kidney cell line (HEK293T), and two for inducible changes in transcription in a human liver cancer cell line (HepG2). One assay using a human liver cancer cell line (HepaRG) was active for CYP2B6 induction, which is considered to be a marker for PXR-mediated metabolism. The remaining assays indicated activity for PPARα and PPARγ, PPRE, and PXR response element, all of which are indicative of nuclear receptor activation.

Finally, PFOA and APFO were both active in three assays mapped to KC10, “alters cell proliferation, cell death, or nutrient supply”, with a fourth assay end-point for which APFO was active with less than 50% efficacy, and PFOA perfluorooctanoate was active without flags in two of them. Two assay end-points measured cell viability and one measured reduced mitochondrial membrane potential, all in HepG2 cells. The fourth assay end-point was only active without flags for the effect of PFOA on cell viability in a human cervical cell line (ME-180).

4.3.2 PFOS

After mapping against the KCs of carcinogens, the ToxCast/Tox21 database contained 292 assay end-points in which PFOS was tested and 289 assay end-points in which potassium perfluorooctanesulfonate was tested (US EPA, 2023b). Of these, PFOS and potassium perfluorooctanesulfonate were found to be active and without caution flags in 32 and 31 assay end-points, respectively, relevant to the KCs of carcinogens, with 24 assay end-points active without flags for either (see Tables 4.34 and 4.35), the results of which are summarized below.

PFOS and potassium perfluorooctanesulfonate were active in two assay end-points mapped to KC2, “is genotoxic”, with a third assay end-point being active with flags for PFOS and without flags for potassium perfluorooctanesulfonate. The first two of these were in HepG2 cell lines and related to p53 activation, but at different time points (24 and 72 hours, respectively),

Table 4.33 High-throughput in vitro screening data for PFOA

Assay ID	Assay name	PFOA		APFO	
		AC_{50} (µM)	Flags	AC_{50} (µM)	Flags
KC5: Induces oxidative stress					
1110	TOX21_ARE_BLA_agonist_ratio	29.06		43.91	
97	ATG_NRF2_ARE_CIS_up	47.89		116	
KC8: Modulates receptor-mediated effects					
786	TOX21_ERa_BLA_Antagonist_ratio	51.93		46.87	
117	ATG_ERa_TRANS_up	7.561		44.7	
75	ATG_ERE_CIS_up	10.36		162.6	
132	ATG_PPARa_TRANS_up	21.83		14.23	
134	ATG_PPARg_TRANS_up	124.1		114.7	
719	NVS_NR_hPPARg	23.35	[a]	26.36	
969	LTEA_HepaRG_CYP2B6_up	23.76		5.641	
142	ATG_RXRb_TRANS_up	65.13		37.67	
103	ATG_PXRE_CIS_up	35.28		40.99	
102	ATG_PPRE_CIS_up	116.2		86.49	
KC10: Alters cell proliferation, cell death, or nutrient supply					
2066	TOX21_HRE_BLA_Agonist_viability	23.43		48.35	[b]
64	ATG_AP_1_CIS_up	139.3		124.7	
51	APR_HepG2_MitoMembPot_72h_dn	116.1		111.2	
45	APR_HepG2_CellLoss_72h_dn	123.2		114.2	[c]

AC_{50}, concentration that elicits a half-maximal response; APFO, ammonium perfluorooctanoate; ID, identifier; KC, key characteristic of carcinogens; PFOA, perfluorooctanoic acid.

[a] Less than 50% efficacy.

[b] Only the highest concentration above baseline, active, < 50% efficacy.

[c] Unspecified flag.

and the third assay was related to DNA repair in a chicken lymphoblast cell line (DT40). Additionally, activity for PFOS was reported for one active and unflagged assay end-point for KC4, “induces epigenetic alterations”. This was also performed in HepG2 cells and was related to transcription factor activity of the cis-acting elements in the reporter *Pax* genes. The same assay end-points were reported to be active, but only for one concentration above baseline for potassium perfluorooctanesulfonate.

PFOS was active in two assays mapped to KC5, “induces oxidative stress”. Both of these assays were performed in HepG2 cells. One indicated transcriptional activation of the NRF2 promoter, which regulates antioxidant response elements, and this was also active for potassium perfluorooctanesulfonate. The other indicated transcriptional activation that induces metallothioneins, and was reported to be active, but only for one concentration above baseline for potassium perfluorooctanesulfonate. Potassium perfluorooctanesulfonate was also reported to be active for a third assay end-point indicating increased stress kinases in HepG2 cell line, in which PFOS was not tested.

In addition, PFOS was active without flags in 10 assays mapped to KC8, “modulates receptor-mediated effects”, seven of which were also active, without flags, for potassium perfluorooctanesulfonate. Three assays for PFOS were active for ER/PR, including progesterone antagonism in a kidney cell line (HEK293T) and two for transcriptional activation in a human liver cancer cell

Table 4.34 Number of assays available for the evaluation of high-throughput in vitro screening data for PFOS data

		PFOS	
		Active (no flag)	Active with flag or inactive
KPFOS	Active (no flag)	24	7
	Active with flag or inactive	8	249

KPFOS, potassium perfluorooctanesulfonate; PFOS, perfluorooctanesulfonic acid.

Table 4.35 High-throughput in vitro screening data for PFOS

Assay ID	Assay name	PFOS		KPFOS	
		AC_{50} (µM)	Flags	AC_{50} (µM)	Flags
KC2: Is genotoxic					
2131	TOX21_DT40_657	67.02	[a]	65.28	
60	APR_HepG2_p53Act_72h_up	5.435		111	
40	APR_HepG2_p53Act_24h_up	109.3		123.6	
KC4: Induces epigenetic alterations					
100	ATG_Pax6_CIS_up	76.27		231.1	[b]
KC5: Induces oxidative stress					
97	ATG_NRF2_ARE_CIS_up	10.22		30.7	
91	ATG_MRE_CIS_up	41.98		165.9	[b]
62	APR_HepG2_StressKinase_72h_up	NA		112	
KC8: Modulates receptor-mediated effects					
804	TOX21_TR_LUC_GH3_Antagonist	86.54	[a]	65.05	
2127	TOX21_PR_BLA_Antagonist_ratio	35.5		63.53	[a, c]
75	ATG_ERE_CIS_up	150.3		32.63	
117	ATG_ERa_TRANS_up	23.05		35.92	[b]
132	ATG_PPARa_TRANS_up	58.85		88.24	
719	NVS_NR_hPPARg	20.9		20.28	
963	LTEA_HepaRG_CYP1A1_up	21.51		NA	
102	ATG_PPRE_CIS_up	179.7		179.5	
135	ATG_PXR_TRANS_up	18.01		14.01	
103	ATG_PXRE_CIS_up	11.32		30.38	
134	ATG_PPARg_TRANS_up	167		73.43	
KC10: Alters cell proliferation, cell death, or nutrient supply					
2066	TOX21_HRE_BLA_Agonist_viability	41.58		26.33	
1195	TOX21_PPARd_BLA_Agonist_viability	57.77		32.44	
1121	TOX21_FXR_BLA_antagonist_viability	31.45		31.86	
2120	TOX21_ERb_BLA_Antagonist_viability	30.28		42.46	
2116	TOX21_ERb_BLA_Agonist_viability	25.45		42.18	
1188	TOX21_FXR_BLA_agonist_viability	30.13		29.07	[d]
2128	TOX21_PR_BLA_Antagonist_viability	32.85		55.94	
2082	TOX21_RT_HEK293_FLO_40hr_viability	27.66	[c]	29.09	
2080	TOX21_RT_HEK293_FLO_32hr_viability	27.93	[c]	28.81	
2078	TOX21_RT_HEK293_FLO_24hr_viability	28.14	[c]	29.16	

Table 4.35 (continued)

Assay ID	Assay name	PFOS		KPFOS	
		AC_{50} (µM)	Flags	AC_{50} (µM)	Flags
2077	TOX21_RT_HEK293_FLO_16hr_viability	29.81	c, d	23.96	
2124	TOX21_PR_BLA_Agonist_viability	27.65		40.5	
64	ATG_AP_1_CIS_up	18.83		23.71	
55	APR_HepG2_NuclearSize_72h_dn	8.097		105.1	
54	APR_HepG2_MitoticArrest_72h_up	113.8		107.5	
49	APR_HepG2_MitoMass_72h_dn	104.3		112.9	
45	APR_HepG2_CellLoss_72h_dn	110.6		110.9	
1326	TOX21_p53_BLA_p4_viability	81.43		45.36	
29	APR_HepG2_MitoMass_24h_dn	114.2		120.2	
251	BSK_hDFCGF_Proliferation_down	10		60	b, f
112	ATG_TGFb_CIS_up	107.6		101.5	

AC_{50}, concentration that elicits a half-maximal response; ID, identifier KC, key characteristic; KPFOS, potassium perfluorooctanesulfonate; NA, not tested or not available; PFOS, perfluorooctanesulfonic acid.

[a] Only the highest concentration above baseline, active.

[b] Only one concentration above baseline, active.

[c] Efficacy, < 50%.

[d] Borderline active.

[e] Multiple points above baseline, inactive.

[f] Hit-call potentially confounded by overfitting.

line (HepG2), although for potassium perfluorooctanesulfonate two of these had activity flags. Multiple assays were active for other nuclear receptors, including transcriptional activation of PPARα in HepG2 cells, PPARγ in HepG2 cells and a biochemical (cell-free, using extracted gene-proteins) assay, CYP1A1 induction in HepaRG cells, and the transcriptional activation of PXR in HepG2 cells. Additionally, thyroid hormone modulation was indicated in one assay end-point performed in a rat pituitary gland cell line (GH3) for potassium perfluorooctanesulfonate, but only at the highest concentration of PFOS.

Finally, PFOS and potassium perfluorooctanesulfonate were active without flags in 17 and 18 assay end-points, respectively, mapped to KC10, "alters cell proliferation, cell death, or nutrient supply", 14 of which overlapped. Many of these assay end-points measured cell viability in ME-180, HEK293T, HepG2, and HCT116 cells. Reduced proliferation, which is also indicative of a loss of viability under pro-inflammatory conditions, was detected in one assay for PFOS using human foreskin fibroblasts, with potassium perfluorooctanesulfonate being active at only one concentration above baseline. Reduced nuclear size 72 hours after treatment, reduced mitochondrial mass after 24 and 72 hours, and increased mitotic arrest after 72 hours were also detected in HepG2 cells. Increased transactivation of TGFβ in HepG2 cells was also detected in one assay.

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5. SUMMARY OF DATA REPORTED

5.1 Exposure characterization

Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are per- and polyfluoroalkyl substances (PFAS) with a carbon chain length of eight carbons. The carbon–fluorine bond is one of the strongest bonds known in nature, making PFOA and PFOS extremely resistant to degradation in the natural environment. Both PFOA and PFOS exist as linear and branched isomers, and their salts exhibit different physicochemical properties to those of the pure acid form. PFOA and PFOS and their respective salts will be in an acid–base equilibrium in aqueous solutions such as in the human body and are present mainly as their conjugate bases perfluorooctanoate and perfluorooctane sulfonate, respectively. All isomeric forms and their salts should be considered as included within the definition of the agents reviewed in the present monograph.

The production of PFOA and PFOS began in the 1940s and steadily increased until the late 1990s, and companies located in the USA, Europe, and Japan were responsible for most of the manufacturing. However, in the early 2000s, there was a geographical shift in the production of PFOA and PFOS to other parts of the world (primarily in emerging Asian economies) and a shift towards production of other PFAS.

PFOA and PFOS have unique properties (e.g. hydrophobicity and oleophobicity, surface-active properties, chemical stability, and thermal resistance). They may be present in products as main ingredients, or as unreacted raw materials, undesired reaction by-products, or cross-contaminants along the production and supply chains. Ammonium perfluorooctanoate (APFO) – a salt of PFOA – has been used extensively to manufacture fluoropolymers, such as polytetrafluoroethylene (PTFE). Applications for fluoropolymers, as well as direct uses for PFOA, include household products with non-stick coatings; textiles for outdoor or personal protection applications; personal care products; seals and gaskets; coatings for cables and wires; electronics, solar panels and electrolyte fuel cells; carpets; cleaning and impregnating agents; construction materials; and surface coatings for conferring stain, oil and water resistance on carpets, textiles, leather products, and paper or cardboard for food and feed packaging.

With some applications that overlap those of PFOA, such as waxes, carpets, and food and feed packaging, PFOS has additionally been used in the semiconductor industry; as a hydraulic fluid additive; as an etchant and antireflective coating in photolithography processes; in the fabrication of imaging devices; as a mist suppressant in electroplating operations; in building and construction materials, including paints and varnishes; in

insulation; in dyes and ink; and in wetting, levelling, and dispersing agents. PFOS has been used extensively in class B firefighting foams known as aqueous film-forming foams (AFFFs).

PFOA and PFOS occur in the whole ecosystem, including air, water, dust, soil, and food, but levels vary greatly in different geographical regions due to pollution sources such as industrial sites, firefighter-training areas, waste deposits, and contaminated wastewater. The transport of PFOA and PFOS in air and surface water leads to their deposition in oceans, soil, and groundwater.

Foods are contaminated with PFOA and PFOS through atmospheric deposition and uptake from water and soil, including from use of biosolids as fertilizer. Animal-based foods are contaminated through water, feed, soil, and air. The highest concentrations have been measured in fish, seafood, and eggs.

Occupationally exposed populations have some of the highest exposure to PFOA and PFOS, with the leading route of exposure consisting of inhalation, as well as potentially dermal absorption and ingestion of dust. Biomonitoring data indicate exposure in diverse occupational settings, with the highest levels in primary manufacturing (up to median values of thousands of nanograms per millilitre of serum) and lower levels in secondary manufacturing, public safety, and services. Not all occupations have been characterized for PFOA and PFOS exposure. Measures in the work environment such as air frequently indicate that concentrations of PFOA and PFOS are higher in facilities manufacturing or using PFAS-laden products than in other occupational environments.

PFOA and PFOS are detected in blood samples in all populations worldwide who have been tested. The general population in non-polluted communities is mainly exposed to PFOA and PFOS via the diet and drinking-water. Additional exposure via consumer products and building materials may occur. In communities located in the proximity of polluted sites, the general population is mainly exposed via drinking-water. Biomonitoring in general populations mainly in North America and Europe has shown serum concentrations in the low nanograms per millilitre range and that concentrations have decreased since the early 2000s. Median concentrations in serum samples collected in contaminated communities have been measured in the hundreds of nanograms per millilitre range.

The term “precursor compounds” refers to PFAS known to break down or transform into PFOA or PFOS in the environment or biota, including in humans. Although estimates vary according to exposure scenario, it has been estimated that a substantial proportion of the body burden of PFOA and PFOS may originate from exposure to precursors. Direct exposure to PFOA and PFOS may decline as a result of regulation or voluntary efforts; however, production and use of precursors may contribute to ongoing exposure.

International, national, and regional authorities have developed occupational exposure thresholds for PFOA, PFOS, and/or related compounds, restrictions on the use of PFOA and PFOS in consumer products, and regulatory standards or guidance values for these PFAS in environmental media. PFOA and its salts and PFOS and derivatives are listed in Annex A (elimination) and Annex B (restriction), respectively, in the Stockholm Convention on Persistent Organic Pollutants. Drinking-water is a major focus for the regulation of PFOA and PFOS. Additional restrictions, regulations, and guidance values continue to be developed and have generally become more stringent over time.

5.2 Cancer in humans

More than three dozen studies were available for the evaluation of the carcinogenicity of PFOA and PFOS in humans; this represents a substantial increase over the number available during the previous evaluation of PFOA in Volume

110 of the *IARC Monographs* (Some Chemicals used as Solvents and in Polymer Manufacture). Most of these were cohort studies (including nested case–control and case–cohort studies), but there were also some population-based or hospital-based case–control studies. The studies were conducted within three different types of populations: (i) workers exposed to high levels of PFOA and/or PFOS during employment at industrial plants manufacturing or using these chemicals; (ii) general populations of residents exposed to high environmental levels of PFOA and/or PFOS, primarily through drinking-water near sites contaminated by chemical production or use; and (iii) populations exposed to background levels of these compounds primarily through food and drinking-water. The studies were conducted mainly in the USA and Europe, although several studies were carried out in China. Exceptionally, the Working Group performed an ecological analysis of the association between average serum concentrations of PFOA and the rates of orchiectomies for a set of 21 municipalities in the Veneto region of northern Italy, where drinking-water had been heavily contaminated by pollution from a local chemical plant, described below. Orchiectomies were found to be a highly reliable surrogate for testicular cancer in this region.

Despite the overall large number of available studies, for most cancer types there were fewer than 10 studies that examined risk for the type. The most informative studies for the evaluation were large cohort and nested case–control studies from all three exposure scenarios described above. There were three occupational cohorts from the USA: at a PFOA-manufacturing plant in Minnesota, a fluoropolymer-manufacturing plant (using PFOA) in West Virginia, and a fluorochemical plant in Alabama where PFOS was extensively used. There was an additional small occupational cohort of workers in another fluorochemical plant (with mainly PFOA exposure) in Veneto, Italy. This plant was the source of the contamination in that area. The most informative occupational cohort was from the facility in West Virginia. The Mid-Ohio Valley (West Virginia and Ohio) general population (part of the C8 Science Panel Cohort and exposed to high background levels of PFOA), a prospective cohort based on the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial participants, and other prospective cohorts of populations exposed to background levels of PFOA or PFOS exposure were also considered highly informative.

An important consideration in the evaluation of the human cancer evidence was the quality of the exposure assessment methodology used in the studies. The highest-quality studies used pre-diagnostic, targeted serum analyses for individual PFAS compounds, based on samples collected at least several years before cancer diagnosis. These were features of most of the nested case–control and case–cohort studies. One potential concern was the fact that most studies in the general population relied on a single time point measurement of PFOA or PFOS in serum, and it was unclear how representative such single time point exposure measures were for long-term exposure assessment in relation to cancer. This concern was allayed by the Working Group's evaluation of the possible impact of such exposure misclassification, given the long serum half-life of the compounds in humans and the resulting high correlation between repeated time point measures of exposure available for two of the studies. The Working Group therefore concluded that only minor bias towards the null would probably result from this source of exposure uncertainty, at least over a 5–8-year time period.

The main concern across the set of studies related to the potential for co-exposure to other potentially carcinogenic PFAS compounds (e.g. PFOA and PFOS together, or with other PFAS compounds). In the most informative studies, the researchers adjusted statistically for the effects

of the other compounds, or the Working Group concluded that exposure to one of the compounds –either PFOA or PFOS – was predominant (this was generally the case for the occupational and high-environmental-exposure studies). Other types of confounding were not a major concern in the set of studies, particularly for kidney and testicular cancer, as relatively few strong risk factors are known, and correlations of these risk factors with occupational or environmental exposures to PFOA or PFOS are anticipated to be low. In addition, for some other cancer types, e.g. breast, estimates were well adjusted for important potential confounders.

Among the occupational cohort studies, in the Minnesota and Alabama cohorts, findings were mostly null, but the studies and case numbers were small and there were limitations related to potential survivor bias and/or weaknesses in exposure assessment, which would be expected to cause bias downwards or towards the null. The Veneto occupational cohort was small and showed some evidence (albeit weak) of positive findings for a few cancer sites.

5.2.1 PFOA

The cancer sites with the strongest evidence of an association with PFOA were kidney and testis. For kidney cancer, two independent studies were considered most informative: the set of three partly overlapping studies of workers and residents in West Virginia and Ohio (the Mid-Ohio Valley Study), and the general population case–control study nested within the PLCO cohort. The studies set in the Mid-Ohio Valley consistently showed increased risk of kidney cancer related to PFOA exposure. A clear increase in risk of renal cell carcinoma (which accounts for 80–90% of kidney cancers) with indication of an exposure–response relationship was seen in the PLCO cohort, which had much lower exposure than the Mid-Ohio Valley study. In contrast, no increase in kidney cancer incidence or mortality was seen in the occupational studies in Minnesota or in the International TFE (tetrafluoroethylene) cohort, which were considered to be less informative because they were small, were subject to survivor effects, and/or had exposure assessment limitations. Two other prospective studies in general population cohorts provided equivocal evidence for renal cell carcinoma: in the Multiethnic Cohort (MEC), which was considered informative, positive findings were seen only in White participants, but not overall or in African-American, Japanese-American, Latino, or Native Hawaiian participants; and in the Lifelink subcohort of the Cancer Prevention Study II cohort, for which there were concerns about survivor bias, positive findings were seen only among women exposed to PFOA. Taken together, the body of epidemiological evidence indicated that a positive association between PFOA and renal cell carcinoma is credible, but positive findings have not been consistently observed among the most informative studies, and chance, bias, and confounding by other PFAS in some of the studies could not be ruled out with reasonable confidence. For other subtypes of kidney cancer, no conclusions could be drawn about an association with PFOA.

For cancer of the testis, the most informative studies for the evaluation of PFOA were the set of Mid-Ohio Valley studies, a study of Air Force servicemen with exposure levels similar to those in the general population of the USA, and the ecological analysis of orchiectomies in relation to average serum PFOA concentrations, conducted in the Veneto region, Italy, among municipalities with different levels of PFOA contamination. A positive finding was observed in the Mid-Ohio Valley study and in the Veneto ecological analysis, but not in the Air Force study overall. Mortality studies were deemed to be less informative, because of the high survivability of testicular cancer and the unknown impact of determinants of survival. In summary, there were indications in two independent populations for

an increased risk of testicular cancer associated with PFOA serum concentrations in residents exposed at high levels. In the third informative study, a null association was seen, but exposure levels were at background in this population, which meant that a low exposure contrast existed in the population, making a positive effect, if present, difficult to detect, and did not preclude effects at higher levels of exposure. Overall, the Working Group concluded that a positive association between PFOA and testicular cancer is credible; however, chance and/or bias could not be ruled out as explanations for these findings, given the small number of cases in the few available studies, concerns about co-exposure to other PFAS compounds, and the fact that one of the positive studies was of ecological design.

For breast cancer, most epidemiological studies gave generally null results for all types of breast cancer combined. However, the epidemiological studies with prospective serum samples for PFOA showed a slightly elevated but uncertain association with PFOA. The two most informative studies were null overall but were the only prospective studies that examined postmenopausal breast cancer cases by estrogen receptor/progesterone receptor (ER/PR) status. Both found nonlinear positive associations with ER-negative and PR-negative postmenopausal breast cancer. The statistical power was low in studies examining associations with specific tumour subtypes or stratified by levels of endogenous hormone levels (pre- or postmenopausal cancer), limiting the ability to identify causal associations. Moreover, there were few data on risk of breast cancer above background levels of PFOA exposure. Overall, the available epidemiological evidence was not considered consistent enough to permit a conclusion about the presence of a causal association between exposure to PFOA and breast cancer.

For other cancer types, there was little consistent evidence of an association with PFOA, and the results were considered inconclusive regarding the presence or absence of a causal association.

5.2.2 PFOS

For PFOS, there were fewer available studies than for PFOA. The evidence was suggestive but sparse or inconsistent for three cancer sites: the testis, thyroid gland, and breast. For breast cancer, there was little evidence of an association between PFOS exposure and all types of breast cancer combined. However, the two most informative studies, one from France and one from the USA, which were the only prospective studies to examine the association by hormone receptor breast tumour subtype, found an imprecise but increased risk of hormone receptor-positive breast cancers associated with higher levels of PFOS. However, there were null findings among postmenopausal women in two cohorts from China and the USA, for which there was no stratification by receptor status (most postmenopausal breast cancers are hormone receptor-positive). Given the inconsistencies across studies, the Working Group considered that the available evidence on risk of breast cancer conferred by PFOS exposure was inconclusive.

For testicular cancer, the only informative studies were conducted among the Air Force servicemen in the USA and in the population exposed to contaminated drinking-water near a military airfield in Ronneby, Sweden. In the Air Force study, overall a positive but imprecise association was observed for PFOS exposure, after controlling for exposure to PFOA and other PFAS compounds. For the Ronneby study (in which PFOS levels were much higher than PFOA levels), a positive association was observed for testicular cancer, but co-exposure to perfluorohexanesulfonic acid (PFHxS) was a concern. For thyroid cancer, some positive evidence related to PFOS

exposure came from the less-informative occupational studies; among women in the Ronneby Register cohort study in Sweden; and in a hospital-based case–control study in New York, USA, in which exposure was at background levels. But in a well-conducted population-based study conducted among women in Finland who were exposed at background levels, findings for PFOS were null after adjusting for other PFAS compounds. There was evidence of an inverse association in two case–control studies in China that were considered less informative. For kidney cancer, there were several informative studies, but the findings were largely null. Overall, the evidence for all cancer types was considered to be inconclusive for PFOS exposure.

5.3 Cancer in experimental animals

5.3.1 PFOA

Treatment with PFOA caused an increase in the incidence of an appropriate combination of benign and malignant neoplasms, in both sexes of a single species, in a well-conducted study that complied with Good Laboratory Practice (GLP).

PFOA was administered by oral administration (in feed) in one well-conducted study that complied with GLP, in male and female Sprague-Dawley rats. In males, there was a significant increase in the incidence of hepatocellular adenoma (includes multiple), with a significant positive trend. There was a significant positive trend in the incidence of hepatocellular carcinoma. There was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined), with the incidence being significantly increased. There was a significant positive trend in the incidence of acinar cell adenoma of the pancreas (includes multiple), with the incidence being significantly increased. There was a significant positive trend in the incidence of acinar cell adenomas or adenocarcinoma (combined) of the pancreas, with the incidence being significantly increased. In females, there was a significant increase in the incidence of adenocarcinoma of the uterus. There was significant positive trend in incidence of pancreatic acinar cell adenoma or adenocarcinoma (combined).

In another well-conducted study that complied with GLP, PFOA was administered in the feed of male and female Sprague-Dawley rats. PFOA increased the incidence of testicular Leydig cell adenoma in males.

In a non-GLP study on oral administration (in feed) in male Sprague-Dawley rats only, PFOA increased the incidence of hepatocellular adenoma, Leydig cell tumours, and pancreatic acinar cell adenoma. In a study in female CD-1 mice treated by gavage, there was a positive trend in the incidence of liver haemangiosarcoma in females. PFOA was shown to promote hepatocarcinogenesis in two feeding studies in male Wistar rats and two feeding studies in rainbow trout.

5.3.2 PFOS

Treatment with PFOS caused an increase in the incidence of an appropriate combination of benign and malignant neoplasms in one sex (female) of a single species (rat) in a well-conducted study that complied with GLP.

PFOS was administered by oral administration (in feed) in one study that complied with GLP, in male and female Sprague-Dawley rats. In males, there was a significant positive trend in the incidence of hepatocellular adenoma, with the incidence being significantly increased. In females, there was a significant positive trend and significant increase in the incidence of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined).

PFOS was also shown to promote hepatocarcinogenesis in one feeding study in male and female rainbow trout.

5.4 Mechanistic evidence

5.4.1 PFOA

Regarding the absorption, distribution, metabolism, and excretion of PFOA, data were available from studies in humans and from experimental systems. Studies in experimental animals demonstrated high bioavailability after oral exposure, which was presumed to be similar in humans. Absorption via dermal and inhalation routes has been demonstrated in rodents; in humans, there is some evidence that these exposure routes may also be relevant. On the basis of its structure and physicochemical properties, PFOA is unlikely to readily diffuse across cellular membranes; membrane transporters mediate tissue distribution and cell uptake. PFOA can bind to specific proteins, including albumin in serum and liver-type fatty acid-binding protein (L-FABP). Partitioning of PFOA to the liver and kidney can differ across species. There is no evidence in humans or experimental animals that PFOA is biotransformed; PFOA is eliminated by excretion. PFOA undergoes enterohepatic recirculation. Biliary and urinary excretion are the major elimination pathways in humans, with women of reproductive age also eliminating PFOA via blood loss during menstruation, placental transfer to the fetus, and lactational transfer to infants. Urinary excretion is predominant in rodents. In humans, half-lives are in the order of years; half-lives in experimental animals range from hours to months. The basis of species differences in distribution and elimination is not well understood.

There was consistent and coherent evidence that PFOA exhibits key characteristics of carcinogens.

PFOA induces epigenetic alterations. Consistent and coherent evidence came from numerous studies in exposed humans showing that exposure to PFOA alters DNA methylation. Several studies using umbilical cord and peripheral blood leukocytes, or dried blood spots from exposed humans, showed associations between blood PFOA and gene-specific methylation. A robust human epigenome-wide association study showed persistence of PFOA-associated 5′-C-phosphate-G-3′ dinucleotide (CpG) methylation between birth and adolescence. This study was of great importance as it investigated developmental reprogramming that may influence human cancer susceptibility. In additional studies in exposed humans, alterations were found in the expression of cancer-related microRNAs (miRNAs) in relation to PFOA exposure. There were no data in primary human cells. Consistent and coherent evidence from experimental systems, both in vivo and in vitro, suggested that PFOA induced changes in DNA methylation, histone modifications, or miRNA expression in multiple tissues, including the liver or kidney.

PFOA is immunosuppressive. Consistent and coherent evidence from multiple well-conducted studies in different populations of exposed humans, including children and adults, demonstrated that exposure to PFOA is associated with increased risk of infectious disease and decreased vaccine response to diverse antigens. These findings were corroborated by consistent and coherent evidence from studies in primary human cells showing that PFOA decreases the production of cytokines and reduces lymphoproliferation. Additionally, consistent and coherent evidence from multiple studies in rodents has demonstrated that PFOA administration alters antibody responses to T-cell dependent antigens. In some studies in rodents, alterations in leukocyte populations were reported.

PFOA induces oxidative stress. The few available studies in exposed humans were not informative. There was consistent and coherent evidence in human primary cells that PFOA exposure increases reactive oxygen species (ROS) production, alters antioxidant function, or increases markers of lipid peroxidation. Consistent and

coherent evidence from experimental systems showed induction of oxidative stress by PFOA, including increased levels of oxidatively damaged DNA in cell lines or 8-oxo-2′-deoxyguanosine (8-oxodG) in the urine and liver in rodents. Several studies in experimental systems showed that biomarkers of oxidative stress induced by PFOA were reduced by co-treatment with antioxidants.

PFOA modulates receptor-mediated effects. Data were available for peroxisome proliferator–activated receptors alpha and gamma (PPARα, PPARγ), constitutive androstane receptor/pregnane X receptor (CAR/PXR), hepatocyte nuclear factor 4 alpha (HNF4α), aryl hydrocarbon receptor (AHR), estrogen, androgen, thyroid, progesterone, and glucocorticoid pathways. In exposed humans, the data were suggestive of an association between PFOA exposure and modulation of thyroid, androgen, and progesterone pathways. Data for the remaining receptor pathways in exposed humans were sparse or absent. Consistent and coherent evidence in human primary cells showed that PFOA modulates the action of PPARα and CAR/PXR. Data for human primary cells suggested that PFOA modulates the action of both estrogen and PPARγ. Data for the remaining receptor pathways in exposed human primary cells were sparse. There was consistent and coherent evidence from numerous studies performed in experimental systems, including human cell lines, that exposure to PFOA modulates the activity of PPARα and CAR/PXR, as well as PPARγ. There was suggestive evidence that PFOA alters serum estradiol and testosterone concentrations in rodents. There was a paucity of information for PFOA in other receptor pathways in experimental systems.

PFOA alters cell proliferation, cell death, or nutrient supply. The evidence in PFOA-exposed humans was suggestive on the basis of high-throughput metabolomic analyses showing alterations in pathways related to nutrient and energy supply. Evidence from primary human cells suggested that PFOA increases cell proliferation. Transcriptomic analyses from primary human cells suggested that PFOA modulates gene signalling pathways involved in cell proliferation and oncogenesis. Metabolomic analyses from primary human cells suggested that PFOA increases activity in glycolytic pathways. Consistent and coherent evidence in multiple experimental systems showed that PFOA induces cell proliferation, migration, or invasion in human cell lines and cell proliferation or hyperplasia in multiple tissues in rodents, including in PPARα-null mice.

There was suggestive evidence that PFOA is genotoxic. A single study in exposed humans reported increased levels of DNA strand breaks; results from other studies using less-relevant end-points were mixed. The results of studies in human primary cells were negative. Evidence in experimental systems suggested that PFOA causes DNA damage. Available studies in rodents in vivo showed largely negative results for DNA damage and micronucleus assays.

There was suggestive evidence that PFOA induces chronic inflammation. Data in exposed humans were not informative. In most studies in human primary cells, decreased production of pro-inflammatory markers occurred after PFOA exposure. The results of several studies in rodents suggested that PFOA induces small increases in severity or incidence of chronic inflammation in the stomach, liver, or pancreas. The results of studies of inflammatory markers in experimental systems were mixed, with results differing depending on the model, tissue, and assay.

There was a paucity of data for the following key characteristics: is electrophilic or metabolized to an electrophile, alters DNA repair or genomic instability, or causes immortalization.

PFOA and its ammonium salt were tested in the Toxicology Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes. However, the analytical

purity quality control rating for these data was labelled "unknown/inconclusive".

5.4.2 PFOS

Regarding the absorption, distribution, metabolism, and excretion of PFOS, data were available from studies in humans and from experimental systems. Studies in experimental animals demonstrated high bioavailability after oral exposure, which was presumed to be similar in humans. Absorption via dermal and inhalation routes has been demonstrated in rodents; in humans, these exposure routes may also be relevant. On the basis of its structure and physicochemical properties, PFOS is unlikely to readily diffuse across cellular membranes; membrane transporters mediate tissue distribution and cell uptake. PFOS can bind to specific proteins, including albumin in serum and L-FABP. Partitioning of PFOS to the liver can differ across species. There is no evidence in humans or experimental animals that PFOS is biotransformed; PFOS is eliminated by excretion. PFOS undergoes enterohepatic recirculation. Biliary and urinary excretion are the major elimination pathways in humans, with women of reproductive age also eliminating PFOS via blood loss during menstruation, placental transfer to the fetus, and lactational transfer to infants. Urinary excretion is predominant in rodents. In humans, half-lives are on the order of years; half-lives in experimental animals range from weeks to months. The basis of species differences in distribution and elimination is not well understood.

There was consistent and coherent evidence that PFOS exhibits key characteristics of carcinogens.

PFOS induces epigenetic alterations. Consistent and coherent evidence from numerous studies in exposed humans showed that exposure to PFOS alters DNA methylation. Several studies using umbilical cord and peripheral blood leukocytes, or dried blood spots from exposed humans, showed associations between blood PFOS and gene-specific methylation. A robust human epigenome-wide association study showed persistence of PFOS-associated CpG methylation between birth and adolescence. This study was of great importance as it investigated developmental reprogramming that may influence human cancer susceptibility. In additional studies in exposed humans, alterations were found in the expression of cancer-related miRNAs in relation to PFOS exposure. There were no data in primary human cells. Consistent and coherent evidence from studies in experimental systems, both in vivo and in vitro, suggested that PFOS induced changes in DNA methylation, histone modifications, or miRNA expression in multiple tissues, including the liver or kidney.

PFOS is immunosuppressive. Consistent and coherent evidence from multiple well-conducted studies in different populations of exposed humans, including children and adults, demonstrated that exposure to PFOS is associated with increased risk of infectious disease and decreased vaccine response to diverse antigens. These findings were corroborated by consistent and coherent evidence from studies in primary human cells showing that PFOS decreases production of cytokines and reduces lymphoproliferation. Additionally, consistent and coherent evidence from multiple studies in rodents has demonstrated that PFOS administration alters antibody responses to T-cell dependent antigens. In some studies in rodents, alterations in leukocyte populations were reported. One study in mice showed that PFOS increased morbidity and mortality after influenza A infection.

PFOS induces oxidative stress. There was suggestive evidence in exposed humans that PFOS induces oxidative stress, with several studies showing associations between PFOS and various oxidative stress markers in serum or urine. Two of three studies that measured urinary 8-oxodG with high specificity gave positive results. There

was consistent and coherent evidence in human primary cells that PFOS exposure increases ROS production, alters antioxidant function, or increases markers of lipid peroxidation. One study in human primary cells showed that biomarkers of oxidative stress induced by PFOS were reduced by co-treatment with antioxidants. There was consistent and coherent evidence from experimental systems that PFOS induces oxidative stress. In cell lines, PFOS increased levels of ROS production. PFOS increased markers of lipid peroxidation and altered antioxidant function in rodent tissues. Several studies in experimental systems showed that biomarkers of oxidative stress induced by PFOS were reduced by co-treatment with antioxidants.

PFOS modulates receptor-mediated effects. Data were available for PPARα, PPARγ, CAR/PXR, HNF4α, AHR, estrogen, androgen, thyroid, progesterone, and glucocorticoid pathways. In exposed humans, the data were suggestive of an association between PFOS exposure and modulation of thyroid, estrogen, androgen, progesterone, and glucocorticoid pathways. Data for the remaining receptor pathways in exposed humans were sparse or absent. Consistent and coherent evidence in human primary cells showed that PFOS modulates the action of PPARα and CAR/PXR. Data from human primary cells suggested that PFOS modulates the PPARγ pathway. Data for the remaining receptor pathways in human primary cells were sparse. Consistent and coherent evidence came from numerous studies in experimental systems, including human cell lines, and showed that exposure to PFOS modulates the activity of PPARα and CAR/PXR. Consistent and coherent evidence from experimental systems showed that PFOS modulates the androgen and thyroid pathways. Evidence from human cell lines and receptor assays suggested that PFOS modulates the PPARγ pathway. Several studies in experimental systems suggested that PFOS modulates the estrogen pathway. There was a paucity of information for PFOS in other receptor pathways in experimental systems.

PFOS alters cell proliferation, cell death, or nutrient supply. The evidence in PFOS-exposed humans was suggestive on the basis of high-throughput metabolomic analyses showing alterations in pathways related to nutrient and energy supply. Evidence from primary human cells suggested that PFOS increases cell proliferation, migration, or invasion. Transcriptomic analyses from primary human cells suggested that PFOS modulates gene signalling pathways involved in cell proliferation and oncogenesis. Metabolomic analyses from primary human cells suggested that PFOS increases activity in glycolytic pathways. Consistent and coherent evidence in multiple experimental systems showed that PFOS induces cell proliferation, migration, or invasion in human cell lines and cell proliferation or hyperplasia in multiple tissues in rodents.

There was suggestive evidence that PFOS is genotoxic. Results from the few studies in exposed humans were mixed. The results of studies in human primary cells were negative. Evidence in experimental systems suggested that PFOS causes DNA damage. Studies in rodents showed mixed results for DNA damage and micronucleus assays.

There was suggestive evidence that PFOS induces chronic inflammation. Data in exposed humans were not informative. In most studies in human primary cells, decreased production of pro-inflammatory markers occurred after PFOS exposure. The results of studies in rodents suggested that PFOS increases inflammation. The results of studies of inflammatory markers in other experimental systems were mixed, with results differing depending on the model, tissue, and assay.

There was a paucity of data for the following key characteristics: is electrophilic or metabolized to an electrophile, alters DNA repair or genomic instability, or causes immortalization.

PFOS and its potassium salt were tested in the assay battery of the Tox21 and ToxCast research programmes. However, the analytical purity quality control rating for these data was labelled "unknown/inconclusive".

6. EVALUATION AND RATIONALE

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of perfluorooctanoic acid (PFOA). Positive associations have been observed between PFOA and renal cell carcinoma and cancer of the testis.

There is *inadequate evidence* in humans regarding the carcinogenicity of perfluorooctanesulfonic acid (PFOS).

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of PFOA.

There is *limited evidence* in experimental animals for the carcinogenicity of PFOS.

6.3 Mechanistic evidence

There is *strong evidence* that PFOA exhibits multiple key characteristics of carcinogens in exposed humans, in human primary cells, and in experimental systems.

There is *strong evidence* that PFOS exhibits multiple key characteristics of carcinogens in exposed humans, in human primary cells, and in experimental systems.

6.4 Overall evaluation

PFOA is *carcinogenic to humans* (Group 1).

PFOS is *possibly carcinogenic to humans* (Group 2B).

6.5 Rationale

6.5.1 PFOA

The Group 1 evaluation for PFOA is based on the combination of *sufficient evidence* for cancer in experimental animals and *strong* mechanistic evidence of key characteristics of carcinogens in exposed humans. The evidence for cancer in experimental animals was *sufficient* because exposure to PFOA caused an increase in the incidence of an appropriate combination of benign and malignant neoplasms in both sexes of a single species (rat) in one study that complied with GLP. The mechanistic evidence was *strong* in exposed humans because PFOA induces epigenetic alterations and is immunosuppressive. In exposed humans, PFOA induces epigenetic alterations in the form of gene-specific methylation and cancer-related miRNAs. These effects are supported by evidence of epigenetic alterations in multiple experimental systems. In exposed humans, PFOA is immunosuppressive, increasing risk of infectious disease and decreasing vaccine response to diverse antigens. These effects are supported by evidence

of immunosuppression in human primary cells and experimental systems. In addition, in human primary cells and experimental systems, PFOA induces oxidative stress and modulates receptor-mediated effects. Additionally, in experimental systems, PFOA alters cell proliferation, cell death, or nutrient supply.

Also, for PFOA, the evidence for cancer in humans was found to be *limited* for renal cell carcinoma and cancer of the testis. Despite the increase in the number of available human cancer studies since the previous evaluation by the *IARC Monographs*, the results were somewhat inconsistent across the studies. For renal cell carcinoma, positive findings were observed in three studies conducted in partly overlapping occupationally and environmentally exposed populations and in a fourth population with background exposure. However, positive findings were not observed overall in two other background-exposed populations. For testicular cancer, there were two studies with positive findings: one cohort study and a second ecological study that had limitations. For other cancer types, there were only sporadic positive findings in the informative studies (e.g. breast), and for all these other cancer types, the evidence was *inadequate*.

6.5.2 PFOS

The Group 2B evaluation for PFOS is based on *strong* mechanistic evidence. There is *strong* evidence that PFOS exhibits multiple key characteristics of carcinogens in exposed humans, human primary cells, and experimental systems. There is strong evidence that PFOS in exposed humans induces epigenetic alterations in the form of gene-specific methylation and cancer-related miRNAs. These effects are supported by evidence of epigenetic alterations in multiple experimental systems. In exposed humans, PFOS is immunosuppressive, increasing risk of infectious disease and decreasing vaccine response to diverse antigens. These effects are supported by evidence of immunosuppression in human primary cells and experimental systems. In human primary cells and experimental systems, PFOS modulates receptor-mediated effects and induces oxidative stress. Additionally, in experimental systems, PFOS alters cell proliferation, cell death, or nutrient supply.

In addition, the evidence for cancer in experimental animals was *limited*. Exposure to PFOS caused an increase in the incidence of an appropriate combination of benign and malignant neoplasms in one sex (female) of a single species (rat) in a study that complied with GLP. The evidence regarding cancer in humans was found to be *inadequate*, because among the relatively few available studies, positive findings were seen only sporadically and inconsistently for a few cancer sites (i.e. breast, testis, and thyroid).

LIST OF ABBREVIATIONS

2-AAF	2-acetylaminofluorene
ACGIH	American Conference of Governmental Industrial Hygienists
ACOX	acyl-coenzyme A oxidase
ACS	American Cancer Society
ADME	absorption, distribution, metabolism, or excretion
AFB_1	aflatoxin B_1
AFFF	aqueous film-forming foam
AHR	aryl hydrocarbon receptor
AOR	adjusted odds ratios
APFO	ammonium perfluorooctanoate
AR	androgen receptor
ARE	antioxidant responsive element
ASTM	American Society for Testing and Materials
ATBC	Alpha-Tocopherol, Beta-Carotene Cancer Prevention
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BMI	body mass index
BrdU	bromodeoxyuridine
bw	body weight
cAMP	cyclic adenosine monophosphate
CAR	constitutive androstane receptor
CAT	catalase
CBD	cannabidiol
CEPA	Canadian Environmental Protection Act
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CGA	chlorogenic acid
CHDS	Child Health and Development Studies
CHO	Chinese hamster ovary
CI	confidence interval
CIOB	Chemicals in Our Bodies
CNBCSP	Chinese National Breast Cancer Screening Program
CoA	coenzyme A
ConA	concanavalin A

CONTAM	European Food Safety Authority Panel on Contaminants in the Food Chain
CpG	5′-C-phosphate-G-3′-dinucleotide
CPS	Cancer Prevention Study
CRP	C-reactive protein
CS	collagen sandwich
CTS	California Teachers Study
CV	coefficient of variation
CYP	cytochrome P450
DCF	2′,7′-dichlorofluorescein
DCF-DA	2′,7′-dichlorofluorescein diacetate
DCFH-DA	2′,7′-dichlorodihydrofluorescein diacetate
DEN	diethylnitrosamine
DFTJ	Dongfeng-Tongji
dG	2′-deoxyguanosine
DHEA	dehydroepiandrosterone
diPAP	polyfluoroalkyl phosophate diester
DMBA	7,12-dimethylbenz[*a*]anthracene
DMR	differentially methylated region
DMSO	dimethyl sulfoxide
DNMT	DNA methyltransferase
DoD	Department of Defense
DoDSR	Department of Defense Serum Repository
DOX	doxycycline
DTH	delayed-type hypersensitivity
EC	European Commission
EC_{50}	half-maximal effective concentration
ECA	Environment and Childhood Asthma
ECCC	Environment and Climate Change Canada
ECF	electrochemical fluorination
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
EPCRA	Emergency Planning and Community Right-to-Know Act
ER	estrogen receptor
ERS	endoplasmic reticulum stress
ESI	electrospray ionization
N-EtFOSAA	*N*-ethyl-perfluorooctane sulfonamido acetic acid
EU	European Union
EWAS	epigenome-wide association analysis study
FDR	false discovery rate
FMC	Finnish Maternity Cohort
Fpg	formamidopyrimidine-DNA glycosylase
FSANZ	Food Standards Australia New Zealand
FT3	free triiodothyronine
FT4	free thyroxine
FTOH	fluorotelomer alcohol
GBCA	Genetic and Biomarkers study for Childhood Asthma
GC	gas chromatography
GDP	guanosine diphosphate

GF	glomerular function
GFR	glomerular filtration rate
GGT	gamma-glutamyl transferase
GJIC	gap junctional intercellular communication
GLP	Good Laboratory Practice
GM	geometric mean
GM-CSF	granulocyte-macrophage colony-stimulating factor
GPx	glutathione peroxidase
GRULAC	Group of Latin America and the Caribbean
GSH	glutathione
GSPE	grape seed proanthocyanidin extract
GSR	glutathione reductase
GSSG	oxidized glutathione
GST	glutathione *S*-transferase
γH2AX	phosphorylated H2A histone family member X
HBM	Human Biomonitoring
HBM4EU	Human Biomonitoring for Europe
HCC	hepatocellular carcinoma
HETE	hydroxyeicosatetraenoic acid
HFD	high-fat diet
HGF	hepatocyte growth factor
Hib	*Haemophilus influenza* type b
HMC	human mast cell
HMVEC	human microvascular endothelial cells
HO-1	haem oxygenase 1
HOME	Health Outcomes and Measures of the Environment
HPLC	high-performance liquid chromatography
HR	hazard ratio
HUVEC	human umbilical vein endothelial cell
IARC	International Agency for Research on Cancer
IC_{50}	half-maximal inhibitory concentration
ICC	intraclass correlation coefficient
IFN-γ	interferon gamma
Ig	immunoglobulin
IKIDS	Illinois Kids Development Study
IL	interleukin
INHAND	International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice
iNOS	inducible nitric oxide synthase
INSR	insulin receptor
i.p.	intraperitoneal
IQR	interquartile range
IRR	incidence rate ratio
IsoF	isofuran
IsoP	isoprostane
ITRC	Interstate Technology and Regulatory Council
i.v.	intravenous
JEM	job-exposure matrix
KC	key characteristic of carcinogens
KEEP	Korean Elderly Environmental Panel
KLH	keyhole limpet haemocyanin

LC_{50}	median lethal concentration
LC-MS/MS	liquid chromatography-tandem mass spectrometry
L-FABP	liver fatty acid-binding protein
LH	luteinizing hormone
LINE-1	long interspersed nuclear element 1
LLE	liquid–liquid extraction
LMIC	low- or middle-income country
LOD	limit of detection
LOQ	limit of quantification
LOX	lipoxygenase
LPS	lipopolysaccharide
LRTI	lower respiratory tract infection
LTL	leukocyte telomere length
LWBC	Laizhou Wan (Bay) birth cohort
MCP-1	monocyte chemoattractant protein
MDA	malondialdehyde
MDL	method detection limit
MEC	Multiethnic Cohort
MHC	major histocompatibility complex
miRNA	microRNA
mRNA	messenger RNA
MLQ	method limit of quantification
MNNG	*N*-methyl-*N′*-nitro-*N*-nitrosoguanidine
MRL	minimum reporting level
MRP	multidrug resistance protein
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NAC	*N*-acetylcysteine
NADPH	nicotinamide adenine dinucleotide phosphate
NAFLD	non-alcoholic fatty liver disease
NAR	naringin; 4′,5,7-trihydroxyflavonone-7-rhamnoglucoside
NASEM	National Academies of Sciences, Engineering, and Medicine
NDAA	National Defense Authorization Act
NDI	National Death Index
NESHAP	National Emissions Standards for Hazardous Air Pollutants
NeuroP	neuroprostane
NGF	nerve growth factor
NHANES	National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NHMRC	National Health and Medical Research Council
NICHD	National Institute of Child Health and Human Development
NK	natural killer
NO	nitric oxide
NOx	nitrogen oxides
8-NO_2Gua	8-nitrosoguanine
NOAEL	no observed adverse effect level
NPDES	National Pollutant Discharge Elimination System
NTCP	Na+/taurocholate cotransporting polypeptide
NTP	National Toxicology Program

OAT	organic acid transporter
OATP	organic anion transporting polypeptide
OCC	Odense Child Cohort
OCM	organotypic culture model
8-OHdG	8-hydroxy-2′-deoxyguanosine
OR	odds ratio
8-oxodG	8-oxo-2′-deoxyguanosine
PAH	polycyclic aromatic hydrocarbon
PanIN	pancreatic intraepithelial neoplasia
PBDE	polybrominated diphenyl ether
PBMC	peripheral blood mononuclear cells
PBPK	physiologically based pharmacokinetic
PCB	polychlorinated biphenyl
PCDD	2,3,7,8-substituted polychlorinated dibenzodioxin
PCDF	2,3,7,8-substituted polychlorinated dibenzofuran
PCNA	proliferating cell nuclear antigen
PCO	palmitoyl coenzyme A oxidase
PCR	polymerase chain reaction
PFAA	perfluoroalkyl acid
PFAS	perfluoroalkyl and polyfluoroalkyl substances
PFHpS	perfluoroheptane sulfonate
PFHxS	perfluorohexanesulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
PGF	prostaglandin
PHA	phytohaemagglutinin
PKA	protein kinase A
PKC	protein kinase C
PLCO	Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial
PM	particulate matter
$PM_{2.5}$	particulate matter with diameter < 2.5 μm
POP	persistent organic pollutant
POSF	perfluorooctane sulfonyl fluoride
PPAR	peroxisome proliferator-activated receptor
ppb	parts per billion
PPE	personal protective equipment
ppm	parts per million
PR	progesterone receptor
PTFE	polytetrafluoroethylene
PUF	polyurethane foam
PWS	public water systems
PXR	pregnane X receptor
qPCR	quantitative polymerase chain reaction
QuEChERS	quick, easy, cheap, effective, rugged, safe
RACK-1	receptor for activated C kinase 1
RBC	red blood cell
RCC	renal cell carcinoma
RD	human embryonal rhabdomyosarcoma cell line
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals

RL	reporting limit
RNA	ribonucleic acid
tRNA	transfer RNA
RNS	reactive nitrogen species
ROS	reactive oxygen species
RONS	reactive oxygen and nitrogen species
RPE	retinal pigment epithelial
RR	rate ratio
RT-PCR	reverse transcription-polymerase chain reaction
RSV	respiratory syncytial virus
SCAP	sterol regulatory element-binding protein cleavage-activating protein
SD	standard deviation
SEER	Surveillance, Epidemiology, and End Results
SEM	systemic evidence map
SHBG	sex hormone-binding globulin
SIR	standardized incidence ratio
SMBCS	Sheyang Mini Birth Cohort Study
SMR	standardized mortality ratio
SNUR	Significant New Use Rules
SOD	superoxide dismutase
SPE	solid-phase extraction
SRBC	sheep red blood cell
StAR	steroidogenic acute regulatory
STEL	short-term exposure limit
$T_{1/2}$	half-life
TAC	total antioxidant capacity
TAD	total administered dose
TBARS	thiobarbituric acid-reactive substance
TCA	tricarboxylic acid
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TCR	T-cell receptor
TDAR	T-cell-dependent antibody response
TDI	tolerable daily intake
TFE	tetrafluoroethylene
TGCT	testicular germ cell tumour
TH	thyroid hormone
TK	toxicokinetic
TL	telomere length
TNF-α	tumour necrosis factor alpha
TNP	trinitrophenyl
TPOAb	thyroid peroxidase antibody
TR	thyroid hormone receptor
TRI	Toxics Release Inventory
TSH	thyroid-stimulating hormone
TSCA	Toxic Substances Control Act
TT3	total triiodothyronine
TT4	total thyroxine
TTR	transthyretin
TWA	time-weighted average
TWI	tolerable weekly intake

UBA	Umweltbundesamt (German Environment Agency)
UCMR 3	Third Unregulated Contaminant Monitoring Rule
UK	United Kingdom
UNEP	United Nations Environment Programme
UPR	unfolded protein response
US	United States
USA	United States of America
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
V_d	volume of distribution
vP	very persistent
WBC	white blood cell
WT	wildtype

ANNEX 1. SUPPLEMENTARY MATERIAL FOR SECTION 1, EXPOSURE CHARACTERIZATION

These supplementary online-only tables are available from: https://publications.iarc.who.int/636.

Please report any errors to imo@iarc.who.int.

The following tables were produced in draft form by the Working Group and were subsequently fact-checked but not edited:

Table S1.11	Occurrence of PFOA and PFOS in drinking-water, surface water, groundwater, snow, and ice
Table S1.15	Occupational exposure to PFOA and PFOS measured in biological matrices
Table S1.22	Exposure assessment review and critique for epidemiological studies on cancer in humans exposed to PFOA and PFOS
Table S1.23	Exposure assessment review and critique for mechanistic studies on cancer and exposure to PFOA and PFOS

The following tables were produced in draft form by the Working Group, fact-checked, and edited:

Table S1.13	Occurrence of PFOA in food
Table S1.14	Occurrence of PFOS in food

ANNEX 2. ACTIONS AND REGULATIONS FOR THE ELIMINATION OF PFAS WORLDWIDE

In the European Union (EU), the Stockholm Convention on Persistent Organic Pollutants (POPs) places restrictions on perfluorooctanesulfonic acid (PFOS) and has targeted global elimination of perfluorooctanoic acid (PFOA); these mandates are implemented through the Persistent Organic Pollutants (POPs) Regulation (ECHA, 2023c). The first POP regulation (European Commission, EC) 850/2004 was published in 2004, but only in the 2019 recast (POP Regulation (EU) 2019/1021) was PFOS included in Annex 1 of the regulation. Annex 1 is dedicated to substances that should be allowed to be manufactured and used only as closed-system site-limited intermediates if an annotation to that effect is expressly entered in the relevant Annex and if the manufacturer demonstrates to the Member State concerned that the substance is manufactured and used only under strictly controlled conditions (EU, 2019). In the scope of the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation, PFOA and ammonium perfluorooctanoate (APFO) are identified as Substances of Very High Concern under Article 57 I and (d) (ECHA, 2023a). These two per- and polyfluoroalkyl substances (PFAS) already have a harmonized classification and labelling under the Classification, Labelling and Packaging (CLP) Regulation (ECHA, 2023c). In 2020, the European Commission published its Chemicals Strategy for Sustainability, which states that the use of PFAS is to be phased out in the EU, unless the use is proven to be essential for society (European Commission, 2023; OECD, 2023). A proposal submitted in January 2023, by Germany, Denmark, the Netherlands, Norway, and Sweden that would further restrict the manufacture, placing on the market, and use of a broader group of more than 10 000 PFAS is under consideration by the scientific Committee for Risk Assessment (RAC) and the Committee for Socio-Economic Analysis (SEAC) of the European Chemicals Agency (ECHA) (ECHA, 2023c).

In Canada, both PFOS and its salts and PFOA and its precursors are listed on the Canadian Environmental Protection Act (CEPA) Schedule 1 – List of Toxic Substances. The 2012 Prohibition of Certain Toxic Substances Regulation from Environment and Climate Change Canada (ECCC) restricts the manufacture, use, sale, or import of products containing PFAS (including PFOA and PFOS), such as aqueous film-forming foam (AFFF) and personal care products. In 2022, changes were proposed to this regulation that would remove some exemptions and accommodations (ECCC, 2023).

Under the Emergency Planning and Community Right-to-Know Act (EPCRA) in the United States of America (USA), the Toxics Release Inventory (TRI) collects information reported by companies manufacturing, processing, or

otherwise using 100 lbs or more of PFOA or PFOS or other listed PFAS. Additional reporting is required under the Toxic Substances Control Act (TSCA), and there are multiple recent Significant New Use Rules (SNURs) restrictions. Discharge of PFOA and PFOS is controlled under the Clean Water Act National Pollutant Discharge Elimination System (NPDES) permitting system of the United States Environmental Protection Agency (US EPA). In 2022, the US EPA proposed that PFOA and PFOS be designated as hazardous substances under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) (Office of the Federal Register, 2022). Several states in the USA have also designated PFAS as a hazardous substance and proposed or enacted restrictions on use or limits in products and in the environment (ITRC, 2023a).

In 2020, Australia issued a National PFAS Position Statement that was added to the 2018 Intergovernmental Agreement on a National Framework for Responding to PFAS Contamination. This Position Statement describes a national stance on an intentional phase-out of PFAS from use (Australian Government, 2020).

In 2016, the United States Food and Drug Administration (US FDA) revoked regulations authorizing the remaining uses of PFOA and PFOS in food packaging (81 FR 5, 4 January 2016 and 81 FR 83672, 22 November 2016; US FDA, 2022). Since then, multiple states in the USA have enacted bans on PFAS in food packaging materials (ITRC, 2023b). There are also state reporting requirements for PFOS and PFOA in children's products, regulations on PFAS in carpets, and regulations broadly governing the sale of products containing PFAS (ITRC 2023a, b). At present, the most comprehensive law regulating PFAS in consumer products in the USA has been established in Maine. This law includes reporting requirements for manufacturers of products to which certain PFAS (including PFOS and PFOA) are intentionally added, as well as bans on the sale of carpets, rugs, and fabric treatments containing PFAS (Maine DEP, 2023). However, other states, such as Minnesota, have proposed banning all "nonessential use" of PFAS in products. If enacted, this would prohibit the sale of certain consumer products containing PFAS, including carpets, cleaning products, cookware, cosmetics, dental floss, fabric treatments, juvenile products, menstruation products, textile furnishings, ski wax, and upholstered furniture (MNPCA, 2023).

The class B firefighting foam known as AFFF is the subject of numerous legislative efforts. In the EU, a 2022 proposal to the ECHA to restrict the use of PFAS in firefighting foams is moving forward, with combined opinions from the RAC and SEAC committees to review by the European Commission. This proposal would implement a gradual ban on PFAS in foams in the EU, if adopted, and could reduce PFAS emissions into the environment by around 13 200 tonnes over 30 years (ECHA, 2023b). In Canada, the Prohibition of Certain Toxic Substances Regulations currently allow for certain uses of AFFF, including AFFF that contains PFOA, AFFF contaminated with PFOS in military vessels or firefighting vehicles returning from foreign operations, and AFFF containing residual levels of PFOS (≤ 10 ppm) (ECCC, 2017). Legislation enacted under the 2019–2022 National Defense Authorization Act (NDAA) requires the United States Department of Defense to take certain actions, including surveying technology for AFFF replacement to be included on the approved list for military use (ITRC, 2023b); this list is also used by commercial airports in the USA. Numerous states in the USA have also enacted partial bans on the sale and distribution of AFFF, as well as AFFF collection or buy-back programmes and restrictions on firefighting training with AFFF (ITRC, 2023b). In Australia, some states, including Queensland, South Australia, and New South Wales, have implemented phased restrictions on AFFF, including banning the use

of AFFF during training or demonstration and restrictions on the use or sale of AFFF products (NSW EPA, 2023).

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ANNEX 3. SUPPLEMENTARY ANALYSES USED IN REVIEWING EVIDENCE ON CANCER IN HUMANS

A3.1 How well does the PFOA concentration in a single serum sample represent long-term exposure in a population with low exposure?

Introduction

Several of the epidemiology studies on perfluoroalkyl and polyfluoroalkyl substance(s) (PFAS) and cancer were cohort studies in the general population, or nested case–control studies within such cohorts, and used a single serum sample per participant to assess exposure. There was little information on how well the PFAS measurement in a single serum sample (typically at baseline) represents longer-term exposure, which is important for studying chronic diseases. In this analysis, summary statistics from two cohorts with repeated measurements of serum perfluorooctanoic acid (PFOA) for the participants in the control groups were used to evaluate the potential impacts of using a single serum sample to represent chronic exposure for each participant.

Methods

The first study, by Rhee et al. (2023a), was a nested case–control study on prostate cancer, with 675 cases and 675 controls from within the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) cohort. In this study, three repeat serum samples were collected from 60 control participants at baseline, 1 years, and 5 years (in 1996, 1997, and 2001). The second study, by Purdue et al. (2023), was a nested case–control study on testicular cancer among Air Force servicemen, with 530 cases and 530 controls. Purdue et al. (2023) had available a second prediagnostic serum sample from 187 case–control pairs. Of these, summary statistics for repeat samples were available from 84 controls for which the dates of first and repeat sampling were the furthest apart (collected ≥ 4.7 years apart) (mean for years of sampling, 1999 and 2007). Serum PFOA concentrations in these populations were similar to those in the general US population as measured by the US National Health and Nutrition Examination Survey (NHANES). The actual analysis by Rhee et al. (2023a) and the main analysis by Purdue et al. (2023) used only the single (or first) sample for each subject.

The summary statistics from these repeat samples are posted on a National Cancer Institute GitHub project (NCI, 2024). Summary statistics for the repeated serum PFOA measurements for the controls in two cohorts were used to generate plausible serum concentrations for each participant at each time point, taking within-subject correlations into account. Five data sets of controls were generated for each cohort, with

Table A3.1 Descriptive statistics for participants with repeated samples (*n* = 60) from the study by Rhee et al. (2023a)[a]

Parameter[b]	Sample 1 (T0)			Sample 2 (T1)			Sample 3 (T5)			All samples (T0, T1, T5)		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Observed PFOA concentration (ng/mL)	3.88	3.63	1.8	3.87	3.53	1.99	4.69	4.53	2.43	4.15	NR	1.91
Simulated PFOA concentration (ng/mL)	3.85	3.88	1.82	3.82	3.81	1.96	4.63	4.61	2.4	4.1	4.09	1.81

NR, not reported; PFOA, perfluorooctanoic acid; SD, standard deviation.

[a] An analysis of prostate cancer in the PLCO study. The data reported here are posted in the National Cancer Institute GitHub project (NCI, 2024).

[b] Spearman correlations: T0–T1 observed, 0.78; simulated, 0.77; T1–T5 observed, 0.60; simulated, 0.60; T0–T5 observed, 0.62; simulated, 0.60.

the same number of controls as in the original studies for each simulated data set. The statistics used to generate simulated data were the mean and standard deviation (SD) for the PFOA serum concentrations among controls at each time point, as well as the Spearman correlation between each set of samples (three correlations for Rhee et al. (2023a) for three samples, one for Purdue et al. (2023) for two samples).

The samples appeared to have an approximately normal distribution, judging by the small differences between means and medians. From the three samples from the PLCO study used in Rhee et al. (2023a), the means and medians were 3.88 ng/mL and 3.63 ng/mL, 3.87 ng/mL and 3.53 ng/mL, and 4.69 ng/mL and 4.53 ng/mL, respectively (SDs, 1.8, 1.99, and 2.43) (Table A3.1). For the samples from Air Force servicemen in Purdue et al. (2023), the mean and median for the first sample were 6.8 ng/mL and 6.1 ng/mL (SD, 3.0), respectively, while for the second sample they were 5.5 ng/mL and 5.1 ng/mL (SD, 2.3), respectively (Table A3.2). For normally distributed data, Pearson and Spearman correlations are similar (de Winter et al., 2016), and we used Spearman correlation coefficients between samples to generate the simulated data, as an approximation of the Pearson correlations (Rhee et al., 2023a; ρ for sample T0–T1, 0.78; ρ for samples T1–T5, 0.60; ρ for samples T0–T5, 0.62); (Purdue et al., 2023; ρ for samples 1 and 2, 0.32) (Table A3.1, Table A3.2).

The distributions were generated using an R package (mvtnorm library) for generating multivariate normal samples with known means, standard deviations, and (Pearson) correlations between different sets of samples (Genz and Bretz, 2009). The mean across the five simulations for each control was then used to represent the simulated data for each sample.

Having generated simulated serum PFOA concentrations for each control at each time point (three time points for Rhee et al., 2023a, two time points for Purdue et al., 2023), mean concentrations across samples for each control in each study were used as an estimate of long-term exposure. For the controls in each study, the first samples were then compared with the long-term average exposure, the latter taken as the “true” exposure and the former as the “misclassified” exposure.

Exposures were categorized into quintiles, as in the original published analysis of Rhee et al. (2023a), and used to determine the extent of misclassification across exposure categories using serum concentrations at the first time point versus the long-term exposure. “True” or long-term exposure values were then also generated

Table A3.2 Descriptive statistics for participants with repeated samples (*n* = 84) from the study by Purdue et al. (2023) [a]

Parameter[b]	Sample 1			Sample 2			Both samples
	Mean	Median	SD	Mean	Median	SD	Mean[c]
Observed PFOA concentration (ng/mL)	6.8	6.1	3.0	5.5	5.1	2.3	6.1
Simulated PFOA concentration (ng/mL)	6.8	6.8	2.9	5.5	5.5	2.3	6.1

PFOA, perfluorooctanoic acid; SD, standard deviation.
[a] A study of testicular cancer in the United States Air Force cohort. The data reported here are posted in the National Cancer Institute GitHub project (NCI, 2024).
[b] Spearman correlation between samples 1 and 2: observed, 0.32; simulation, 0.30.
[c] Restricted to those with a second sample collected > 4.7 years (the median for controls sampled twice) after the first sample. The mean time between first and second samples for these 84 subjects was 7.8 years.

for a set of hypothetical cases for each study, such that there was a monotonic increasing trend across quintiles, and an approximate rate ratio of 1.5 for the highest versus the lowest quintile, using long-term exposure. The misclassification rates used for the hypothetical cases were the same as those observed in the controls (i.e. assuming non-differential exposure misclassification) to simulate "misclassified" exposure at the first time point for the hypothetical cases. Finally, epidemiological effect estimates (odds ratios) were computed across quintiles for cases and controls, using the long-term ("true") versus first sample ('misclassified') data.

Results

The simulated data corresponded well with the observed means and standard deviations for the original data, and the Spearman correlations between repeated samples in each study from the simulated data closely resembled the same correlation from the observed data. For example, for Rhee et al. (2023a), the observed Spearman correlations (ρ) between samples T0–T1, T1–T5, and T0–T5 were 0.78, 0.60, and 0.62, respectively, while the Spearman correlations in the simulated data were 0.77, 0.60, and 0.60 (Table A3.1). The Spearman correlations between first and second samples for Purdue et al. (2023) in the simulated and observed data were 0.30 and 0.32, respectively (Table A3.2).

Comparing long-term "true" exposure (the mean across samples) with "misclassified" exposure (for the first sample alone), epidemiological results were quite similar, with a relatively small bias to the null when using only a single serum sample per participant (bias to the null is expected for non-differential misclassification, see Weinberg et al., 1994). For the data from Rhee et al. (2023a), the odds ratios (ORs) by quintile, using the long-term average, or "true", data were 1.00, 1.14, 1.29, 1.43, and 1.57 (*P* for trend, 0.007), while the odds ratios by quintile using the first serum sample only were 1.00, 1.12, 1.24, 1.35, and 1.42 (*P* for trend, 0.007) respectively, indicating only a slight bias to the null (Table A3.3). Similarly for the data from Purdue et al. (2023), odds ratios by quintile using the long-term average were 1.00, 1.12, 1.23, 1.35, and 1.47 (*P* for trend, 0.005), whereas odds ratios by quintile using the first sample only were 1.00, 1.07, 1.13, 1.17, and 1.31 (*P* for trend, 0.02), again indicating only a slight bias towards the null (Table A3.4).

Table A3.3 Hypothetical "true" (long-term) and "misclassified" (first sample only) for cases (*n* = 675) and controls (*n* = 675) with a positive exposure–response relation for PFOA, based on the PLCO data in Rhee et al. (2023a)

Analysis	Exposure metric	PFOA quintile (ng/mL)	Cases	Controls	Odds ratio
True	Mean (T0, T1, T5) [a]	≤ 3.45	105	135	1.00
		> 3.45 to ≤ 3.90	120	135	1.14
		> 3.90 to ≤ 4.31	135	135	1.29
		> 4.31 to ≤ 4.77	150	135	1.43
		> 4.77	165	135	1.57
					Trend-test *P* value, 0.007
Misclassified	T0 only	≤ 3.45	177	210	1.00
		> 3.45 to ≤ 3.90	123	130	1.12
		> 3.90 to ≤ 4.31	145	138	1.24
		> 4.31 to ≤ 4.77	124	109	1.35
		> 4.77	105	88	1.42
					Trend-test *P* value, 0.007

OR, odds ratio; PFOA, perfluorooctanoic acid; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial.
[a] The quintile cut-points used here stem from the data generated via multivariate normal distributions based on summary statistics from the controls in each study (posted in the National Cancer Institute GitHub project; NCI, 2024) with repeat samples, and differ from the cut-points in the original papers, which are based on all cases and controls in the original studies.

Table A3.4 Hypothetical "true" (long-term) and "misclassified" (first sample only) cases and controls with positive exposure–response relation for PFOA, based on the Air Force data in Purdue et al. (2023)

Analysis	Exposure metric	PFOA quintile (ng/mL)	Cases	Controls	Odds ratio
True	Mean (samples 1, 2)[a]	≤ 5.38	86	106	1.00
		> 5.38 to ≤ 5.93	96	106	1.12
		> 5.93 to ≤ 6.39	106	106	1.23
		> 6.39 to ≤ 6.81	116	106	1.35
		> 6.80	126	106	1.47
		Totals	530	530	Trend-test *P* value, 0.005
Misclassified	Sample 1 only	≤ 5.38	58	70	1.00
		> 5.38 to ≤ 5.93	47	53	1.07
		> 5.93 to ≤ 6.39	61	65	1.13
		> 6.39 to ≤ 6.81	70	72	1.17
		> 6.80	294	270	1.31
		Totals	530	530	Trend-test *P* value, 0.02

[a] The quintile cut-points used here stem from the data generated via multivariate normal distributions based on summary statistics from the controls in each study (posted in the National Cancer Institute GitHub project; NCI, 2024) with repeat samples, and differ from the cut-points in the original papers, which are based on all cases and controls in the original studies.

Discussion

Single (first) samples represented rather well the mean of repeated samples taken an average of 5 and 8 years apart in two cohort studies of populations with background levels of exposure to PFOA (Spearman correlations, 0.87 and 0.83, for PLCO and Air Force data respectively). Others have demonstrated that changes in PFOA exposure estimates after correcting for measurement error cause little change in epidemiological findings in a high-exposure population in which the rank order of exposure among participants changes little with modest group-level misclassification (Avanasi et al., 2016). The same results were found here, for individual-level misclassification in low exposure populations more typical of the general population. Individual serum PFOA concentrations changed somewhat over time in these two cohorts, but the reported within-subject correlations were high, so the relative ranking of exposure remained approximately the same over time. This implied that tests for trend in disease risk in studies relying on only one serum sample might not differ markedly from those using a more accurate estimate of long-term exposure, i.e. the average for repeated samples over time. Such relative rankings might remain relatively constant in the case of the legacy PFAS, such as PFOA, because these chemicals have relatively long half-lives, and because local sources in the environment where the participants live (e.g. drinking-water, consumer products, and diet) may be relatively stable over time, at least during the decade of serum sampling represented by these two cohorts, despite longer-term secular trends in environmental levels and serum concentrations.

The limitations of these findings were the restriction to only a few repeated samples over relatively short time periods, before the production of PFOA and perfluorooctanesulfonic acid (PFOS) was phased out in the USA. However, another study with five repeated samples collected over almost 40 years in a general population sample in Norway also reported a relatively high within-subject correlation between samples over time (Nøst et al., 2014). Another limitation here was that all the data in the studies by Rhee et al. (2023a) and Purdue et al. (2023) came from men. However, while there are important differences between men and women, e.g. women's serum levels of PFAS change during pregnancy and after menopause (Dhingra et al., 2017; Steenland et al., 2018), there is no a priori reason to think that the findings regarding the consistency of relative rankings across time would be radically different for men and women. Other limitations included the assumption of normality, the use of Spearman instead of Pearson coefficients, and a relatively small number of simulations. However, it is not expected that changing these assumptions would have a substantial impact on the findings of the present analysis.

A3.2 Summary of the Working Group's ecological analysis of PFOA and orchiectomy among men aged 15–54 years in 21 municipalities of the Veneto region in Italy, 1997–2014

A3.2.1 Background on PFOA exposure in the Veneto region

The Trissino factory in the Veneto region, Italy, produced PFOA from 1968 to 2014 (Girardi and Merler, 2019). When PFOA production started in 1968, production was estimated to be about 12 tonnes per year in 1968–1970 and then increased over time until the 2000s, when the annual production of PFOA and its ammonium salt was on average 250 tonnes, peaking at 460 tonnes in 2007. PFOS production also occurred at the site but at much lower volumes, with an average of 36.6 tonnes per

year in 2001–2011, peaking at 88 tonnes in 2004 (Girardi and Merler, 2019). As reported by Pitter et al. (2020):

> "…based on general information on production practices, it is believed that the plant produced long-chain PFAS only, particularly PFOA and PFOS, from 1968 until 2001. PFOA reached the highest concentrations both in drinking-water and serum, consistent with previous reports from the Mid-Ohio Valley (Frisbee et al., 2009). PFBA and PFBS were found in high concentrations in drinking-water but were detected only in a minority of serum samples at relatively low concentration, whereas PFOS and PFHxS, which were scarcely represented in drinking-water, were detected in almost 100% of serum samples. This discrepancy may be explained by the exposure to PFOS and PFHxS from other sources, as demonstrated for the general population".

Releases of PFOA from the factory resulted in contamination of ground and surface water used for drinking in the region. The groundwater contamination plume extended over an area of 190 km^2 and affected both public waterworks and private wells. The municipalities in the area of maximum exposure (referred to as the "red area") are further divided into "red area A", which includes municipalities served by the contaminated waterworks that are also located on the groundwater contamination plume; and "red area B", which includes municipalities served by the contaminated waterworks but not located on the groundwater contamination plume. Initially, the red area was composed of 21 municipalities, with 126 000 inhabitants. In 2018, nine additional municipalities were added, some of which were only partially supplied by the contaminated waterworks. Currently, the red area is 595 km^2 wide and has a total population of approximately 140 000 people.

Biomonitoring has been conducted in this community since 2015 (Ingelido et al., 2018; Pitter et al., 2020). In 2015–2016, Ingelido et al. (2018) measured PFOA and PFOS and other PFAS in the serum of 257 individuals, aged 20–51 years, residing in municipalities in the affected areas (Altavilla, Brendola, Creazzo, Lonigo, Montecchio Maggiore, Sarego, and Sovizzo) and in 250 individuals living in uncontaminated areas. In each area, participants were selected and stratified by sex and age. Each participant had resided in an area for at least 10 years. Serum levels of PFOA were much higher in the contaminated areas (median, 13.77 ng/g; maximum, 754.50 ng/g) than in uncontaminated areas (median, 1.64 ng/g; maximum, 27.88 ng/g); similarly, PFOS levels were higher in the exposed group (median, 8.69 ng/g; maximum, 70.27 ng/g) than in the non-exposed (median, 5.84 ng/g; maximum, 118.58 ng/g). The Spearman correlation for PFOA and PFOS in serum was 0.743 in the exposed and 0.619 in the unexposed. In 2015–2016, Pitter et al. (2020) conducted a larger study of 18 345 participants aged 14–39 years at recruitment; 63.5% agreed to participate in the surveillance programme; serum results for people who had lived in the red area for < 1 year were excluded. The PFAS with the highest serum concentrations were PFOA (median, 44.4 ng/mL; interquartile range, IQR, 19.3–84.9 ng/mL), PFOS (median, 3.9 ng/mL; IQR, 2.6–5.8 ng/mL), and PFHxS (median, 3.9 ng/mL; IQR, 1.9–7.4 ng/mL). Within the red areas, median PFOA levels varied by community, ranging from 10.9 ng/mL in Terrazo to 73.3 ng/mL in Asigiliano-Veneto.

Individuals in this contaminated area of the Veneto region are exposed to a mixture of PFAS, including PFOA and PFOS; but information on production volumes, water levels, and biomonitoring data are consistent with PFOA being the PFAS present at highest concentrations throughout the region (Ingelido et al., 2018; Mastrantonio et al., 2018; Girardi and Merler, 2019; Pitter et al., 2020; Giglioli et al., 2023).

Taken as a whole, these data provided extensive evidence for PFOA contamination in the region, with both water and biomonitoring data showing differences in concentrations within the region. While other PFAS, notably PFOS and PFHxS, are correlated with PFOA, they are present at levels that are substantially lower than those of PFOA.

A3.2.2 Working Group analysis of orchiectomy data

The Working Group conducted an ecological analysis comparing biomonitoring data from Pitter et al. (2020) with data from an investigation on the frequency of orchiectomies in 21 municipalities in this region between 1997 and 2014 (Sistema Epidemiologico Regionale, 2016, summarized in English by Saugo et al., 2024). Orchiectomy was used as a proxy for diagnosis of testicular cancer [sensitivity and positive predictive values of 91.7% (95% CI, 88.0–95.4%) and 92.8% (95% CI, 89.3–96.2%), respectively, in this region]. Orchiectomies were ascertained using information in hospital discharge records, including address of residence, which included the main medical procedures from hospital stays and were completed for the purpose of reimbursement from the Italian national health system. As shown in Table A3.5 below, standardized incidence ratios (SIRs) for orchiectomy were estimated for each of the 21 municipalities separately by comparing the observed orchiectomies (n = 70, overall) versus expected numbers based on rates in the region overall that were standardized on age by 5-year age groups from 15 to 54 years (Sistema Epidemiologico Regionale, 2016). A strong correlation was observed between median serum PFOA concentration and the rate of orchiectomy by municipality (Spearman correlation, 0.57; P = 0.006). The Working Group also conducted a Poisson regression of observed orchiectomy counts regressed on median PFOA levels across the 21 municipalities. The Poisson regression was done using the log of expected events as an offset and correcting for dispersion. The rate ratio for each unit (ng/mL) increase of PFOA was 1.018 (95% CI, 1.006–1.031; P = 0.003).

The SAS code used in this analysis is presented in Table A3.6.

A3.3 Working Group meta-analysis of studies on kidney cancer to estimate rate ratio per unit (linear) increase in serum PFOA concentration

The Working Group conducted a meta-analysis that included estimates from the studies of Steenland and Woskie (2012), Barry et al. (2013), Vieira et al. (2013), Shearer et al. (2021), Rhee et al. (2023b), and Winquist et al. (2023). The studies by Barry et al. (2013), Vieira et al. (2013), and Steenland and Woskie (2012) were included, although they overlap to an unknown extent, under the assumptions that: (i) they are largely independent; and (ii) the mortality rate ratio in Steenland and Woskie (2012) is roughly equivalent to what would have been obtained for an incidence rate ratio. The kidney cancer results from Raleigh et al. (2014) were not included, given that the exposure assessment in this study was based on air measurements, nor were those from Consonni et al. (2013), in which there were no serum data to permit the pooling of a comparable cumulative dose–response estimate with the other studies, or from Mastrantonio et al. (2018), because of its ecological design and lack of data on serum levels.

The Working Group used the approach of the meta-analysis by Bartell and Vieira (2021). This approach uses categorical rate ratios based on contrasting the upper category (usually quartiles) with the referent, together with the assumed midpoints of the upper category and referent, to regress the log of the rate ratios on the midpoints to obtain a single linear continuous coefficient that estimates the change in log rate

Table A3.5 Data used by the Working Group for an ecological analysis of PFOA and orchiectomy among men aged 15–54 years in 21 municipalities of the Veneto region, Italy, 1997–2014

Municipality (red area A or B)	Serum PFOA concentrations, by municipality[a]		Orchiectomy data, by municipality[b]		
	n (%) of samples	Median serum PFOA concentration (ng/mL)	Observed N	SIR	95% CI
Albaredo D'Adige (B)	767 (4.2%)	29	1	0.34	0.01–1.90
Alonte (A)	346 (1.9%)	62.6	1	1.13	0.03–6.27
Arcole (B)	899 (5.0%)	29.5	2	0.58	0.07–2.11
Asigliano Veneto (A)	161 (0.9%)	73.3	1	2.15	0.05–11.98
Bevilacqua (B)	216 (1.2%)	56.2	1	0.97	0.02–5.43
Bonavigo (B)	279 (1.5%)	29.8	1	0.87	0.02–4.85
Boschi Sant'Anna (B)	206 (1.1%)	38.4	0	0	0.00–3.77
Brendola (A)	1007 (5.6%)	41	6	1.60	0.59–3.48
Cologna Veneta (A)	1208 (6.7%)	53.9	2	0.44	0.05–1.60
Legnago (B)	2945 (16.3%)	22.2	11	0.83	0.42–1.49
Lonigo (A)	2569 (14.2%)	61.8	16	1.84	1.05–2.98
Minerbe (B)	628 (3.5%)	55.2	3	1.18	0.24–3.46
Montagnana (A)	1146 (6.3%)	67.6	8	1.54	0.67–3.04
Noventa Vicentina (A)	1410 (7.8%)	46.4	3	0.62	0.13–1.80
Pojana Maggiore (A)	767 (4.2%)	67.5	3	1.18	0.24–3.46
Pressana (A)	365 (2.0%)	58.8	1	0.69	0.02–3.82
Roveredo Di Guà (A)	263 (1.4%)	55.8	0	0	0.00–3.61
Sarego (A)	1124 (6.2%)	47.5	3	0.81	0.17–2.38
Terrazzo (B)	288 (1.6%)	10.9	0	0	0.00–2.51
Veronella (B)	778 (4.3%)	48.2	4	1.58	0.43–4.04
Zimella (A)	750 (4.1%)	49.9	3	1.10	0.23–3.21

CI, confidence interval; PFOA, perfluorooctanoic acid; SIR, standardized incidence ratio.
[a] Data from Pitter et al. (2020).
[b] Data from Sistema Epidemiologico Regionale (2016).

ratio per unit of (linear) PFOA. In addition, for Steenland and Woskie (2012), Barry et al. (2013), and Vieira et al. (2013), which used cumulative exposure, the Working Group divided the midpoints of exposure by the assumed average duration of exposure. In the case of Vieira et al. (2013), there were 10 years of cumulative exposure for cases and controls, so the Working Group divided by 10. In the case of Barry et al. (2013), the Working Group used the average duration of follow-up, which was 33 years (the average length of follow-up in the study), as the divisor. In the case of Steenland and Woskie (2012), the average length of follow-up was 30 years, so the Working Group divided the cumulative exposure by 30. The studies by Steenland and Woskie (2012), Barry et al. (2013), and Rhee et al. (2023b) did not have midpoints for the upper categories. For the studies by Steenland and Woskie (2012) and Barry et al. (2013), we multiplied the upper cut-point by 4, based on the observed midpoint in Vieira et al. (2013) (who studied a similar population), being about 4 times the lower level of the uppermost category. For Rhee et al. (2023b), the Working Group multiplied the upper cut-point by 2.5, based on the observed midpoint for the two other general population studies by Shearer

Table A3.6 SAS code used in the Working Group analysis of orchiectomy data

```
data one;
input medianpfoa sir numsamples obs exp estgeomean;
*estgeomean comes from Pitter Table 2 regression;
logexp=log(exp);
lnmedianpfoa=log(medianpfoa);
lnestgeomean=log(estgeomean);

cards;
29 0.34 767 1 2.94 28.3
62.6 1.13 346 1 0.89 36.6
29.5 0.58 899 2 3.43 31.5
73.3 2.15 161 1 0.47 28.5
56.2 0.97 216 1 1.03 34.4
29.8 0.87 279 1 1.15 29.4
38.4 0 206 0 0.80 32.9
41 1.6 1007 6 3.76 24.5
53.9 0.44 1208 2 4.51 37.4
22.2 0.83 2945 11 13.19 20.4
61.8 1.84 2569 16 8.71 38.4
55.2 1.18 628 3 2.53 46.3
67.6 1.54 1146 8 5.18 39.7
46.4 0.62 1410 3 4.87 29.0
67.5 1.18 767 3 2.53 39.6
58.8 0.69 365 1 1.46 40.6
55.8 0 263 0 0.83 37.4
47.5 0.81 1124 3 3.68 32.4
10.9 0 288  0 1.19 10.9
48.2 1.58 778 4 2.54 48.0
49.9 1.1 750  3 2.73 36.6
;
*proc univariate plot; *var sir medianpfoa estgeomean;

proc corr spearman; var medianpfoa sir; run;
proc freq; tables medianpfoa; run;

proc genmod; model obs=medianpfoa / dist=poisson link=log offset=logexp pscale; *best AIC;
proc genmod; model obs=lnmedianpfoa / dist=poisson link=log offset=logexp pscale;
run;
proc genmod; model obs=estgeomean / dist=poisson link=log offset=logexp pscale;
run;
proc genmod; model obs=lnestgeomean / dist=poisson link=log offset=logexp pscale;
run;
```

et al. (2021) and Winquist et al. (2023) for the upper category.

Once the continuous linear coefficient for each study was obtained, the Working Group then used an R package (metagen) to calculate random weights (inverse variance weights, where the variance is the sum of the within and between variance across studies) using the formulae from restricted maximum likelihood (REML) (Veroniki et al., 2016). The Working Group used random weights, given the high heterogeneity of the linear coefficient across studies (I^2 value, 0.91)

The meta-analysis described above gave the result for an increase in the rate ratio per increase of 10 ng/mL in PFOA as 1.15 (95% CI, 0.97–1.37), with an I^2 value of 91%.

We then also conducted, as a sensitivity analysis, a meta-analysis of Winquist et al. (2023), Shearer et al. (2021), Rhee et al. (2023b), and Barry et al. (2013), to avoid the overlapping nature of Barry et al. (2013) with Steenland and Woskie (2012), and Vieira et al. (2013), and choosing Barry et al. (2013) because it was an incidence study that also had the best exposure estimation.

This sensitivity analysis gave the result for an increase in the rate ratio per increase of 10 ng/mL PFOA as 1.21 (95% CI, 0.94–1.57) with an I^2 value of 95%.

As a general limitation to the meta-analysis, we noted the assumption of a linear exposure–response relation, although we know that, in studies with continuous exposure coefficients (Barry et al., 2013; Shearer et al., 2021; Winquist et al., 2023; Rhee et al., 2023b), a log-linear model (i.e. log-transformed PFOA) seemed to fit the data better than did a linear model (i.e. untransformed PFOA). Other main limitations were: (i) the estimate of the linear coefficient using assumed midpoints of only two categories (uppermost and lowest); (ii) the use of average duration of exposure to transform cumulative exposure in Barry et al. and Viera et al. to an assumed average exposure; and (iii) the assumption in the studies by Rhee et al. (2023b), Shearer et al. (2021), and Winquist et al. (2023) that a single PFOA measurement is a good estimate of long-term lifetime average exposure (beyond a 5–8-year duration, discussed Section A3.1 of the present Annex). Given these limitations, as well as the high heterogeneity across studies with different strengths and weaknesses, the Working Group chose to not rely primarily on the meta-analysis of exposure–response relations to determine the hazard identification for kidney cancer in humans.

The R code used for these estimations is presented in Table A3.7.

Table A3.7 R code[a] used for the Working Group's meta-analysis of kidney cancer to estimate rate ratio per unit (linear) increase in serum PFOA concentration

```
library(meta)
#####Outcome: Summary RR based on 10 ng/mL increase
#function to get increase per unit
trendp = function(datalist){
 lapply(datalist, function(df) {
 se1 = (log(df$upper)-log(df$RR))/qnorm(.975) # se of log RR for each dose category
 se2 = (log(df$RR)-log(df$lower))/qnorm(.975)
 se = (se1 + se2) / 2
 scores = 0:(length(se)-1)
 if(se[1] == 0) {
 lm1 = lm(log(RR) ~ 0 + scores, weights = 1/se^2, data=df, subset=se>0)
 lm2 = lm(log(RR) ~ 0 + mids, weights = 1 / se^2, data=df, subset=se>0)
 p1 = summary(lm1)$coef[1,4]
 p2 = summary(lm2)$coef[1,4]
 slope = summary(lm2)$coef[1,1]
 se = summary(lm2)$coef[1,2]
 } else {
 lm1 = lm(log(RR) ~ scores, weights = 1 / se^2, data=df)
 lm2 = lm(log(RR) ~ mids, weights = 1 / se^2, data=df)
 p1 = summary(lm1)$coef[2,4]
 p2 = summary(lm2)$coef[2,4]
 slope = summary(lm2)$coef[2,1]
 se = summary(lm2)$coef[2,2]
 }
 return(c(p1,p2,slope,se))
 })
}

#####
# Kidney/PFOA (Including Rhee overall)
#per 10 ng/mL serum

kidney = list(
 shearer = data.frame(
 stlab = c("Shearer et al., 2020","","",""),
 labs = c("0-4 ng/mL","4-5.5 ng/mL", "5.5-7.3 ng/mL", "7.3-27.2 ng/mL"),
 cutpoints = c(0, 4.0, 5.5, 7.3), # max given as 27.2; sub in after lapply
 RR = c(1.0, 1.47, 1.24, 2.63),
 lower = c(1, 0.77, 0.64, 1.33),
 upper = c(1, 2.80, 2.41, 5.20)),
 vieira = data.frame(
 stlab = c("Vieira et al., 2013","","","",""),
 labs = c("0-3.7 ng/mL-yr","3.8-88 ng/mL-yr", "89-197 ng/mL-yr", "198-599 ng/mL-yr", "600-4679 ng/mL-yr"), #this are
categories cut-points taken from Table S1 (cumulative over 10 years)
 cutpoints = c(0, 3.8, 89, 198, 600) / 10, # max is given as 4679, sub in after lapply #divided by 10 because is 10 cumulative
exposure
 RR = c(1, 0.8, 1.2, 2.0, 2.1),
 lower = c(1, 0.4, 0.7, 1.3, 1.1),
 upper = c(1, 1.5, 2.0, 3.2, 4.2)),
 barry = data.frame(
 stlab = c("Barry et al., 2013","","",""),
 labs = c("0-219 ng/mL-yr", "219-812 ng/mL-yr", "812-5358 ng/mL-yr", ">5358 ng/mL-yr"),
 cutpoints = c(0, 219, 812, 5358) / 33, # ng/mL-yr / av age diag (divided by 33 because this is average length follow-up)
```

Table A3.7 (continued)

```
#cutpoints are reported in the meta-analysis by Bartell and Vieira (2021), in the R code within the supplement material but not
#in the original publication
  RR = c(1, 1.23, 1.48, 1.58),
  lower = c(1, 0.70, 0.84, 0.88),
  upper= c(1, 2.17, 2.60, 2.84)),
  steenland = data.frame(
  stlab = c("Steenland and Woskie, 2012","","",""),
  labs = c("0-904 ng/L-years", "904-1520 ng/mL-yr","1520-2700 ng/mL-yr", ">2700 ng/mL-yr"),
  cutpoints = c(0, 904, 1520, 2700) / 30, # Divided by 30 because of average follow-up
  RR = c(1.07, 1.37, 0.005, 2.66), # mortality; 3rd RR is 0 but cannot log
  lower = c(0.02, 0.28, 0.005, 1.15),
  upper= c(3.62, 3.99, 1.42, 5.24)),
  rhee = data.frame(
  stlab = c("Rhee et al., 2023","","",""),
  labs = c("0-3.27 ng/mL","3.27-4.47 ng/mL", "4.47-6.22 ng/mL", ">6.22 ng/mL"),
  cutpoints = c(0, 3.27, 4.47, 6.22), #
  RR = c(1.0, 1.26, 1.26, 1.04),
  lower = c(1, 0.80, 0.78, 0.60),
  upper = c(1, 1.97, 2.05, 1.81)),
  winquist = data.frame(
  stlab = c("Winquist et al., 2023","","",""),
  labs = c("0-3.9 ng/mL","3.9-5.2 ng/mL", "5.2-7.3 ng/mL", ">7.3"),
  cutpoints = c(0, 3.9, 5.2, 7.3), #
  RR = c(1.0, 0.93, 0.83, 1.20),
  lower = c(1, 0.56, 0.49, 0.71),
  upper = c(1, 1.56, 1.40, 2.04))
)

#calculate midpoints of the time-averaged serum PFOA categories within each study
kidney2 = lapply(kidney, function(df) {
  cp = df$cutpoints
  l = length(cp) + 1
  cp[l] = 2.5 * cp[l-1] # assume max is 2.5*last cutpoint
  df$mids = apply(rbind(cp[-l],cp[-1]),2,mean)
  return(df)
})
#in this we assumed that maximum is 2.5*last cutpoints, but in reality for some studies maximum is reported
kidney2$vieira$mids[5] = mean(c(600,4679)) / 10
kidney2$shearer$mids[4] = mean(c(7.3,27.2))
kidney2$winquist$mids[4] = mean(c(7.3,54))
kidney2$steenland$mids[4] = mean(c(2700,10800))/30 #assumed a maximum 4 times the highest cutpoint as more similar to
Vieira
kidney2$barry$mids[4] = mean(c(5358,21432))/33 ##assumed a maximum 4 times the highest cutpoint as more similar to Vieira

#apply ktrend function
(ktrend = trendp(kidney2))

# get RR and CI per 10 ng/mL increase in serum PFOA in each study
lapply(ktrend, function(df) round(exp(10*df[3] + 10*c(0,-1,1)*qnorm(.975)*df[4]),2))
klogRR = c(ktrend$vieira[3], ktrend$barry[3], ktrend$shearer[3], ktrend$steenland[3], ktrend$rhee[3], ktrend$winquist[3])
kse = c(ktrend$vieira[4], ktrend$barry[4], ktrend$shearer[4], ktrend$steenland[4], ktrend$rhee[4], ktrend$winquist[4])

(m2 = metagen(klogRR,kse)) # meta-analysis for kidney
round(exp(10*c(m2$TE.fixed,m2$lower.fixed,m2$upper.fixed)),2)
round(exp(10*c(m2$TE.random,m2$lower.random,m2$upper.random)),2)
```

Table A3.7 (continued)

```
m2$pval.Q
m2$tau2
m2$I2


#sensitivity taking out steenland and vieira
# get RR and CI per 10 ng/mL increase in serum PFOA in each study
lapply(ktrend, function(df) round(exp(10*df[3] + 10*c(0,-1,1)*qnorm(.975)*df[4]),2))
klogRR3 = c(ktrend$barry[3], ktrend$shearer[3], ktrend$rhee[3], ktrend$winquist[3])
kse3 = c( ktrend$barry[4], ktrend$shearer[4], ktrend$rhee[4], ktrend$winquist[4])

(m3 = metagen(klogRR3,kse3)) # meta-analysis for kidney
round(exp(10*c(m3$TE.fixed,m3$lower.fixed,m3$upper.fixed)),2)
round(exp(10*c(m3$TE.random,m3$lower.random,m3$upper.random)),2)

m3$pval.Q
m3$tau2
m3$I2
```

[a] Note that, if the code is copied from this document and pasted directly to R, the user may need to retype the quotation marks for the code to run correctly.

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ANNEX 4. SUPPLEMENTARY MATERIAL FOR SECTION 2, CANCER IN HUMANS

These supplementary online-only tables are available from: https://publications.iarc.who.int/636.

Please report any errors to imo@iarc.who.int.

The following tables were produced in draft form by the Working Group and were subsequently fact-checked but not edited:

Table S2.5	Epidemiological studies on exposure to PFOA or PFOS and cancers of the digestive tract
Table S2.6	Epidemiological studies on exposure to PFOA or PFOS and cancers of the brain, haematolymphoid system, and other cancers
Table S2.7	Epidemiological studies on exposure to PFOA or PFOS and cancers of all sites combined

ANNEX 5. SUPPLEMENTARY MATERIAL FOR SECTIONS 4.1 AND 4.2, MECHANISTIC EVIDENCE

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Please report any errors to imo@iarc.who.int.

The following tables were produced in draft form by the Working Group and were subsequently fact-checked but not edited:

Table S4.1	Calculations to derive dissociation constants, by the Working Group
Table S4.23	End-points relevant to modulation of receptor-mediated effects in humans exposed to PFOA or PFOS
Table S4.24	End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS
Table S4.25	End-points relevant to modulation of receptor-mediated effects in experi mental systems in vivo exposed to PFOA or PFOS
Table S4.26	End-points relevant to modulation of receptor-mediated effects (endocrine) in experimental systems in vivo exposed to PFOA and PFOS

ANNEX 6. SUPPLEMENTARY MATERIAL FOR SECTION 4.3, EVALUATION OF HIGH-THROUGHPUT IN VITRO TOXICITY SCREENING DATA

These supplementary online-only tables (available from: https://www.publications.iarc.who.int/636) contain summaries of the findings (including the assay name, the corresponding key characteristic, the resulting "hit calls" both positive and negative, and any reported caution flags) for those chemicals evaluated in the present volume that have been tested in high-throughput screening assays performed by the United States Environmental Protection Agency (US EPA) and the United States National Institutes of Health. The results were generated by the Working Group using the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) available from https://gitlab.com/i1650/kc-hits.git (Reisfeld et al., 2022), using the US EPA Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) assay data and the curated mapping of key characteristics to assays available at the time of the evaluations performed for *IARC Monographs* Volume 135. Data were available for perfluorooctanoic acid (PFOA), ammonium perfluorooctanoate (APFO), perfluorooctanesulfonic acid (PFOS), and potassium perfluorooctanesulfonate.

Please report any errors to imo@iarc.who.int.

1. Perfluorooctanoic acid (PFOA): ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens
2. Ammonium perfluorooctanoate (APFO): ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens
3. Perfluorooctanesulfonic acid (PFOS): ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens
4. Potassium perfluorooctanesulfonate: ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens

Reference

Reisfeld B, de Conti A, El Ghissassi F, Benbrahim-Tallaa L, Gwinn W, Grosse Y, et al. (2022). kc-hits: a tool to aid in the evaluation and classification of chemical carcinogens. *Bioinformatics*. 38(10):2961–2. doi:10.1093/bioinformatics/btac189 PMID:35561175

SUMMARY OF FINAL EVALUATIONS

Summary of final evaluations for Volume 135

Agent	Evidence stream			Overall evaluation
	Cancer in humans	**Cancer in experimental animals**	**Mechanistic evidence**	
Perfluorooctanoic acid (PFOA)	*Limited*	*Sufficient*	*Strong*[a]	Group 1
Perfluorooctanesulfonic acid (PFOS)	*Inadequate*	*Limited*	*Strong*[a]	Group 2B

[a] The mechanistic evidence was *strong* in exposed humans because PFOA and PFOS were found to induce epigenetic alterations and to be immunosuppressive.

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of two agents, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), and their corresponding isomers and salts.

PFOA and PFOS are per- and polyfluoroalkyl substances (PFAS) that are extremely resistant to degradation. First produced in the 1940s, PFOA has extensive uses, including in fluoropolymer manufacture and applications; in surface coatings conferring stain-, oil-, and water-resistance on household products, carpets, textiles, leather products, and food and feed packaging; in electrics and electronics; and in construction materials. With some similar uses to those of PFOA, PFOS additionally has applications in aqueous film-forming foams used in firefighting; in the fabrication of imaging devices and semiconductors; in photolithography and electroplating; and in insulation, dyes, and ink.

PFOA and PFOS occur ubiquitously in the environment, with high levels at pollution sources such as industrial sites and in firefighter-training areas and waste deposits. They may also be present in contaminated food, especially fish, seafood, and eggs. Occupationally exposed populations can have high levels of exposure, mainly via inhalation. The general population in contaminated areas is mainly exposed via drinking-water, and the general population in communities that are not near pollution sources is mainly exposed via diet and drinking-water.

An *IARC Monographs* Working Group reviewed evidence from epidemiological studies, cancer bioassays in experimental animals, and mechanistic studies to assess the carcinogenic hazard to humans of exposure to these agents and concluded that:

- PFOA is *carcinogenic to humans (Group 1)*;
- PFOS is *possibly carcinogenic to humans (Group 2B)*.

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