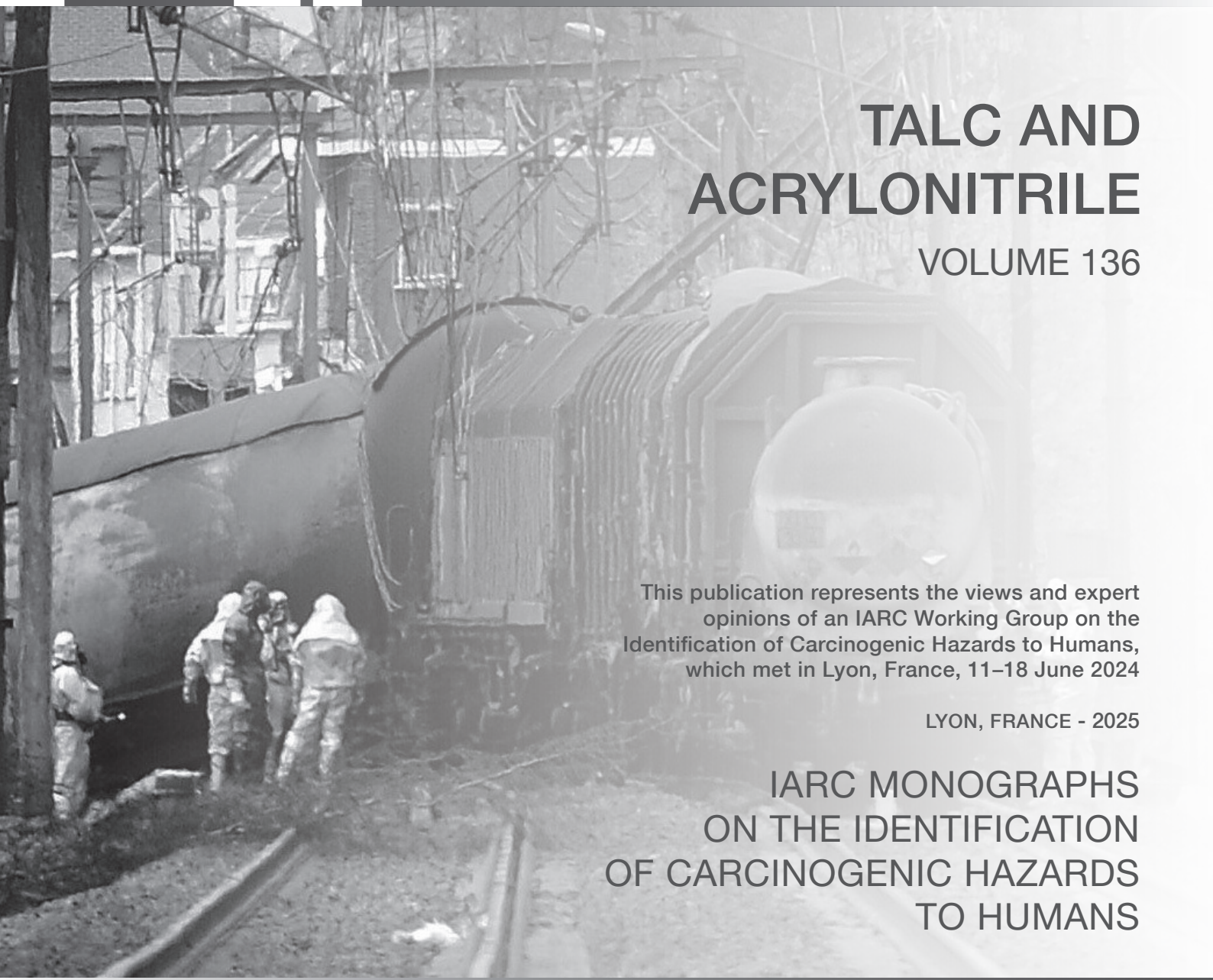
IARC MONOGRAPHS



TALC AND ACRYLONITRILE

VOLUME 136

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, 11–18 June 2024

LYON, FRANCE - 2025

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

ACRYLONITRILE

1. Exposure Characterization

1.1 Identification of the agent

Acrylonitrile was previously evaluated in 1998 when it was classified in Group 2B, *possibly carcinogenic to humans* (Volume 71; IARC, 1999).

In previous evaluations by the *IARC Monographs*, the acrylonitrile-derived copolymers acrylonitrile–butadiene–styrene (ABS) copolymers and styrene–acrylonitrile were each classified in Group 3, *not classifiable as to its carcinogenicity to humans* (Volume 19 and Supplement 7; IARC, 1979, 1987), although these agents were not considered in the present evaluation.

1.1.1 Nomenclature

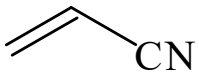
Chem. Abstr. Serv. Reg. No.: 107-13-1 (NCBI, 2024)

EC/List No.: 203-466-5 (ECHA, 2023a)

IUPAC systematic name: prop-2-enenitrile (ECHA, 2023b)

Synonyms: acrylonitrile; 2-propenenitrile; vinyl cyanide; propenenitrile; prop-2-enenitrile; cyanoethylene (ECHA 2023a; NCBI, 2024).

1.1.2 Structural and molecular information



Molecular formula: C_3H_3N (NCBI, 2024)

Relative molecular mass: 53.06 (NCBI, 2024).

1.1.3 Chemical and physical properties

Description: acrylonitrile is a colourless to pale-yellow liquid with a pungent garlic-like odour. The odour threshold values ranged from [3.5 to 47.7 mg/m^3] (AIHA, 2013; NCBI, 2024).

Boiling-point: 77 °C (NCBI, 2024)

Melting-point: −84 °C (NCBI, 2024)

Flash-point: 0 °C at 101.3 kPa [some slightly different values have been reported in the literature] (ECHA, 2023b; NCBI, 2024)

Density: 0.8 g/mL at 20 °C (NCBI, 2024)

Vapour pressure: 11 kPa at 20 °C (NCBI, 2024)

Solubility: 70 g/L in water at 24 °C; very soluble in ethanol, acetone, benzene, and ether (IARC, 1999; NCBI, 2024)

Octanol/water partition coefficient (P): *log* K_{ow} = 0.25 (NCBI, 2024)

Stability: undiluted acrylonitrile is generally stabilized against polymerization with trace

levels of hydroquinone monomethyl ether and water. (NCBI, 2024); acrylonitrile is reported to be stable under standard conditions in several sources (NCBI, 2024; Royal Society of Chemistry, 2024); polymerization may be caused by elevated temperature or strong bases (NCBI, 2024).

[The Working Group used a conversion factor of 1 ppm ≈ 2.17 mg/m^3 at 25 °C in air.]

1.1.4 Technical grade and impurities

Acrylonitrile with a purity of > 99% by weight is available commercially (NCBI, 2024). Reported impurities include acetone, acetonitrile, hydrogen cyanide, methacrylonitrile, aldehydes, iron, and water (ECHA, 2023b; NCBI, 2024). Hydroquinone monomethyl ether is used as a stabilizer to prevent polymerization (NCBI, 2024).

1.2 Production and use

1.2.1 Production process

The French chemist Charles Moureu first synthesized acrylonitrile in 1893 (Moureu, 1893). The most common industrial production method is ammoxidation, which was introduced in the late 1950s. In this method, propylene, ammonia, and air (used as an oxidizer) are passed through a fluidized bed reactor that contains a catalyst at temperatures of 400–510 °C and pressures of 50–200 $kPa_{g,}$ (Gupta and Afshari, 2018), and the reaction is then quenched in sulfuric acid. The resulting aqueous solution is a mixture of acrylonitrile, acetonitrile, hydrocyanic acid, and ammonium sulfate. Water is removed using a recovery column, and acrylonitrile is isolated by distillation (Grasselli, 2014; Grasselli and Trifirò, 2016). Acrylonitrile is currently made from fossil resources (Mack et al., 2020), but several improvements have been implemented in the process, with the aim of exploring and developing more sustainable approaches. Some of these are from renewable feedstocks, such as lignocellulosic biomass, glycerol, or glutamic acid. Fermentation of the lignocellulosic biomass results in propionic acid and 3-hydroxypropionic acid, which are then converted to acrylonitrile by dehydration and ammoxidation (Grasselli and Trifirò, 2016; Karp et al., 2017; Davey, 2018; Trangwachirachai and Lin, 2023). Mack et al. (2020) also described the production of acrylonitrile from biobased propionic acid (which can be produced through fermentation) in only two steps: first, nitrilation of propionic acid to propionitrile, and second, dehydrogenation of propionitrile to acrylonitrile (Mack et al., 2020).

1.2.2 Production volume

Acrylonitrile is a chemical with a high production volume (OECD, 2021; US EPA, 2024). In 2022, the leading exporters of acrylonitrile [probably also related to the main producers in the world], were the USA ([373 527 tonnes]), Republic of Korea ([235 489 tonnes]), China ([217 390 tonnes]), and Germany ([155 259 tonnes]) (World Bank, 2024). In 2019, production of acrylonitrile in the USA was about 1.08 million tonnes (Statista, 2023).

The Asia–Pacific region is the largest producer and consumer of acrylonitrile and accounts for almost 70% of the global installed production capacity of acrylonitrile, followed by North America (18%), western Europe (7%), and the rest of world (nearly 5%) (Shah, 2023). [The Working Group noted that production data by country were not available.]

The global market for acrylonitrile reached approximately [5.9 million tonnes] in 2022. One of the main drivers of the global market is the production of ABS, which is extensively used in the electronics and automotive industries (ChemAnalyst, 2023).

In the European Union, acrylonitrile is registered under the Registration, Evaluation,

Authorisation and Restriction of Chemicals (REACH) regulation and is manufactured in and/or imported to the European Economic Area at volumes of ≥ 1 000 000 to 10 000 000 tonnes per year (ECHA, 2023a).

1.2.3 Uses

Acrylonitrile is mainly used as a monomer to prepare the homopolymer, polyacrylonitrile, and copolymers, such as ABS, styrene–acrylonitrile (SAN), acrylonitrile–styrene–acrylate (ASA), and synthetic rubbers (Royal Society of Chemistry, 2024) such as acrylonitrile–butadiene (NBR). Hydrodimerization of acrylonitrile (Ellis, 1972; Buxton et al., 1979) provides adiponitrile for the synthesis of certain polyamides, and acrylonitrile is also a precursor in the manufacture of acrylamide and acrylic acid (Brazdil, 2012).

Acrylonitrile is used extensively in the manufacture of synthetic fibres, being the primary component in the production of acrylic fibres used in clothing, carpets, and other textiles. In the production of plastics, acrylonitrile is a key component in the production of ABS, which is used in a wide range of consumer products, including toys, electronics, and automotive parts. Acrylonitrile is used in the production of adhesives, which are used in a variety of industrial applications. Acrylonitrile is also used in the production of resins, which are used in the construction, marine, and aerospace industries (IARC, 1999; NTP, 2016). Acrylic acid–acrylonitrile copolymer membranes produced by molecular imprinting technology are used for clinical applications, e.g. blood filtration devices (Cristallini et al., 2004). ABS is used also for filament fabrication (FFF) in three-dimensional (3D) printing techniques (Chohan et al., 2020).

In the 1940s, acrylonitrile mixed with carbon tetrachloride was used as a pesticide to fumigate food and to control pests in stored cereals (ATSDR, 2021). However, all pesticide uses of acrylonitrile have since stopped; for example, this use ceased in 1976 in Canada (Environment and Climate Change Canada, 2000; ILO/WHO, 2002).

The uses mentioned have different demands for acrylonitrile, as described in Fig. 1.1.

In Europe, acrylonitrile is registered for use in the following areas: formulation of mixtures and/or re-packaging, health services, and scientific research and development. More specifically, acrylonitrile is registered for the following uses: manufacture of chemicals, rubber products, and plastic products (ECHA, 2023a).

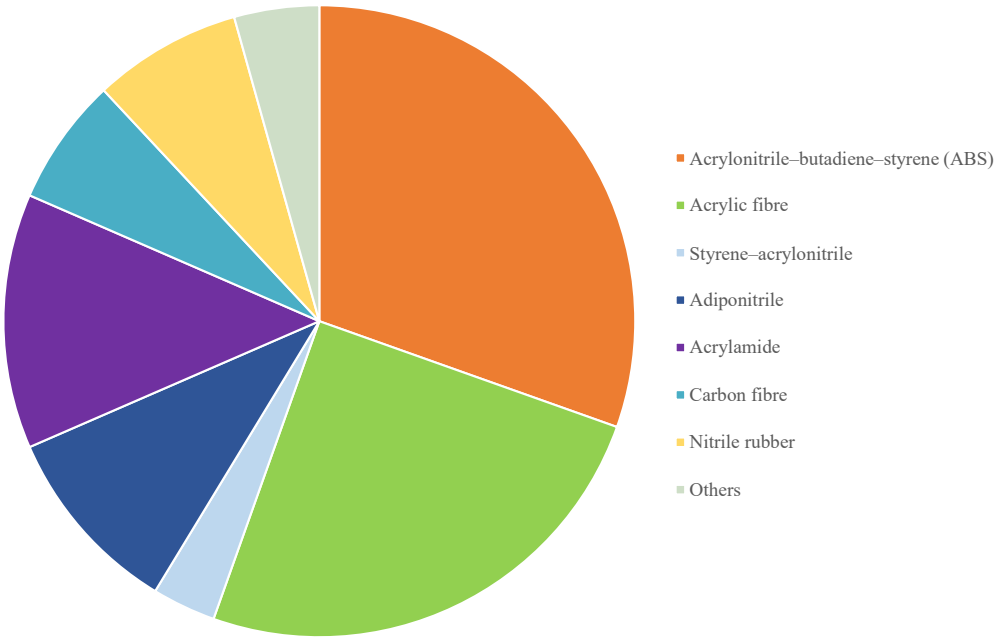
1.3 Detection and quantification

1.3.1 Air

The quantification of acrylonitrile in air involves collection of air using a sampler, followed by processing and analysis in a laboratory (time-integrated sampling) or real-time monitoring in situ. As shown in Table 1.1, method limits of detection (LODs) for early sorbent-tube sampling methods were up to 2200 μg/m^3 (Baxter, 1979). [The Working Group noted that a method LOD of 2200 μg/m^3 is similar to or higher than current exposure limits in many countries (see Section 1.5).] By the 1990s to early 2000s, standard methods were developed for sorbent-tube sampling followed by solvent extraction and flame ionization detection (FID) or nitrogen/phosphorous detection (NPD), with method LODs of approximately 25–50 μg/m^3 (NIOSH, 1994; OSHA, 2001). More recently, a method was published that uses porous polymer sorbent-tube sampling followed by thermal desorption and FID or mass spectrometry (MS) detection, with a method LOD of 4.2 μg/m^3 (Tschickardt et al., 2023). Method LODs are lowest for time-integrated evacuated canister sampling followed by preconcentration and MS detection (0.002–0.21 μg/m^3).

As summarized in Table 1.1, for time-integrated sampling, air is: (i) drawn into an

Fig. 1.1 Demand for acrylonitrile by type of use, as a proportion of market share



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evacuated canister by pressure difference relative to the atmosphere (Ochiai et al., 2003; Jia and Foran, 2013; He et al., 2015; Hsu et al., 2022; Leníček et al., 2022; Han et al., 2023); (ii) drawn into a bag (Rössner et al., 2002; Hsu et al., 2007); (iii) pulled through a tube that contains a sorbent such as silica gel (Sakurai et al., 1978), charcoal (Baxter, 1979; Cicolella et al., 1981; Borders et al., 1986; Major et al., 1998; Perbellini et al., 1998; Zey et al., 2002; Sajedian et al., 2023), unspecified resin (Baxter, 1979), porous polymer beads (Campbell and Moore, 1979; Benson and Boyce, 1981; Zey et al., 2002; Cirla et al., 2007), a dual bed of porous polymer beads and porous carbon molecular sieve (Borders et al., 1986), or carbonized polymer beads (Borders et al., 1986) using a sampling pump; or (iv) passively diffused onto a sorbent such as porous polymer beads (Benson and Boyce, 1981; Benson et al., 1981), carbon (Muto et al., 1992; Perbellini et al., 1998), or unspecified sorbent (Tuček et al., 2002; Zey et al., 2002; Leníček et al., 2022). Evacuated canister sampling has generally been used for environmental monitoring because it does not require the use of a pump, thereby permitting continuous sampling for many hours (Ochiai et al., 2003; Jia and Foran., 2013; He et al., 2015; Hsu et al., 2022; Leníček et al., 2022; Han et al., 2023). Drawing air into a bag for subsequent laboratory analysis was used for stack sampling of acrylonitrile emissions into the atmosphere (Hsu et al., 2007). Sorbent tubes and passive samplers were most often used in occupational settings to determine short-term or time-weighted average (TWA) personal breathing zone exposures and area air concentrations of acrylonitrile.

Regardless of the technique used for time-integrated sample collection (canister, bag, sorbent tube, passive sampler), quantification of acrylonitrile was achieved using gas chromatography (GC) coupled to a detector such as MS (Tuček et al., 2002; Ochiai et al., 2003; Jia and Foran., 2013; He et al., 2015; Hsu et al., 2022; Leníček et al., 2022; Han et al., 2023), NPD (Benson et al., 1981; Borders et al., 1986; Zey et al., 2002), or FID (Campbell and Moore, 1979; Benson and

Table 1.1 Analytical methods for the measurement of acrylonitrile in air

Sample preparation	Analytical method (LOD, µg/m³)	Comment	Reference
Evacuated canisters			
Preconcentration	GC-MS (0.01)	Evaluated method validity	Ochiai et al. (2003)
Preconcentration	GC-MS (0.21)		He et al. (2015)
Preconcentration	GC-MS (0.05)	Polished stainless-steel canister	Hsu et al. (2022)
Preconcentration	GC-MS (0.002)	Canister with inert lining	Han et al. (2023)
Preconcentration	GC (NR)	US EPA Method TO-14	US EPA (1999a)
Preconcentration	GC-MS (NR)	US EPA Method TO-15	US EPA (1999b)
Preconcentration	GC-MS (NR)		Leníček et al. (2022)
Bag sampling			
NR	GC (NR)	No information on collection or analysis	Rössner et al. (2002)
NR	GC-FID (NR)	Bag made of inert material	Hsu et al. (2007)
Direct injection	GC (NR)	US EPA Method 18	US EPA (2019)
Sorbent tubes			
Water extraction	GC (NR)	Silica gel sorbent	Sakurai et al. (1978)
Carbon disulfide extraction	GC ([2200])	Charcoal sorbent	Baxter (1979)
Thermal desorption	GC ([220])	Resin sorbent	Baxter (1979)
Thermal desorption	GC-FID (NR)	Porous polymer sorbent	Campbell and Moore (1979)
Thermal desorption	GC-FID (NR)	Porous polymer sorbent	Benson and Boyce (1981)
Methanol extraction	GC-FID (NR)	Charcoal sorbent	Cicolella et al. (1981)
Acetone/carbon disulfide extraction	GC-FID (NR)	Charcoal sorbent	Borders et al. (1986)
Acetone/carbon disulfide extraction	GC-NPD (NR)	Charcoal sorbent	Borders et al. (1986)
Thermal desorption	GC-FID (NR)	Porous polymer/carbon molecular sieve	Borders et al. (1986)
Acetone/carbon disulfide extraction	GC-FID (NR)	Carbonized polymer sorbent	Borders et al. (1986)
Acetone/carbon disulfide extraction	GC-NPD (NR)	Carbonized polymer sorbent	Borders et al. (1986)
Thermal desorption	GC-FID (NR)	HSE Method 72 (porous polymer sorbent)	HSE (1993)
Acetone/carbon disulfide extraction	GC-FID (1 µg/sample)	NIOSH Method 1604 (charcoal sorbent)	NIOSH (1994)
Acetone extraction	GC-FID (NR)	Charcoal sorbent	Major et al. (1998)
Ethanol extraction	GC-FID (NR)	Charcoal sorbent	Perbellini et al. (1998)
Acetone extraction	GC-NPD (26)	OSHA Method 37 (charcoal sorbent)	OSHA (2001)
Methanol extraction	GC-NPD (NR)	Charcoal sorbent	Zey et al. (2002)
NR	NR (NR)	Porous polymer sorbent	Zey et al. (2002)
Thermal desorption	GC-FID (10)	Porous polymer sorbent	Cirla et al. (2007)
Benzene extraction	GC-FID (NR)	NIOSH Method 1604 (charcoal sorbent)	Sajedian et al. (2023)
Thermal desorption	GC-FID or GC-MS (4.2)	Porous polymer sorbent	Tschickardt et al. (2023)
Diffusion			
Thermal desorption	GC-FID (NR)	Porous polymer sorbent	Benson and Boyce (1981)
Thermal desorption	GC-NPD (NR)	Porous polymer sorbent	Benson et al. (1981)
Carbon disulfide extraction	GC (NR)	Carbon wafer	Muto et al. (1992)

Table 1.1 (continued)

Sample preparation	Analytical method (LOD, µg/m³)	Comment	Reference
Ethanol extraction	GC-FID (NR)	Three carbon filters	Perbellini et al. (1998)
Thermal desorption	GC-FID (NR)	NR	Tuček et al. (2002)
Thermal desorption	GC-MS (200)	NR	Tuček et al. (2002)
NR	NR (NR)	NR	Zey et al. (2002)
Thermal desorption	GC-MS (NR)	US EPA calculator for diffusion coefficients	Leníček et al. (2022)
Real-time monitoring			
No preparation (online analysis)	GC ([2200])	Spot samples of workplace air	Sakurai et al. (1978)
No preparation (online analysis)	GC-FID (NR)	Spot samples of workplace air	Cicolella et al. (1981)
No preparation (online analysis)	GC (NR)	Spot samples of workplace air	Kaneko and Omae (1992)
No preparation (online analysis)	OP-FTIR (NR)	NR	Malachowski et al. (1994)
No preparation (online analysis)	OP-FTIR (NR)	Spot and continuous samples of workplace air	Chan et al. (2006)
No preparation (online analysis)	OP-FTIR (NR)	US EPA Method TO-16	US EPA (1999c)

GC, gas chromatography; GC-FID, gas chromatography-flame ionization detection; GC-MS, gas chromatography-mass spectrometry; GC-NPD, gas chromatography-nitrogen/phosphorous detection; HSE, Health and Safety Executive; LOD, limit of detection; MS, mass spectrometry; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; OP-FTIR, open-path Fourier transform infrared; OSHA, Occupational Safety and Health Administration; US EPA, United States Environmental Protection Agency.

Boyce, 1981; Benson et al., 1981; Cicolella et al., 1981; Borders et al., 1986; Major et al., 1998; Perbellini et al., 1998; Tuček et al., 2002; Cirla et al., 2007; Hsu et al., 2007; Sajedian et al., 2023). Air collected in canisters or bags was preconcentrated then transferred to a GC system (Ochiai et al., 2003; Jia and Foran, 2013; He et al., 2015; Hsu et al., 2022; Leníček et al., 2022; Han et al., 2023) or injected directly into a GC system (Hsu et al., 2007). Extraction from sorbent tubes or passive samplers was carried out with water (Sakurai et al., 1978) or an organic solvent such as carbon disulfide (Baxter, 1979; Muto et al., 1992), acetone (Major et al., 1998), ethanol (Perbellini et al., 1998), benzene (Sajedian et al., 2023), or methanol (Cicolella et al., 1981; Zey et al., 2002); or thermally desorbed (Baxter, 1979; Campbell and Moore, 1979; Benson and Boyce, 1981; Benson et al., 1981; Borders et al., 1986; Tuček et al., 2002; Cirla et al., 2007; Leníček et al., 2022) and then injected into a GC system for analysis.

Existing standard validated methods for the quantification of acrylonitrile in workplace air include United States (US) National Institute for Occupational Safety and Health (NIOSH) Method 1604 – charcoal tube sampler and GC-FID analysis (NIOSH, 1994), US Occupational Safety and Health Administration (OSHA) Method 37 – charcoal tube sampler and GC-NPD analysis (OSHA, 2001), and United Kingdom (UK) Health and Safety Executive (HSE) Methods for Determination of Hazardous Substances 72 – porous polymer tube sampler and GC-FID analysis (HSE, 1993). Most recently, the Expert Committee on Raw Materials and Chemical Industry of the German Social Accident Insurance developed a method that uses a porous polymer sorbent-tube sampler and GC-FID or GC-MS analysis, which was

validated by the Working Group of the DFG Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, or MAK commission (Tschickardt et al., 2023). Existing standard validated methods for the quantification of acrylonitrile in ambient air include United States Environmental Protection Agency (US EPA) Method TO-14 – evacuated canister sampler and GC analysis with appropriate detector (US EPA, 1999a), US EPA Method TO-15 – evacuated canister sampler and GC-MS analysis (US EPA, 1999b), and US EPA Method 18 – evacuated bag sampler and GC analysis with appropriate detector (US EPA, 2019).

Quantification of acrylonitrile can also be achieved on a continuous basis using real-time or near real-time monitoring in situ. A standard validated method for the quantification of acrylonitrile in ambient air by portable GC is US EPA Method TO-16 (US EPA, 1999c). Cicolella et al. (1981) used a portable GC-FID to measure acrylonitrile levels in real time at factories in France. Previously, Sakurai et al. (1978) then Kaneko and Omae (1992) used portable GC systems to measure acrylonitrile levels in real time at acrylic fibre-manufacturing factories in Japan. Others have used open path-Fourier transform infrared spectroscopy for ambient (outdoor) real-time monitoring of acrylonitrile in air at a chemical processing plant (Malachowski et al., 1994) and at a petrochemical complex (Chan et al., 2006).

1.3.2 Water and other matrices

Examples of methods for the analysis of acrylonitrile in water, food, and food-contact materials are presented in Table 1.2.

Generally, the sampling of acrylonitrile in water is performed by drawing a defined volume of water through an adsorbent. Depending on the adsorbent, the acrylonitrile is eluted from the adsorbent by liquid extraction or thermodesorption. Acrylonitrile is then separated and detected by GC techniques. These methods obtained LODs in the range of 0.22 to 0.5 µg/L. For the determination of acrylonitrile in solid materials, e.g. food packaging and solid foods, headspace techniques are applied for the transfer from the material to a chromatographic system. For these methods, LODs in the range of 0.1 to 0.5 µg/kg raw material were reported. Special guidelines are available to counteract the challenges posed by complex matrices (Marano et al., 1978; Vassilaros et al., 1991).

1.3.3 Human biospecimens

Human exposure to acrylonitrile can be monitored by measuring the specific metabolites *S*-(2-cyanoethyl) mercapturic acid (CEMA, *N*-acetyl-*S*-(2-cyanoethyl)cysteine) and *S*-(1-cyano-2-hydroxyethyl) mercapturic acid (CHEMA, *N*-acetyl-*S*-(1-cyano-2-hydroxyethyl) cysteine) in urine or *N*-(2-cyanoethyl)valine (CEV), which represents a specific acrylonitrile adduct at the terminal position of the globin chains in haemoglobin (Jäger et al., 2021; Christ et al., 2023). 2-Hydroxyethylmercapturic acid (HEMA, *N*-acetyl-*S*-(2-hydroxyethyl)-L-cysteine), another metabolite of acrylonitrile, and also of other compounds such as vinyl chloride, is less specific for acrylonitrile but is used to assess acrylonitrile exposure in combination with more specific markers such as CEMA. Details on the absorption, distribution, metabolism, and elimination of acrylonitrile, including the aforementioned metabolites and adducts, are described in Section 4.1. [In the literature, CEMA is also referred to as CYMA, CNEMA, CYEMA, or 2CyEMA. CHEMA is also referred to as 1CyHEMA. Abbreviations for HEMA include 2HEMA.] CEMA in urine and CEV in blood represent well-validated biomarkers of exposure to acrylonitrile because of their high specificity and sensitivity. The European Commission recommends the use of the adduct CEV in blood because of its high specificity (ECHA, 2018). Additionally, this adduct has a lifespan of

Table 1.2 Selected analytical methods for the measurement of acrylonitrile in different matrices

Sample matrix	Sample procedure	Analytical method	LOD or LOQ	Reference
Water	Purge (inert gas); trap (porous polymer); thermodesorption	GC-FID	0.5 µg/L (LOD)	US EPA (1996) (Method 603)
	Addition of internal standard; purge (inert gas); trap (porous polymer); thermodesorption	GC-MS	0.22 µg/L (LOD)	US EPA (1995) (Method 524.2)
Food packaging	Dispersion of the bulk material in *N,N*-dimethylacetamide; transfer to headspace vial	Headspace-GC	0.5 mg/kg (LOD)	ASTM International (2001) (Method D 4322-96)
	Raw granules; transfer to headspace vial	Dynamic headspace-trapping-GC-FID	0.1 µg/kg (LOD)	Forero et al. (2023)
Solid foods	Cutting in chilled blender; transfer to headspace vial	Headspace-GC-NPD	2 µg/kg (LOD)	Page and Charbonneau (1985)

GC, gas chromatography; GC-FID, gas chromatography-flame ionization detection; GC-MS, gas chromatography-mass spectrometry; GC-NPD, gas chromatography-nitrogen/phosphorus detection; LOD, limit of detection; LOQ, limit of quantification.

100–130 days (the lifespan of erythrocytes in the blood), which means that samples do not need to be collected immediately after an exposure event (Huizer et al., 2014).

Exposure monitoring by CEMA analysis requires the collection of a urine sample at the end of the exposure interval (Jäger et al., 2021). The German MAK commission has not set a biological monitoring guidance value (as it considered acrylonitrile to be a carcinogen) but has published a correlation between air and adduct levels and biological reference values for both parameters (Jäger et al., 2021). A quality assurance scheme for analytical laboratories that measure CEV in blood and CEMA in urine is available through the German External Quality Assessment Scheme (G-EQUAS) based on the guidelines of the German Medical Association (G-EQUAS, 2024).

Several reliable analytical procedures that are validated for the determination of CEMA in urine have been developed; all of them are based on liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) combined with isotope dilution (Table 1.3). Most of the procedures monitor the resulting ions in negative mode. For sample preparation, either column-switching solid-phase extraction (online-SPE) (Schettgen et al., 2009, 2012; Hou et al., 2012; Zhang et al., 2014; Chiang et al., 2015; Pluym et al., 2015) or a dilute-and-shoot procedure (Alwis et al., 2012; Wu et al., 2012; Xiaotao et al., 2014; Frigerio et al., 2019) has been applied. In one procedure, a coupling of liquid–liquid extraction, SPE, and derivatization with analysis by LC-MS/MS was performed (Scherer et al., 2010). All procedures offered high reliability and adequate sensitivity. The limits of quantification (LOQs) ranged between 0.1 and 1 ng/mL in general. One of these methods also included the determination of CHEMA in urine and provided an LOQ of 1 ng/mL for this parameter (Wu et al., 2012).

Exposure monitoring by CEV analysis requires blood sampling followed by timely separation and washing of the erythrocyte fraction (Tavares et al., 1996). The washed erythrocyte fraction can be stored until further processing and transport. Sampling time is less critical for CEV because of the slow kinetics of this parameter (Huizer et al., 2014). The next step of sample processing is the isolation of globin by precipitation. For the determination of CEV, specific cleavage of the adduct-carrying terminal amino

Table 1.3 Selected methods for biomonitoring of acrylonitrile

Parameter	Sample procedure	Analytical method	LOD or LOQ	Reference
CEMA in urine	Online-SPE	LC-ESI(–)-MS/MS	0.077 µg/L (LOD)	Chiang et al. (2015)
	Online-SPE	LC-ESI(–)-MS/MS	0.25 µg/L (LOQ)	Pluym et al. (2015)
	Online-SPE	LC-ESI(–)-MS/MS	0.015 µg/L (LOQ)	Zhang et al. (2014)
	Online-SPE	LC-ESI(–)-MS/MS	0.003 µg/L (LOQ)	Hou et al. (2012)
	Online-SPE	LC-ESI(–)-MS/MS	1 µg/L (LOQ)	Schettgen et al. (2009)
	Online-SPE	LC-ESI(–)-MS/MS	1 µg/L (LOQ)	Schettgen et al. (2012)
	Dilute-and-shoot injection	LC-ESI(–)-MS/MS	0.9 µg/L (LOQ)	Frigerio et al. (2019)
	Dilute-and-shoot injection	LC-ESI(–)-MS/MS	0.01 µg/L (LOD)	Xiaotao et al. (2014)
	Dilute-and-shoot injection	LC-ESI(–)-MS/MS	0.5 µg/L (LOD)	Alwis et al. (2012)
	Dilute-and-shoot injection	LC-ESI(+)-MS/MS	0.1 µg/L (LOQ)	Wu et al. (2012)
	LLE, SPE, derivatization	LC-ESI(+)-MS/MS	1.5 µg/L (LOQ)	Scherer et al. (2010)
CHEMA in urine	Dilute-and-shoot injection	LC-ESI(+)-MS/MS	1 µg/L (LOQ)	Wu et al. (2012)
CEV in blood	Isolation of erythrocytes; precipitation of globin; Edman degradation	GC-NCI-MS/MS	0.5 pmol/g globin (LOD)	Schettgen et al. (2010)
	Isolation of erythrocytes; precipitation of globin; Edman degradation	GC-NCI-MS/MS	1.5 pmol/g globin (LOQ)	Schettgen et al. (2016)
	Isolation of erythrocytes; precipitation of globin; Edman degradation	GC-NCI-MS/MS	60 pmol/g globin (LOQ)	Christ et al. (2023)

+ or –, positive or negative ionization mode; CEMA, *S*-(2-cyanoethyl) mercapturic acid; CEV, *N*-(2-cyanoethyl)valine; CHEMA, *S*-(1-cyano-2-hydroxyethyl) mercapturic acid; ESI, electron spray ionization (either in positive or negative mode); GC, gas chromatography; LLE, liquid–liquid extraction; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NCI, negative chemical ionization; SPE, solid-phase extraction.

acid by reaction with pentafluorophenyl isothiocyanate (Edman degradation) has been established (Osterman-Golkar et al., 1994; Schettgen et al., 2010, 2016; Christ et al., 2023). Moreover, the standard procedure features calibration with a dipeptide anilide reference compound, an isotope dilution, as well as the analytical determination of the reaction products by gas chromatography-negative chemical ionization-tandem mass spectrometry (GC-NCI-MS/MS) (Schettgen et al., 2010, 2016; Christ et al., 2023). The LOQs for CEV range between 0.5 and 60 pmol/g globin (see Table 1.3). [The Working Group noted that the latter LOQ may not be sufficiently sensitive for the detection of general background exposure in non-smokers.]

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

(a) Air

The presence of acrylonitrile in ambient air and indoor environments is mainly attributed to anthropogenic sources, but it can also occur naturally as a product of biomass combustion (ATSDR, 2023).

(i) Ambient air

Sources of acrylonitrile in ambient air include vehicle exhaust emissions, industrial emissions, solid waste facilities, and biomass burning (e.g. tobacco smoking, wildland fires) (see Tables 1.4 and 1.5). Acrylonitrile in air reacts primarily with hydroxyl radicals in the atmosphere (Atkinson et al., 1982), and its half-life

Table 1.4 Concentrations of acrylonitrile in ambient air

Location and site	Period	No. of samples	Concentration (μg/m³)			Comment	Reference
			Average (± SD)	Minimum	Maximum		
Japan Air-monitoring network	2004–2012	NR	0.073–0.11	NR	NR	Annual averages	Tsai (2016)
South-western Memphis, USA	2008–2010	NR	0.55	NR	26.11	60% of samples < LOD	Jia and Foran (2013)
Tokyo, Japan Suburban roadside	2002	14 2	[0.09] [0.08]	[0.05] NR	[0.13] NR	24-h daily value Weekly value	Ochiai et al. (2003)
Hong Kong Special Administrative Region, China Roadside	2013	60 60	[0.86 ± 0.09] [0.70 ± 0.20]	NR NR	[1.48] [1.34]	Morning rush hour (t = 2 h) Afternoon rush hour (t = 2 h)	Han et al. (2023)
Metropolitan Chicago, USA	2011	NR	0.13	NR	NR	36% of samples < LOD	Strum and Scheffe (2016)
Roxbury, MA, USA	2011	NR	0.011	NR	NR	98% of samples < LOD	
Underhill, VT, USA	2011	NR	0.006	NR	NR	90% of samples < LOD	
Richmond, VA, USA	2011	NR	0.002	NR	NR	100% of samples < LOD	
Taipei City, Taiwan, China	2021–2022	4 4	0.012 ± 0.009 0.004 ± 0.001	NR NR	NR NR	Level 3 COVID-19 warning Level 2 COVID-19 alert	Yen et al. (2023)
Taiwan, China Petrochemical complex	1997–1999	NR	[95.8]	NR	[9248]	Outdoor low estimate[a]	Chan et al. (2006)
Taiwan, China Petrochemical complex	2005–2009	NR	[< 1]	NR	NR	Simulated annual average	Chen et al. (2016)
Taiwan, China Petrochemical complex	2015–2018	NR	ND [0.13 ± 0.62] [1.37 ± 2.58] [0.02 ± 0.48] [0.02 ± 0.18] [1.30 ± 4.25] [0.02 ± 0.29]	NR ND ND ND ND ND ND	NR [11.0] [13.2] [7.0] [2.64] [36.1] [3.74]	Site A (1 station) Site B (8 stations) Site C (1 station) Site D (1 station) Site E (1 station) Site F (1 station) Site G (4 stations)	Hsu et al. (2022)
Spain Tarragona North Industrial Complex	2017–2018	NR	1.26 1.14 2.30 1.96 15.78 47.11 96.97	0.46 0.51 0.80 0.75 2.17 12.36 19.33	2.3 2.1 4.2 4.4 56.5 126.9 142.4	Zone A (P1 site) Zone A (P2 site) Zone A (P3 site) Zone A (P4 site) Zone A (P5 site) Zone A (P6 site) Zone A (P7 site)	Vallecillos et al. (2019)

Table 1.4 (continued)

Location and site	Period	No. of samples	Concentration (µg/m³)			Comment	Reference
			Average (± SD)	Minimum	Maximum		
			24.20	8.45	73.2	Zone A (P8 site)	Vallecillos et al. (2019) (cont.)
			32.48	1.71	118.6	Zone B (P9 site)	
			0.75	0.21	1.9	Zone B (P10 site)	
			0.85	0.13	2.7	Zone B (P11 site)	
			0.70	0.09	2.1	Zone B (P12 site)	
			7.85	0.58	21.9	Zone B (P13 site)	
			4.69	0.66	13.4	Zone B (P14 site)	
			8.31	1.05	21.3	Zone B (P15 site)	
			4.52	0.60	12.0	Zone B (P16 site)	
Sarnia, ON, Canada NBR-production plant	NR	6	< 52.9	NR	NR	Outside fence line	WHO (2002)
Cobourg, ON, Canada Chemical manufacturing	1993	NR	NR	0.12	0.28	Near facility (t = 6 days)	
Cobourg, ON, Canada Chemical manufacturing	1993		NR	< 251	100 763	Stack sample	
ON, Canada Urban air-monitoring sites	1990	11	NR	NR	1.9	91% of samples < LOD	
Windsor, ON, Canada	1991	7	ND	NR	NR	Industrialized area	
Toronto, ON, Canada	NR	16	ND	NR	NR	Downtown	
	NR	7	ND	NR	NR	Residential	
South-western China Rural solid waste transfer station	NR	10	627.5	NR	NR	Five monitoring sites	Chai et al. (2022)
China Domestic waste landfills	NR	NR	1690	NR	NR	Dumping area (south)	Li et al. (2023)
			5525	NR	NR	Leachate pool (south)	
			NR	NR	NR	Dumping area (north)	
			80.2	NR	NR	Leachate pool (north)	
Western states, USA	2018	346	[0.2]	[0.01]	[10]	Young wildfire smoke	O'Dell et al. (2020)
		463	[0.1]	[0.01]	[0.8]	Medium wildfire smoke	
		94	[0.05]	[0.005]	[0.08]	Old wildfire smoke	

ABS, acrylonitrile–butadiene–styrene; COVID-19, coronavirus disease; h, hour(s); LOD, limit of detection; MA, Massachusetts; NBR, nitrile–butadiene rubber; ND, not detected; NR, not reported; ON, Ontario; t, sample duration; SD, standard deviation; USA, United States of America; VA, Virginia; VT, Vermont; [ ] value extrapolated from figure in article or calculated by the Working Group.

[a] Measurements of less than the LOD were replaced with "0".

Table 1.5 Concentrations of acrylonitrile in indoor environments

Location	Indoor space	Concentration (μg/m³)			Comment	Reference
		Average (± SD)	Minimum	Maximum		
USA	Homes	0.5	NR	NR	Modelled daily value	Nazaroff and Singer (2004)
		1.2	NR	NR	Modelled daily value	
USA	Homes	0.49	NR	NR	Modelled daily value	Miller et al. (1998)
USA	18 m³ laboratory room	100.2	NR	NR	20 min after smoking	Sleiman et al. (2014)
		4.2	NR	NR	2 h after smoking	
		2.2	NR	NR	18 h after smoking	
	Home	ND	NR	NR	8 h after smoking	
NR	72 m³ room	< 0.27[a]	NR	NR	"Residential" (THS)	Mitova et al. (2016)
		3.61[a]	NR	NR	"Residential" (cigarette)	
		< 0.27[a]	NR	NR	"Office" (THS)	
		2.61[a]	NR	NR	"Office" (cigarette)	
		< 0.27[a]	NR	NR	"Hospitality" (THS)	
		1.36[a]	NR	NR	"Hospitality" (cigarette)	
Germany	Restaurants	1.1	0.4	3.3	11 businesses	Bolte et al. (2008)
	Bars/pubs	2.2	0.4	10.6	7 businesses	
	Dance clubs	3.8	1.1	7.5	10 businesses	
USA	Home 1	[0.05][b]	NR	[0.51]	Summer	Lunderberg et al. (2021)
		[0.05]	NR	[0.12]	Winter	
	Home 2	[0.07]	NR	[0.26]	Winter	
Taiwan, China	26 homes	0.057 ± 0.057	NR	NR	Level 3 (personal samples)	Yen et al. (2023)
	24 homes	0.050 ± 0.056	NR	NR	Level 2 (personal samples)	
Canada	4 homes	ND	NR	NR	Overnight samples	Bell et al. (1991)

h, hour(s); Level 3, strict COVID-19 control measures; Level 2, loosened COVID-19 control measures; min, minute(s); ND, not detected (limit not given); NR, not reported; SD, standard deviation; THS, tobacco heating system; USA, United States of America.

[a] Median.

[b] [ ], approximately (values read from figure or calculated by the Working Group).

has been estimated to be 4–189 hours (US EPA, 1984; WHO, 2002). In Canada, it was reported that in 1999 the total release of acrylonitrile to air in the province of Ontario was 8.2 tonnes (Alberta Environment, 2002). The Toxic Release Inventory (TRI) of the US EPA found in 2007 that 94 facilities in the USA released approximately 7 million pounds [3175 tonnes] of acrylonitrile, with >90% released into underground injection wells for hazardous waste (NTP, 2021). In 2021, it was estimated from TRI data that 87 facilities in the USA released approximately 0.29 million pounds [133 tonnes] of acrylonitrile into the air (ATSDR, 2023). The Agency for Toxic Substances and Disease Registry (ATSDR) noted that these TRI data were based on reporting requirements for only some types of facilities and were not an exhaustive account of emissions (ATSDR, 2023). Chen et al. (2016) estimated that 21 chemical and petrochemical companies located in an industrial park in southern Taiwan, China, released [11.0] tonnes of acrylonitrile per year.

As summarized in Table 1.4, ambient concentrations of acrylonitrile have been reported in several countries. In Japan, based on air-monitoring network data, the average annual ambient concentration of acrylonitrile during the period 2004–2012 was 0.073–0.11 μg/m³ (Tsai, 2016). Jia and Foran (2013) monitored levels of organic chemicals at a central location in south-western Memphis, Tennessee, USA, an economically depressed residential region surrounded by heavy industry and vehicle traffic. The average ambient concentration of acrylonitrile was 0.55 μg/m³ (maximum, 26.11 μg/m³) (Jia and Foran, 2013). Two studies reported concentrations of acrylonitrile at roadsides in urban areas. Ochiai et al. (2003) monitored levels at a roadside in suburban Tokyo for 24 hours per day over 1 week and found an average concentration of 0.08 μg/m³. Han et al. (2023) monitored concentrations of acrylonitrile during morning and afternoon rush hour periods in Hong Kong Special Administrative Region, China. The overall average concentration (± standard deviation, SD) for 1 month was [0.78 ± 0.13] μg/m³, with means of [0.86 ± 0.09] and [0.70 ± 0.20] μg/m³ during the morning and afternoon rush hour periods, respectively. Average concentrations of acrylonitrile in an area without traffic (i.e. background) were [0.09 ± 0.09] μg/m³ (maximum, [0.20] μg/m³) during the morning rush hour period and [0.04 ± 0.09] μg/m³ (maximum, [0.15] μg/m³) during the afternoon rush hour period.

Sirithian et al. (2018a) found acrylonitrile emissions of 34.3 μg/m³ from maize residue burning in a combustion chamber. Modelling of the dispersion of volatile organic compounds (VOCs) from open crop-residue burning predicted air concentrations of acrylonitrile of between 0.8 and 3.1 μg/m³, depending on the scenario considered (Sirithian et al., 2018b).

Strum and Scheffe (2016) evaluated air-monitoring network data in the USA from 1990 to 2013. Based on data for 2011, the average ambient concentration of acrylonitrile in metropolitan Chicago, Illinois, was 0.13 μg/m³ compared with 0.011, 0.006, and 0.002 μg/m³ in the less urbanized cities of Roxbury, Massachusetts; Underhill, Vermont; and Richmond, Virginia, respectively (Strum and Scheffe, 2016). Statistically significant trends from 2003 to 2014 were found in three of 24 areas in the USA; there were upward trends in Chicago (Spearman correlation coefficient, 0.7) and Underhill, Vermont (Spearman correlation coefficient, 0.6) and a downward trend in Pinellas County, Florida (Spearman correlation coefficient, –1.0). The remaining cities had no significant trend. In Ontario, Canada, in 1990, at six urban air-monitoring stations, just one of 11 samples contained measurable concentrations of acrylonitrile (1.9 μg/m³); in an industrialized area (city of Windsor), the average concentration of acrylonitrile was <0.64 μg/m³; and in downtown and residential areas in metropolitan Toronto, acrylonitrile was below the method LOD of 0.9 μg/m³ in all samples (WHO, 2002). Yen et al. (2023) monitored the ambient concentration of

acrylonitrile at a university site in Taiwan, China, at different times during the coronavirus disease (COVID-19) pandemic. When Taipei was under a Level 3 warning (strict control measures), the average ambient concentration at the university location was 0.012 ± 0.009 μg/m³, whereas when there was a Level 2 alert (loosened control measures), the average ambient concentration was 0.004 ± 0.001 μg/m³.

Several investigators have reported concentrations of acrylonitrile at petrochemical production complexes in Taiwan, China. Over a 3-year period from 1997 to 1999, the average concentration of acrylonitrile outdoors at a complex was [95.8] μg/m³, with a maximum of [9248] μg/m³ (Chan et al., 2006). Chen et al. (2016) calculated a simulated average annual concentration, and from this the Working Group estimated a concentration of [< 1 μg/m³]. Hsu et al. (2022) analysed VOC monitoring data from 17 monitoring stations around industrial parks in Taiwan, China. During the period 2015–2018, average annual concentrations of acrylonitrile ranged from non-detectable to [1.37] μg/m³. Vallecillos et al. (2019) monitored VOC concentrations at 16 sampling sites along perimeter fencing of the North Industrial Complex in Tarragona, Spain, which is one of the largest chemical and petrochemical complexes in southern Europe. For the purposes of the study, the complex was divided into two zones, designated A and B. For zone A, the overall average concentrations of acrylonitrile at eight monitoring locations were 1.14–96.97 μg/m³. In zone B, the overall average concentrations of acrylonitrile at eight monitoring locations were 0.70–32.48 μg/m³. In Ontario, Canada, the concentration of acrylonitrile in the vicinity of a nitrile–butadiene rubber-production plant was below the method LOD (52.9 μg/m³), and near a chemical-manufacturing plant it ranged from 0.12 to 0.28 μg/m³ (WHO, 2002). [The Working Group noted the difference between the method LOD and the substantially lower reported air concentrations. These two sets of data came from different studies and are likely to reflect different sampling and analytical methods.]

Waste disposal facilities were reported to be sources of ambient acrylonitrile in rural areas of China. Chai et al. (2022) monitored VOC levels at five locations at a waste transfer station and up to 100 m downwind of the facility. The average concentration of acrylonitrile at the transfer station was 627.5 μg/m³. At 100 m from the transfer station, the concentrations of acrylonitrile and six other nitrogenous compounds decreased by an average of 87.5%. Li et al. (2023) monitored the concentrations of VOCs at two rural landfills for domestic waste; one facility was in northern China and the other in south-western China. At each landfill, VOCs were monitored at the dumping area and at the leachate storage pool. At the landfill in south-western China, the average concentration of acrylonitrile near the leachate storage pool (5525 μg/m³) was more than a factor of three higher than that in the dumping area (1690 μg/m³).

Overall, the data in Table 1.4 show that average levels of acrylonitrile in ambient air near roadsides and in cities and towns (e.g. from vehicular traffic) ranged from non-detectable to [0.86] μg/m³. Average concentrations tended to be much higher near petrochemical, chemical, polymer, and industrial manufacturing sites (up to 96.97 μg/m³) and even higher at or near solid waste sites (from 80.2 to 5525 μg/m³).

(ii) Indoor air

Concentrations of acrylonitrile reported in indoor air are shown in Table 1.5. Acrylonitrile is formed by tobacco burning (ATSDR, 2023). Studies reported levels of acrylonitrile in tobacco smoke that ranged from 5.10 to 18.5 μg/cigarette (NTP, 2021; ATSDR, 2023). Using published emissions data for tobacco cigarettes, investigators have modelled indoor air concentrations of acrylonitrile in the residences of smokers and estimated concentrations to be 0.5–1.2 μg/m³

(Miller et al., 1998; Nazaroff and Singer, 2004). Sleiman et al. (2014) collected 10- and 20-minute samples for several VOCs in the home of a tobacco smoker 8 hours after three cigarettes had been smoked; acrylonitrile was not detected. [The Working Group noted that the LOD was not reported.] Mitova et al. (2016) measured VOC emissions from a tobacco cigarette and a heat-not-burn device that generated aerosol from tobacco without burning in a room of 72 m^3 under ventilation conditions that simulated a residential setting. The measured concentration of acrylonitrile in the room air from the heat-not-burn device was < 0.27 $\mu g/m^3$ (equivalent to background), and for the tobacco cigarette it was 3.61 $\mu g/m^3$.

Acrylonitrile has been measured in the indoor air of homes where tobacco smoking does not occur. Lunderberg et al. (2021) monitored 200 VOCs in the indoor air of two homes in California, USA. In one home, the average concentration of acrylonitrile was [0.05] $\mu g/m^3$ in both the summer and winter, with maximum values of [0.51] $\mu g/m^3$ (summer) and [0.12] $\mu g/m^3$ (winter). In the other home, the average concentration was [0.07] $\mu g/m^3$, with a maximum of [0.26] $\mu g/m^3$ in the winter. The occurrence of acrylonitrile in both homes was attributed to continuous indoor sources (building materials and building contents), outdoor origins, and cooking. In a study of four residences in metropolitan Toronto, Canada, acrylonitrile was not detected in overnight samples (LOD, 0.9 $\mu g/m^3$) (Bell et al., 1991).

The interior of vehicle cabins is composed of many different types of material, including plastics, that continuously emit VOCs. Yesildagli et al. (2023) measured VOC levels inside the cabin of a new mini-truck-type electric vehicle and reported that levels were 11 times as high as those outdoors. Emission rates for acrylonitrile ranged from 0.04 µg/hour (heater off, windows covered) to 0.85 µg/hour (heater on, windows covered).

Emerging sources of indoor acrylonitrile in air include electronic cigarettes and home hobbyist machines such as 3D printers. Uchiyama et al. (2020) used a smoking machine to generate emissions from two brands of electronic cigarette and reported that the amount of acrylonitrile in emissions (± SD) ranged from 0.2 ± 0.1 to 1.7 ± 0.5 µg per 15 puffs. In another study, Kim et al. (2022) evaluated several different types of tobacco-smoking cessation aids and reported that the release of acrylonitrile from an ignitable cigarette-type of smoking-cessation aid (n = 2) was 0.07 ± 0.01 µg/cigarette.

Laboratory studies indicated that material extrusion-type 3D printers that heat certain types of thermoplastic filament emitted acrylonitrile. Gu et al. (2019) measured concentrations of acrylonitrile in a 3 m^3 chamber during 3D printing with ABS-based plastics. The background concentration of acrylonitrile was 4 $\mu g/m^3$, and the air concentration was 22 $\mu g/m^3$ 1.5 hours after printing (equivalent to an emission rate of 1.4 µg/minute for printing). Wojtyła et al. (2019) reported that, when heated, ABS filament emitted acrylonitrile during 3D printing in a 0.25 m^3 chamber. [The Working Group estimated that the concentration of acrylonitrile was approximately 15 $\mu g/m^3$ after 3 hours of printing.]

Collectively, data presented in Table 1.5 indicated that levels of acrylonitrile in indoor air were up to 3.6 $\mu g/m^3$ in the home of a tobacco smoker but approximately 0.05 $\mu g/m^3$ in the homes of non-smokers. In test chambers where 3D printers were used to extrude ABS-containing plastic, concentrations of acrylonitrile were 15–22 $\mu g/m^3$. Levels of acrylonitrile in homes of tobacco smokers and non-smokers were similar to or higher than in ambient air near roadsides and in cities and towns (up to 0.86 $\mu g/m^3$). Levels of acrylonitrile measured during operation of a 3D printer with ABS plastic were higher than those in the home of a smoker, but lower than those measured at industrial manufacturing

sites (up to 96.97 μg/m^3) or solid waste sites (up to 5525 μg/m^3).

(b) Water

A study investigated groundwater contamination by acrylonitrile in the area surrounding a polyacrylonitrile fibre-production plant in Türkiye where an earthquake had damaged acrylonitrile tanks (Sengör and Unlü, 2013). The peak concentrations of acrylonitrile at the two most exposed pumping wells were about 42 000 mg/L and 30 000 mg/L. After an earthquake in 1999 caused a spill from a tank, acrylonitrile in seawater was measured as 0.157–2.88 μg/L at 10 days after the accident and 0.075–0.178 μg/L at 23 days after the accident (Güven and Gezgin, 2005).

Water samples from wastewater treatment plants in Perth, Western Australia, were analysed for various contaminants, including acrylonitrile (Rodriguez et al., 2012). Acrylonitrile was detected in 50% of the secondary-treatment effluent samples and 83% of post-reverse osmosis samples. The median and maximum concentrations of acrylonitrile were 0.003 and 0.01 μg/L in secondary-treatment effluent samples and 0.01 and 0.02 μg/L in post-reverse osmosis samples, respectively.

Samples of municipal wastewater from spots near industrial zones in two communities in the south-eastern USA were analysed for urinary excreted human metabolites of various VOCs (Kumar et al., 2022). Eight samples of each were withdrawn during a 24-hour period and were analysed for 16 parameters. CEMA, which was used as a marker of acrylonitrile exposure, showed a moderate detection frequency of 38%. The average concentration of CEMA (mean ± SD) was 117 ± 46 ng/L.

(c) Soil and sediments

[The Working Group noted the lack of data on the occurrence of acrylonitrile in soil.] A review article examined the information on the contamination of sewage sludges with organic chemicals (Harrison et al., 2006). The authors identified one study in which acrylonitrile in this matrix was quantified using a bioassay test. The study specified a concentration range of 0.0363 to 82.3 mg/kg dry weight (Jacobs et al., 1987).

(d) Food

[The Working Group noted the lack of data on the occurrence of acrylonitrile in food.] One study evaluated the migration of acrylonitrile from polypropylene granules into liquid food simulants (water and ethanol/water) at two temperatures for up to 6 weeks (Forero et al., 2023). From the experimental data obtained, second-order kinetics were adjusted to represent the migration. For water, equilibrium concentrations of 15.99 and 16.58 μg/kg at 20 °C and 44 °C, respectively, were obtained, whereas for 50% ethanol, values of 15.07 and 16.40 μg/kg, respectively, were obtained for the same temperatures. The authors concluded that the experimental results and the values estimated from the migration kinetics indicated that the maximum concentration of acrylonitrile would not exceed the tolerable specific limit established in regulations (see Section 1.5).

The migration of acrylonitrile from seven commercially available ABS polymers commonly used in typical food-contact applications was investigated using water as food simulant (Lickly et al., 1991). The concentration of monomer residue ranged between 3 and 49 μg/g polymer in the tested materials. The results revealed a linear association between the concentration of acrylonitrile in the polymer and the amount of acrylonitrile that migrated into the food simulant.

(e) Consumer products

The risk of chemical migration of acrylonitrile in ABS-containing plastic toys was explored by Wang et al. (2023). First, the migration was analysed by a simple and sensitive headspace-GC-MS method for detecting residues of

acrylonitrile and its migration. The migration models were then established based on migration data from 5 minutes to 168 hours and verified using 11 ABS samples. The results showed that the predicted values from the models and the experimental values were a good fit. Subsequently, the migration of acrylonitrile in 94 ABS toys was predicted using these models and specific migration times.

Another study investigated the migration of monomers and additives from materials used in cosmetic plastic packaging by using two approaches based on solvent-free extraction coupled with GC-MS (Murat et al., 2020). The application of the method to seven different polymeric packaging materials identified acrylonitrile as the specific and main migrant from a SAN polymer material.

Household laser engravers are used by hobbyists to create images on the surface of a variety of craft materials. The use of these products can result in emissions of acrylonitrile and thus in pollution of the ambient air (see Section 1.4.1(a)). Ko et al. (2021) monitored emissions from a commercially available home laser engraver used in a chamber to make artistic patterns while working with wood, rubber, cork, and leather. Acrylonitrile emissions rates ranged from 0.13 ng/second (rubber) to 16.23 ng/second (leather).

Aerosol emissions from the operation of a fused-deposition modelling 3D printer were investigated and time- and size-resolved emissions of submicrometre-sized aerosols from the printer in a chamber study were modelled (Vance et al., 2017). Raman spectroscopy was used to gain insight into the chemical composition of emitted aerosols, and the potential for exposure to the aerosols generated by 3D printers under real-use conditions in a variety of indoor environments was measured. ABS filament generated the largest number of aerosols, and wood-infused polylactic acid (PLA) filaments generated the smallest amount.

1.4.2 Occupational exposure

Reports of assessments of occupational exposure to acrylonitrile in different settings (i.e. acrylonitrile-monomer production, fibre production, resin production, polymer production, and miscellaneous industries) are presented here. Before the 1970s, most available data on air monitoring were obtained through static sampling (see Section 1.4.2(a)). The abundance of data published since that time come mainly from personal air sampling (see Section 1.4.2(b)), and most data come from the fibre-, resin-, and polymer-production industries. Since the 1990s, biomonitoring has also been increasingly used to assess workers' exposure to acrylonitrile (see Section 1.4.2(c)).

(a) Air monitoring through static sampling

Numerous studies have reported air-monitoring data obtained through static sampling for acrylonitrile in a range of industries and workplaces (see Table S1.6, Annex 1, Supplementary material for Section 1, online only, available from: https://publications.iarc.who.int/646).

(i) Monomer production

Cicolella et al. (1981) surveyed 11 factories in France. [The Working Group noted that tabulated measurement data were presented for only nine factories (designated as A, B, C, D, E, F, G, H, and J).] Of these nine factories, one was a monomer-production facility (designated factory A). The authors reported that acrylonitrile concentrations in factory A ranged from [5.2 to 44.1] mg/m^3.

(ii) Fibre production

Several studies have reported average air concentrations of acrylonitrile in acrylic fibre-production factories in Japan. Mean TWA concentrations of acrylonitrile were [0.6–30.6] mg/m^3 (Sakurai et al., 1978; Kaneko and Omae, 1992; Muto et al., 1992). In a study in France, two of the nine factories were engaged

in acrylic fibre-production (designated factories B and C) (Cicolella et al., 1981). Acrylonitrile concentrations ranged from non-detectable to 542 mg/m³ (leaking equipment). Major et al. (1998, 1999) evaluated exposures to acrylonitrile at a viscose rayon-production factory in Hungary; levels ranged from non-detectable (limit not reported) to 17.6 mg/m³.

(iii) Resin production

Cicolella et al. (1981) performed static sampling at three resin-production factories (designated D, E, and F) in France. At factory D, workers made ABS and SAN resins and concentrations of acrylonitrile ranged from [29.3 to 48.4] mg/m³. At factory E, the concentrations of acrylonitrile at an ABS flocculation (coalescing) station and near a waste drain were both [5.0] mg/m³. At factory F, the concentration of acrylonitrile at feeding pumps was [6.3] mg/m³.

(iv) Polymer production

Cicolella et al. (1981) surveyed three nitrile rubber-production factories (designated factories E, F, and G) in France. At factory E, one sample was collected at a flocculation station; the acrylonitrile concentration was [0.2] mg/m³. At factory F, acrylonitrile concentrations ranged from [6.3] mg/m³ (below a reactor during cleaning) to [12.6] mg/m³ at a drying station. At factory G, concentrations ranged from [0.9] mg/m³ (flocculation station in workshop 1) to [67.9] mg/m³ (slurry sample collection). Two factories (designated H and J) produced acrylic copolymers. At both factories, the concentration of acrylonitrile near unventilated tanks was approximately [76] mg/m³, but higher near leaking stuffing boxes (factory J, range, [97.7–197.5] mg/m³).

At a synthetic rubber factory, concentrations of acrylonitrile ranged from [13.0 to 19.5] mg/m³ (Ivanov et al., 1993). In two studies, air levels of acrylonitrile were evaluated through static sampling during a polymerization process at an India rubber factory in Czechia; concentrations ranged from 0.05 to 0.7 mg/m³ (Sram et al., 2004; Beskid et al., 2006).

(v) Chemical production

Rössner et al. (2002) stated that acrylonitrile concentrations in a factory in Czechia ranged from 0.05 to 0.3 mg/m³. Xu et al. (2003) reported that the average concentration (± SD) of acrylonitrile at operation sites in a factory was 0.8 ± 0.25 mg/m³. [The Working Group inferred that this factory was in China, based on the affiliation of some authors and the research funding source.]

(vi) Other industries and workplaces

From 1976 to 1980, NIOSH conducted health hazard evaluations at downstream-user facilities (tyre-, aircraft-, and helmet-manufacturing) in the USA (NIOSH, 1976, 1979, 1980). The concentration of acrylonitrile under a Banbury mill at a tyre manufacturer was [0.3] mg/m³; samples collected through static sampling for acrylonitrile showed levels below the respective LODs for all other studies.

At a rubber footwear-manufacturer in the Russian Federation, air concentrations of acrylonitrile were up to 2.2 mg/m³ (Solionova et al., 1992). Volkova and Bagdinov (1969) reported that acrylonitrile concentrations in a factory in the Russian Federation that produced butadiene–styrene footwear ranged from [1 to 11] mg/m³.

Cirla et al. (2007) measured levels of acrylonitrile in 12 ABS plastic moulding factories in Italy; all measurements were < 0.01 mg/m³.

Two studies evaluated emissions during electrosurgical procedures at hospitals. In a study at a hospital in the Republic of Korea, the authors reported that the average mass of acrylonitrile was 1.62 mg in 45 L of air, which the Working Group equated to [36.0] mg/m³ (Chung et al., 2010). In the second study, the level of acrylonitrile in surgical smoke in a hospital in China was below the LOD of 0.02 mg/m³ (Liu et al., 2021).

[The Working Group noted the discrepancy between the values reported in the two studies.]

Leng and Gries (2014) reported that, during a fire at a chemical plant in Germany, a tank that contained acrylonitrile was intentionally vented to air to prevent pressure build-up. The average concentration of acrylonitrile in the vicinity of the tank was [15.2] mg/m^3 8 hours after the event and [3.5] mg/m^3 120 hours after the event.

He et al. (2015) evaluated levels of organic chemicals in plastics-recycling workshops in China. Samples were collected in workshops and from extrusion processes. They reported that in workshops, average levels of acrylonitrile ranged from 8.4 mg/m^3 (polycarbonate) to 43 mg/m^3 (polyamide). In samples collected near extrusion processes, levels ranged from 0.01 mg/m^3 (polycarbonate) to 25 mg/m^3 (ABS).

At a 3D-printing centre at a university in the Republic of Korea, it was reported that the concentration of acrylonitrile in air was [0.007] mg/m^3 while five machines extruded PLA thermoplastic (Youn et al., 2019). [The Working Group noted that PLA is a biopolymer, so it was unlikely that the detected acrylonitrile originated from this polymer. It was more likely that acrylonitrile was present in air subsequent to previous 3D printing with petroleum-based thermoplastics such as ABS filament.]

Eun et al. (2022) monitored one cycle of a dry-cleaning process that used a petroleum-based solvent. The average concentration of acrylonitrile was [0.008] mg/m^3. [The Working Group inferred that this study was performed in the Republic of Korea, based on the affiliation of the authors and the research funding source.]

[The Working Group noted that data presented in Table S1.6 (see Annex 1, Supplementary material for Section 1, online only, available from: https://publications.iarc.who.int/646) indicated that levels of acrylonitrile in workplace air were higher in production industries than in other downstream users (e.g. plastic moulding and 3D printing). No clear pattern was observed that indicated that monitoring levels of acrylonitrile obtained through static sampling have declined over time. Values reported by Cicolella et al. (1981) indicated that levels of acrylonitrile in workplace air were highest for tasks near non-ventilated or leaking equipment (see for example, results for factory A and factory C). In general, the levels of acrylonitrile presented in Table S1.6 tended to be orders of magnitude higher than those for the general public (see Section 1.4.3).]

(b) Air monitoring through personal sampling

(i) Monomer production

Cicolella et al. (1981) evaluated 11 factories in France (see Section 1.4.2(a)). Factory A was engaged in monomer production. Two short-term personal samples were collected from workers engaged in unloading and loading tank cars of acrylonitrile; concentrations ranged from [4.1 to 6.1] mg/m^3.

(ii) Fibre production

Sakurai et al. (1978) measured air levels of acrylonitrile using personal sampling for production workers at six acrylic fibre-production factories in Japan. Concentrations were categorized into three groups according to the mean concentrations of acrylonitrile in the air at the workplace: [4.6] mg/m^3 (group A), [16.1] mg/m^3 (group B), and [30.6] mg/m^3 (group C).] Cicolella et al. (1981) monitored exposures to acrylonitrile at a factory (designated factory B) that produced acrylic fibres in France. Short-term personal samples were collected from workers with different tasks. Exposure was [6.5] mg/m^3 for a shift worker, [41.2] mg/m^3 for a reactor manual cleaner, and [71.6] mg/m^3 for a worker unloading and loading acrylonitrile. Guirguis et al. (1984) evaluated exposures using personal sampling in factories that produced acrylic fibres in Canada. The exposure levels for 118 workers who performed unloading, reactor work, wet spinning, maintenance and cleaning, and processing were below the LOD [< 2.2] mg/m^3 in 1980–1981

and did not differ from those in an earlier survey conducted in 1978–1979. Finally, Muto et al. (1992) evaluated exposures to acrylonitrile among production workers in seven acrylic fibre factories in Japan. Personal sampling data were grouped into three categories (designated as groups a, b, and c) for data from 1976 and two categories (groups A and B) for data from 1987. In 1976, average acrylonitrile concentrations were [1.6], [0.7], and [1.1] mg/m^3 in groups a, b, and c, respectively and in 1987, average concentrations were [0.4] and [2.5] mg/m^3 in groups A and B, respectively.

Several reports included exposure measurements for acrylonitrile but did not specify whether results were obtained with personal or static sampling. For workers in a fibre-production factory in Portugal, air concentrations of acrylonitrile were said to not exceed [4.3] mg/m^3 (Borba et al., 1996). Reports from fibre-production factories in Bulgaria indicated seasonal differences in exposure levels, i.e. approximately 4–7 mg/m^3 during summer months and < 0.5 mg/m^3 in winter months (Spassovski, 1976).

(iii) Resin production

Guirguis et al. (1984) used personal sampling to monitor exposures of workers engaged in production of ABS and acrylic resins. ABS-resin workers (n = 148) were engaged in unloading reactors, coagulation and drying, compounding, sample taking, maintenance and cleaning, laboratory work, and control room work; 8-hour time-weighted average (TWA_{8h}) exposure levels were [1.1–3.5] mg/m^3. Acrylic resin workers (n = 44) performed unloading work, reactor work, packaging, and use of product; all exposures were non-detectable ([< 2.2] mg/m^3). These levels were lower than those recorded in an earlier survey conducted at the factories in 1978–1979. Perbellini et al. (1998) evaluated exposures among 34 workers at an acrylic resin-production factory through personal sampling; the average exposure to acrylonitrile was [0.2 ± 0.4] mg/m^3.

Cicolella et al. (1981) reported short-term exposure to acrylonitrile during automated stripper cleaning of a tank at factory E, obtained by personal sampling. [The exposure of this worker was [5.6 mg/m^3], but the Working Group noted that it was unclear whether this person was in the ABS-resin or nitrile-rubber production area.]

(iv) Polymer production

Cicolella et al. (1981) reported exposure measurements through personal sampling of workers at three nitrile rubber-production factories. At factory E, short-term exposure levels ranged from non-detectable (LOD not reported) for a shift worker to [5.6] mg/m^3 for a worker who performed a manual stripper cleaning job. At factory F, short-term exposures to acrylonitrile ranged from [0.2 to 6.3] mg/m^3. At factory G, exposures of workers who manually performed stripper cleaning ranged from [19.1 to 65.1] mg/m^3. Houthuijs et al. (1982) monitored 15 workers exposed to acrylonitrile at a plastic-production factory in the Kingdom of the Netherlands (hereafter, "the Netherlands"); their average TWA_{8h} exposure level was [0.3] mg/m^3. For six workers (no job title given), the average of TWA_{8h} levels was [1.8] mg/m^3 when respiratory protection was worn compared with an average of [0.2] mg/m^3 when not worn; and biomonitoring levels were also higher when respiratory protection was used. The authors mentioned that poor quality of the respiratory protection devices, bad work practices, or inadequate fit of the devices might explain these results.

Guirguis et al. (1984) determined TWA_{8h} exposure levels for 350 workers grouped under four job titles (unloading reactors, sample taking, coagulation and drying, maintenance and drying) at a nitrile rubber-production factory in Canada. Among these job titles, average exposures ranged from [2.2 to 4.3] mg/m^3.

Sajedian et al. (2023) monitored exposures at an ABS plastic factory in the Islamic Republic of Iran. The authors stated that they collected three samples with a duration of 90 minutes using personal sampling from each of 15 workers, for a total of 45 air samples. For all 15 workers, the average exposure to acrylonitrile was 0.7 mg/m^3. The authors reported average exposures for 13 job titles; average exposure levels ranged from [0.003 to 0.2] mg/m^3. [The Working Group noted that the number of samples per job title varied from two to nine, which was not consistent with the statement that three samples were collected per worker.]

(v) Chemical industries

Tuček et al. (2002) measured exposures among workers in 15 factories that made acrylic acid, acrylic acid esters, and acrylate dispersions in Czechia. [The Working Group estimated from Fig. 2 presented in their report that, for the period 1988 to 1999, most samples were below 0.2 mg/m^3.] In occupational hygiene surveys carried out by the Finnish Institute of Occupational Health between 2011 and 2022, mainly in the chemical industry, the average concentration of acrylonitrile in measurements taken from the workers' breathing zone was 0.1 mg/m^3, and the 90th percentile was 0.2 mg/m^3 (FIOH, 2023). Wu et al. (2023) reported low levels of exposure to acrylonitrile among workers using acrylonitrile at three factories in Taiwan, China. Workers in the group with low exposure had non-detectable exposure (< 0.004 mg/m^3), and those in the group with high exposure had a geometric mean exposure of 0.2 mg/m^3 (range, 0.05–11.5 mg/m^3).

(vi) Miscellaneous industries

NIOSH conducted several health hazard evaluations at facilities that use acrylonitrile-containing materials to manufacture products (tyres, aircraft parts, helmets, etc.) (NIOSH, 1976, 1978, 1979, 1980, 1981). The exposure levels in all of these workplaces were below the respective analytical LOD for acrylonitrile in each study. Benson et al. (1981) performed a side-by-side comparison of exposure measurements using active and passive personal sampling techniques in a plastics company. The average exposure levels were the same ([1.0] mg/m^3) for the active and passive sampling techniques. Cirla et al. (2007) measured personal breathing zone exposures among workers engaged in ABS plastic moulding at 12 factories in Italy; acrylonitrile was non-detectable (< 0.01 mg/m^3) for all measurements.

In a study on exposure during commuting (automobile, subway, and/or train) in metropolitan Toronto, Canada, acrylonitrile was not detected (< 0.9 µg/m^3) in any of the samples collected by personal sampling (Bell et al., 1991).

The results of air monitoring obtained through personal sampling, presented in Table S1.7 (Annex 1, Supplementary material for Section 1, online only, available from: https://publications.iarc.who.int/646), indicate that workers performing manual tasks that involve handling large quantities of acrylonitrile have the highest exposures. For example, Cicolella et al. (1981) reported that a worker who was unloading and loading acrylonitrile had an exposure of [71.6] mg/m^3, and that for multiple samples collected during manual reactor cleaning, exposures were approximately [20–65] mg/m^3. Among production industries, personal exposures tended to be lowest for workers in resin and chemical production. For example, among resin-production facilities, Guirguis et al. (1984) and Perbellini et al. (1998) reported that, with the exception of one sample for a reactor unloading task [3.5 mg/m^3], exposures were below approximately 2 mg/m^3. At chemical-production facilities, Tůcek et al. (2002) and Wu et al. (2023) reported that average exposure was 0.2 mg/m^3. Before approximately the year 2000, exposure levels were on the order of a few to tens of milligrams per cubic metre (see, for example,

Cicolella et al., 1981). Thereafter, exposure levels tended to not exceed 0.5 mg/m^3 (Tůcek et al., 2002; Cirla et al., 2007; Sajedian et al., 2023).

(c) Biomonitoring

The literature suggests that acrylonitrile and its metabolites in urine and blood protein adducts are possible biomarkers of exposure (Table 1.8, see Sections 1.3.4 and 4.1.2 for a detailed description of biomarkers). Of these biomarkers, the European Commission recommends only the use of the adduct CEV in the blood (ECHA, 2018). In addition, Frigerio et al. (2019) and Wu et al. (2023) reported that CEMA in the urine is another exposure biomarker for acrylonitrile. Results from studies with data on biomarkers of exposure are presented in Table 1.8.

(i) Fibre production

Sakurai et al. (1978) measured urinary levels of unchanged acrylonitrile and thiocyanate among 102 workers and 62 controls from six acrylic fibre-production plants in Japan. Post-shift urinary levels of acrylonitrile ranged from 3.9 to 359.6 µg/L in production workers and were < 5 µg/L in controls. Urinary levels of thiocyanate were higher in production workers than in controls. Tavares et al. (1996) studied continuous-polymerization workers, maintenance mechanics, and control participants at an acrylic-textile fibre plant in Portugal. Tavares et al. (1996) developed an analytical method for CEV in blood and reported there were no statistically significant differences in CEV levels between continuous-polymerization workers (2276 pmol/g haemoglobin) and maintenance mechanics workers (1984 pmol/g haemoglobin), although levels in both groups were significantly higher than in the control group (31.1 pmol/g haemoglobin). Major et al. (1998) studied 26 workers (13 maintenance and 13 fibre producers) with exposures to acrylonitrile and/or dimethylformamide at a viscose rayon textile plant in Hungary. Post-shift urinary levels of acrylonitrile were approximately double pre-shift levels for maintainers (0–2.94 versus 0–1.61 µg/mmol creatinine) and significantly higher than pre-shift levels for fibre producers (0–36.90 versus 0–63.50 µg/mmol creatinine).

(ii) Resin production

Perbellini et al. (1998) measured urinary levels of acrylonitrile and personal exposures to airborne acrylonitrile among 34 workers and 20 controls at a facility in Italy. Post-shift urinary levels of acrylonitrile were more than twice as high as pre-shift levels (28.8 µg/L versus 12.5 µg/L). Post-shift urinary levels were significantly correlated with acrylonitrile air exposure levels obtained through personal sampling. Tobacco smoking was an important confounder for biomonitoring of acrylonitrile exposures.

(iii) Polymer production

Houthuijs et al. (1982) determined the excretion pattern of acrylonitrile in urine samples from 15 plastic-production workers in the Netherlands. All urine voids during a 7-day work period were collected from production workers and controls. Levels of acrylonitrile in production workers ranged from 5.4 to 50.4 µg/g creatinine compared with 2.0–9.0 µg/g creatinine in controls. For production workers, there was a statistically significant correlation between personal exposure levels to acrylonitrile in air and biological monitoring results. Bergmark et al. (1993) and Calleman et al. (1994) collected blood samples for the measurement of CEV from production workers and controls at a polyacrylamide-production factory (which also made acrylamide) in China. In both studies, blood CEV was present at detectable levels in blood from production workers (polymer synthesis, polymerization, and packaging), but at levels about two orders of magnitude lower (near detection limits) in controls. [The Working Group noted that CEV levels reported for workers and controls differed between studies, although it

Table 1.8 Data on biomonitoring for acrylonitrile exposure, by production industry

Industry	Country Samples	Population	Concentration[a]	Reference
Urinary acrylonitrile				
Acrylic fibre	Japan Urine, post-shift	Production (n = 108) (smokers/non-smokers) Controls (n = 22) (smokers/non-smokers)	3.9–359.6 µg/L < 5 µg/L	Sakurai et al. (1978)
Viscose rayon fibre	Hungary Urine, pre/post-shift	Maintainers (n = 13) Fibre producers (n = 13)	0–1.61 (pre) vs 0–2.94 (post) mg/mmol creatinine 0–36.90 (pre) vs 0–63.50 (post) mg/mmol creatinine	Major et al. (1998)
Acrylic resin	NR Urine, pre/post-shift	Production (n = 34) (smokers/non-smokers)	12.5 (pre) vs 28.8 (post) µg/L	Perbellini et al. (1998)
Plastics polymers	Netherlands (Kingdom of the) Urine, all voids (7 d)	Production (n = 15) (smokers/non-smokers) Controls (n = 41) (smokers/non-smokers)	5.4–50.4 µg/g creatinine 2.0–9.0 µg/g creatinine	Houthuijs et al. (1982)
Thiocyanate				
Acrylic fibre	Japan Urine, post-shift	Production (n = 101) Controls (n = 52)	4.5–11.41 mg/L 4.00 mg/L	Sakurai et al. (1978)
CEMA				
Petrol attendants	Italy Urine, post-shift	Gasoline station attendants (n = 7) (non-smokers) Controls (n = 7) (non-smokers)	1.5 µg/L[b] 1.7 µg/L[b]	Frigerio et al. (2019)
Coke oven workers	Poland Urine, post-shift	Coke workers (n = 49) (non-smokers) Controls (n = 49) (non-smokers)	3.7 µg/g creatinine[b] 1.4 µg/g creatinine[b]	Frigerio et al. (2020)
	Taiwan, China Urine, pre/post-shift	High exposed workers (n = 26) (smokers/non-smokers) Low exposed workers (n = 53) (smokers/non-smokers) Controls (n = 18) (smokers/non-smokers)	24.8 (pre) vs 230 (post) µg/g creatinine 9.88 (pre) vs 14.3 (post) µg/g creatinine 1.10 (pre) vs 1.26 (post) µg/g creatinine	Wu et al. (2023)
HEMA				
Petrol attendants	Italy Urine, post-shift	Gasoline station attendants (n = 7) (non-smokers) Controls (n = 7) (non-smokers)	2.7 µg/L[b] 2.2 µg/L[b]	Frigerio et al. (2019)
	Taiwan, China Urine, pre/post-shift	High exposed workers (n = 26) (smokers/non-smokers) Low exposed workers (n = 53) (smokers/non-smokers) Controls (n = 18) (smokers/non-smokers)	1.16 (pre) vs 4.23 (post) µg/g creatinine 0.51 (pre) vs 0.41 (post) µg/g creatinine 0.61 (pre) vs 0.42 (post) µg/g creatinine	Wu et al. (2023)
CHEMA				
NR	Taiwan, China Urine, pre/post-shift	High exposed workers (n = 26) (smokers/non-smokers) Low exposed workers (n = 53) (smokers/non-smokers)	7.93 (pre) vs 24.8 (post) µg/g creatinine 7.31 (pre) vs 6.71 (post) µg/g creatinine	Wu et al. (2023)

Table 1.8 (continued)

Industry	Country Samples	Population	Concentration[a]	Reference
		Controls (n = 18) (smokers/non-smokers)	1.15 (pre) vs 4.11 (post) µg/g creatinine	Wu et al. (2023) (cont.)
CEV				
Acrylic	Portugal Blood	Continuous polymerization (n = 7) (non-smokers)	2276 pmol/g Hb	Tavares et al. (1996)
		Maintenance mechanics (n = 9) (non-smokers)	1984 pmol/g Hb	
		Controls (n = 11)	31.1 pmol/g Hb	
(Poly) acrylamides	China Blood, 24 h from start	Production (n = 41)	0.02–66 nmol/g Hb	Bergmark et al. (1993)
		Controls (n = 10) (smokers/non-smokers)	0.03–0.14 nmol/g Hb	
(Poly) acrylamides	China Blood, 24 h from start	Production (n = 41)	16.3–19.5 nmol/g Hb	Calleman et al. (1994)
		Controls (n = 10)	0.23 nmol/g Hb	
Surfactants	NR Blood	Production (n = 62) (smokers/non-smokers)	62 pmol/g Hb[b]	Schettgen et al. (2002)
		Controls (n = 10) (smokers/non-smokers)	8 pmol/g Hb[b]	
Laboratory staff	Sweden Blood	Gel workers (n = 40) (smokers/non-smokers)	< 2–24 pmol/g Hb	Bergmark (1997)
		Controls (n = 18) (smokers/non-smokers)	< 2–106 pmol/g Hb	
		1994, unspecified jobs (n = 59) (smokers/non-smokers)	3.7–232.2 µg/L	Thier et al. (1999)
		1995, unspecified jobs (n = 59) (smokers/non-smokers)	2–231 µg/L	
		1994, unspecified jobs (n = 59) (smokers/non-smokers)	65.80–79.25 µg/L	Thier et al. (2001)
		1995, unspecified jobs (n = 59) (smokers/non-smokers)	25.0–81.79 µg/L	
		1994–1999 unspecified jobs (n = 59) (smokers/non-smokers)	15.3–62.3 µg/L[b]	Thier et al. (2002)
Cargo train	Germany			Bader and Wrbitzky (2006)
	Blood, 25 d post-incident	Cleaners (n = 4) (smokers/non-smokers)	566–2020 pmol/g Hb	
	Blood, 25 d post-incident	Emergency responders (smokers/non-smokers)	< 4–135 pmol/g Hb	
	Blood, 85 d post-incident	Cleaners (n = 4) (smokers/non-smokers)	285–1215 pmol/g Hb	
	Blood, 115 d post-incident	Cleaners (n = 4) (smokers/non-smokers)	163–651 pmol/g Hb	
	Blood, 175 d post-incident	Cleaners (n = 4) (smokers/non-smokers)	2–276 pmol/g Hb	
Chemical production	Germany Blood, post-incident	First responders and production workers (n = 863) (smokers/non-smokers)	98 pmol/g Hb	Leng and Gries (2014)

Table 1.8 (continued)

Industry	Country Samples	Population	Concentration[a]	Reference
Cargo train	Belgium Blood, post-incident	First responders (n = 635) (non-smokers)[c]	3.2 pmol/g Hb[b]	Van Nieuwenhuyse et al. (2014)
		First responders (n = 206) (smokers)	140 pmol/g Hb[b]	
Hb–acrylonitrile				
Synthetic rubber	Russian Federation Blood	Production (n = 12)	3.9–33 µmol/g Hb	Ivanov et al. (1993)
MV				
NR	NR Blood	1994, unspecified jobs (n = 59) (smokers/non-smokers)	ND to 18.9 µg/L	Thier et al. (1999)
		1995, unspecified jobs (n = 59) (smokers/non-smokers)	1–19 µg/L	
HEV				
NR	NR Blood	1995, unspecified jobs (n = 59) (smokers/non-smokers)	6–33 µg/L	Thier et al. (1999)

CEMA, *S*-(2-cyanoethyl) mercapturic acid; CEV, *N*-(2-cyanoethyl)valine; CHEMA, *S*-(1-cyano-2-hydroxyethyl) mercapturic acid; d, day(s); h, hour(s); Hb, haemoglobin; HEMA, 2-hydroxyethylmercapturic acid; HEV, *N*-(hydroxyethyl)valine; MV, *N*-(methyl)valine; ND, not detected; NR, not reported; vs, versus.

[a] [ ], approximately (values read from figure or calculated by the Working Group).

[b] Median value.

[c] Value extrapolated by study authors using a kinetic model.

appeared that the studies investigated the same cohort.] Ivanov et al. (1993) measured levels of acrylonitrile in workplace air and haemoglobin-bound acrylonitrile in blood in 12 workers at a synthetic rubber factory in the Russian Federation. Levels in workers (3.9–33 µmol/g haemoglobin) were higher than those in controls (levels not reported). [No comparison was made between levels in the air and biomarker levels.]

(iv) Chemical production

Schettgen et al. (2002) evaluated CEV levels in blood from 62 employees exposed to acrylonitrile during the production of surfactants and from 10 non-exposed controls. Levels of CEV in workers (62 pmol/g haemoglobin) and controls (8 pmol/g haemoglobin) appeared to be mainly accounted for by personal smoking habits and diet.

(v) Other occupations

Bergmark (1997) evaluated CEV levels in the blood of 40 laboratory workers who handled acrylamide powder and 18 controls at a university in Sweden. CEV levels were below the analytical LOD (< 2 pmol/g) for all non-smokers (laboratory workers and controls), and CEV was only detected among smokers (laboratory workers and controls). Levels of CEV correlated with the number of cigarettes smoked per day. Thier et al. (1999, 2001, 2002) reported a series of studies on blood biomonitoring of 59 workers who handled low levels of acrylonitrile at multiple workplaces (industry and country not given) as part of a medical surveillance programme. Thier et al. (1999) reported results from a study of three adducts: CEV, *N*-(methyl)valine (MV), and *N*-(hydroxyethyl)valine (HEV). The authors concluded that CEV (2–232.2 µg/L blood) was a suitable biomarker for medical surveillance in these workplaces; however, levels of MV (not

detected to 19 µg/L blood) and HEV (6–33 µg/L blood) represented background levels and did not appear to be related to occupational exposures (Thier et al., 1999). In follow-on studies (Thier et al., 2001, 2002), only CEV in blood was measured, and levels ranged from 15.3 to 81.79 µg/L blood.

Bader and Wrbitzky (2006) reported on an incident during which a worker at a train depot in Germany who was cleaning a tank used to store acrylonitrile lost consciousness and died. Four co-workers attempted a rescue; one wore a self-contained breathing apparatus and entered the tank while the other three staffed the tank entrance. At 25 days, the four co-workers had CEV levels that exceeded background; by 175 days, levels had declined to background.

Leng and Gries (2014) reported on an incident in Germany during which a fire broke out at a chemical plant and a nearby tank that contained acrylonitrile was vented to air. More than 800 first responders and workers provided blood samples approximately 3 months after the incident. The authors reported that the average concentration of CEV (98 pmol/g haemoglobin) was lower than expected on the basis of the air-monitoring data collected during the incident. Van Nieuwenhuyse et al. (2014) reported that a train derailment in Belgium released acrylonitrile into the air (see the cover of the present volume). Approximately 2–6 weeks after the derailment, 841 first responders gave blood samples; the median concentration of CEV was 3.2 pmol/g haemoglobin among non-smoking first responders and 140 pmol/g haemoglobin among first responders who were tobacco smokers.

Frigerio et al. (2019) monitored urinary CEMA and HEMA levels among petrol station attendants in Italy. They reported no statistical difference in post-shift median concentrations of CEMA (1.7 versus 1.5 µg/L) or HEMA (2.2 versus 2.7 µg/L) between gas station attendants (n = 7) and controls (n = 7). Frigerio et al. (2020) evaluated post-shift urinary levels of CEMA among 49 coke oven workers and 49 controls living in the same area in Poland. Tobacco cigarette smokers were excluded from the study. The median concentration of CEMA in workers (3.7 µg/g creatinine) was statistically significantly higher than in controls (1.4 µg/g creatinine).

Wu et al. (2023) measured personal airborne exposure to acrylonitrile and urinary levels of the mercapturic acids CEMA, HEMA, and CHEMA, and also cotinine (a biomarker of cigarette smoke) in 79 exposed and 18 non-exposed workers at three factories using acrylonitrile (industry not reported) in Taiwan, China. CEMA and CHEMA were detectable in post-shift urinary samples from all exposed workers, despite 53 workers having air exposures below the analytical LOD for airborne acrylonitrile. [The Working Group noted that this detectability might reflect a contribution from dermal exposure or tobacco smoking.] Statistically significant linear correlations were observed between personal airborne exposure levels and urinary levels of CEMA and CHEMA, but not of HEMA.

[The Working Group noted that the levels of CEMA and CEV reported in the studies summarized in Table 1.8 were highly variable, and it was difficult to draw a conclusion with regard to an association with the magnitude of exposure to acrylonitrile through inhalation. The reasons why it was difficult to draw a conclusion included: (i) the absence of information on exposure to acrylonitrile through inhalation in some studies; (ii) differences in types of industry and job responsibilities among studies; (iii) the influence of tobacco smoking on biomarker levels; and (iv) varying dermal exposures.] A comparison with Table 1.9 (Section 1.4.3) indicates that urinary concentrations of CEMA are approximately similar in non-smoking (tobacco) petrol station attendants (1.5 µg/L) and the general public (2.5–3.9 µg/L) in the USA (data from the National Health and Nutrition Examination Survey, NHANES, for 2011–2012). CEMA

concentrations in non-smoking coke oven workers (3.7 µg/g creatinine) are also approximately similar to those in the general public (0.9–2.3 µg/g creatinine) in the USA (2011–2016 NHANES survey data) and the general public in Germany (1.5 µg/g creatinine). Cross-shift CEMA levels in workers (smokers and non-smokers) with "low" or "high" exposure to acrylonitrile in Taiwan, China, were approximately similar to those in smokers in the general populations of the USA and Germany. CEV concentrations in non-smoking fibre-polymerization workers in Portugal were approximately 1980–2280 pmol/g haemoglobin. In contrast, the range of CEV concentrations in the general public (with and without environmental tobacco smoke exposure) was 0.76–1.1 pmol/g haemoglobin for adults in Sweden and 0.5–4.2 pmol/g haemoglobin for adults in Germany. [The Working Group noted that smoking influences CEV levels and that, in general, levels among workers are variable.]

1.4.3 Exposure of the general population

The general population can be exposed to acrylonitrile via cigarette smoking, air pollution, and contact with contaminated consumer products. Biomonitoring of acrylonitrile metabolites and adducts has been used to estimate exposure in different population groups (see Table 1.9, Table 1.10). Most acrylonitrile is metabolized to two specific metabolites, CEMA and CHEMA, which have half-lives of several hours and are eliminated via urine. Acrylonitrile can also penetrate erythrocytes and react with the globin chains of haemoglobin, generating the specific N-terminal haemoglobin adduct CEV, which has an elimination half-life of about 60 days (see also Sections 1.3.4 and 4.1).

(a) Tobacco smoke

A comprehensive analysis of the data from three cycles of the NHANES survey of the USA during 2011–2016 revealed a common exposure of the general population (n = 6181) to acrylonitrile (De Jesús et al., 2021). The median concentration and interquartile range for the acrylonitrile-specific biomarker CEMA in urine samples from all non-smoking individuals were 1.38 and 0.895–2.27 µg/g creatinine, respectively. Acrylonitrile exposure of tobacco smokers was considerably higher than in non-smokers, with a median concentration and interquartile range for urinary CEMA of 145 and 74.9–240 µg/g creatinine, respectively. The descriptive analysis of the data indicated that CEMA values were higher in females than in males and higher in young children than in adults. However, a multiple linear regression model revealed that this phenomenon was caused by the creatinine adjustment and not by differing exposure. The multiple regression model explored possible effects related to the intake of different foodstuffs but did not find any significant dependency on internal exposure to acrylonitrile. Differentiation by smoking frequency revealed that acrylonitrile exposure was higher by 68-fold, 114-fold, and 186-fold in individuals who consumed 1–10, 11–20, or >20 cigarettes per day, respectively, than in non-smokers.

Another comprehensive study showed similar results for 853 adolescents and young adults (age ≤30 years) in the population of Taiwan, China (Lin et al., 2018b). Mean concentrations (± SD) of CEMA in the urine of non-smoking individuals without and with exposure to secondhand smoke were 2.09 ± 6.65 and 1.99 ± 5.99 µg/g creatinine, respectively. Individuals who smoked <10 cigarettes per day excreted CEMA at a concentration of 8.65 ± 8.27 µg/g creatinine, whereas urinary CEMA concentrations in those who smoked 10–19 and ≥20 cigarettes per day were 53.5 ± 2.96 and 76.8 ± 2.35 µg/g creatinine, respectively. CEMA levels were significantly lower in female than in male individuals (2.49 ± 7.37 versus 4.65 ± 8.72 µg/g creatinine) and were also significantly higher in individuals who reported current alcohol consumption than

Table 1.9 Exposure of the general population and subgroups to acrylonitrile according to urinary concentrations of CEMA

Population	Year of sampling	Subpopulation (N)	CEMA concentration	Reference
USA, NHANES	2011–2016	Non-smokers (5440)	1.38 (0.895–2.27) µg/g creatinine[a]	De Jesús et al. (2021)
		Smokers (741)	145 (74.9–240) µg/g creatinine[a]	
Taiwan, China; adolescents and adults (age ≤ 30 yr)	2006–2008	Non-smokers without ETS exposure (381)	2.09 ± 6.65 µg/g creatinine[b]	Lin et al. (2018b)
		Non-smokers with ETS exposure (326)	1.99 ± 5.99 µg/g creatinine[b]	
		Smokers, < 10 CPD (49)	8.65 ± 8.27 µg/g creatinine[b]	
		Smokers, 10–19 CPD (49)	53.5 ± 2.96 µg/g creatinine[b]	
		Smokers, ≥ 20 CPD (36)	76.8 ± 2.35 µg/g creatinine[b]	
Germany, adults	NR	Non-smokers (47)	1.5 (5.9) µg/g creatinine[c]	Schettgen et al. (2012)
		Smokers (36)	148 (393) µg/g creatinine[c]	
USA, PATH	2013–2014	E-cigarette user, non-daily (247)	5.34 µg/g creatinine[d]	Smith et al. (2021)
		E-cigarette user, daily (55)	2.98 µg/g creatinine[d]	
		Tobacco user, non-daily (1.851)	34.8 µg/g creatinine[d]	
		Dual user, non-daily (87)	47.9 µg/g creatinine[d]	
		Tobacco user, daily (560)	147.6 µg/g creatinine[d]	
		Dual user, daily (90)	153.9 µg/g creatinine[d]	
USA, NHANES; adults (age ≥ 20 yr)	2005–2012	Non-smokers/non-users (973)	1.44 (1.34–1.56) µg/L[e]	Wei et al. (2016)
		Marijuana users (47)	15.4 (8.15–29) µg/L[e]	
		Cigarette smokers (99)	24 (15.0–38.5) µg/L[e]	
USA, NHANES	2011–2012	Children, age 6–11 yr (417)	2.1(1.9–2.3) µg/g creatinine[d]	Jain (2015a)
USA, NHANES	2011–2012	Adults, non-smokers (1260)	3.1 (2.5–3.9) µg/L[e]	Jain (2015b)
		Adults, smokers (888)	46.3 (31.6–67.7) µg/L[e]	
USA, NCS	2009–2010	Pregnant women (488)	1.33 (2.22) µg/L[f]	Boyle et al. (2016)
Guatemala, women	2016–2017	Non-smoking, traditional stoves (50)	5.0 (2.8–11.0) µg/g creatinine[a]	Weinstein et al. (2020)
		Non-smoking, LPG stoves (48)	3.1 (1.7–7.7) µg/g creatinine[a]	
Guatemala, women	2012–2013	Entire group (64)	7.8 (5.5–14.7) µg/g creatinine[a]	Weinstein et al. (2017)
		After sauna visit (7)	48.1 (28.2–81.2) µg/g creatinine[a]	
USA, non-smokers	2009–2014	Residence in high-greenery area (71)	2.7 ± 5.0 µg/g creatinine[b]	Yeager et al. (2020)
		Residence in low-greenery area (71)	11.4 ± 37.1 µg/g creatinine[b]	

CEMA, *S*-(2-cyanoethyl) mercapturic acid; CPD, cigarettes per day; e-cigarette, electronic cigarette; ETS, environmental tobacco smoke; LPG, liquefied petroleum gas; NCS, National Children's Study; NHANES, National Health and Nutrition Examination Survey; NR, not reported; PATH, Population Assessment of Tobacco and Health; USA, United States of America; yr, year(s).

[a] Median and interquartile range.

[b] Mean ± standard deviation.

[c] Median (95th percentile).

[d] Geometric mean.

[e] Geometric mean (95% confidence interval).

[f] Median (75th percentile).

Table 1.10 Exposure of the general population and subgroups to acrylonitrile according to concentrations of haemoglobin–acrylonitrile adduct CEV

Population	Year of sampling	Subpopulation (N)	CEV concentration (pmol/g globin) Mean (range)	Comments	Reference
Sweden, Stockholm University, adults	1993	Non-smoking individuals working with PAGE (15)	< 2 (< 2)		Bergmark (1997)
		Smoking individuals working with PAGE (7)	24 (2–72)		
		Non-smoking individuals without contact with PAGE (8)	< 2 (< 2)		
		Smoking individuals without contact with PAGE (10)	106 (25–178)		
Sweden, adults	NR	Non-smokers without ETS exposure (18)	0.76 ± 0.36[a]		Pérez et al. (1999)
		Non-smokers with ETS exposure (3)	1.1 ± 0.6[a]		
		Ex-smokers (2)	0.7		
		Snuff users (3)	1.2 ± 0.5[a]		
		Smokers, 20 CPD (1)	91		
		Smokers, 10–15 CPD (1)	64		
		Party-smokers (3)	8.6		
		Smokers, 1–20 CPD (14)	86.2		
		Party-smoker (1)	8.8		
		Smoker, 1 CPD (1)	8.7		
Germany, adults	NR	Non-smokers without ETS exposure (92)	< 0.5 (< 0.5–4.2)	*P* value for comparison of both groups: 0.07	Schettgen et al. (2010)
		Non-smokers with ETS exposure (12)	1.0 (< 0.5–3.4)		

CEV, *N*-(2-cyanoethyl)valine; CPD, cigarettes per day; ETS, environmental tobacco smoke; NR, not reported; PAGE, polyacrylamide gel electrophoresis.
[a] Mean ± standard deviation.

in abstainers (10.8 ± 10.7 versus 2.83 ± 7.53 µg/g creatinine). [The Working Group noted that the authors did not provide either the distribution of smoking habits by subgroup or a multiple regression model, which may prohibit the elucidation of real determinants.]

Schettgen et al. (2012) explored urinary CEMA levels in 47 non-smokers and 36 smokers in the general population of Germany. Median and 95th percentile concentrations of CEMA in non-smokers were 1.5 and 5.9 µg/g creatinine, respectively, whereas these levels in smokers were 148 and 393 µg/g creatinine. Cotinine content in urine was analysed as a biomarker for the intensity of smoking habits and revealed a strong linear correlation between urinary CEMA and cotinine ($r = 0.87$; $n = 83$).

The Population Assessment of Tobacco and Health (PATH) study in the USA (2013–2014), described as a nationally representative, longitudinal cohort study, explored exposure to nicotine and toxicants in users of tobacco cigarettes and electronic cigarettes (e-cigarettes) and in dual users (Smith et al., 2021). The results of the study suggested that exposure to acrylonitrile was low in e-cigarette users for whom geometric mean urinary concentrations of CEMA were 5.34 and 2.98 µg/g creatinine for non-daily and daily use, respectively. However, users of tobacco cigarettes and dual users had significant exposure

to acrylonitrile; less-than-daily users of tobacco cigarettes and dual users had CEMA concentrations of 34.8 and 47.9 µg/g creatinine, respectively, whereas daily users of tobacco cigarettes and dual users had CEMA concentrations of 147.6 and 153.9 µg/g creatinine, respectively.

Wei et al. (2016) demonstrated that marijuana users had substantial exposure to acrylonitrile in a study investigating CEMA levels in urine samples from a subgroup of the NHANES surveys in 2005–2012. The participants were grouped into non-users (n = 973), marijuana users (who had declared marijuana use within 5 days before sampling) (n = 47), and cigarette smokers (n = 99). The geometric means (95% central interval) were 1.44 (1.34–1.56) µg/L for non-users, 15.4 (8.15–29) µg/L for marijuana users, and 24 (15.0–38.5) µg/L for cigarette smokers, respectively. The CEMA level in non-users was significantly lower than in marijuana users, but levels in marijuana users and cigarette smokers did not differ significantly. Whether the marijuana use was via smoking or via other application routes was not assessed by the authors.

The effect of secondhand smoking on the intake of VOCs was explored in an experimental study with measurements inside of automobiles for a specific indoor secondhand-smoking exposure scenario (St Helen et al., 2014). Non-smokers sat for 1 hour in the righthand back seat of the motor vehicle while one smoker sat in the driver's seat and smoked three cigarettes consecutively during this period. Urine samples from the 14 non-smokers were collected in blocks of 0–4, 4–8, 8–12 and 1–24 hours before the experiment and after the exposure period. The median and interquartile range for CEMA concentrations in the pre-exposure urine samples were 1.12 and 0.77–1.72 µg/g creatinine, respectively. CEMA levels increased significantly within the 8-hour post-exposure interval; the median and interquartile range were 2.31 and 1.94–2.58 µg/g creatinine time-averaged for the whole interval, and the maximum levels were 2.53 and 2.10–2.88 µg/g creatinine during this interval (see Section 1.4.1(a) for additional data on indoor acrylonitrile levels).

The effect of household exposure to tobacco smoke on acrylonitrile exposure in children and non-smoking adolescents (n = 411; age < 18 years) was explored in a study nested in the NHANES cycles of 2013–2014 and 2015–2016 (Kindilien and Goldberg, 2021). The comparative analysis of children in the quartile of highest detected levels of CEMA in urine (n = 101) and the combined three quartiles of lower detected CEMA levels indicated that there was a significantly higher frequency of smokers in the homes of highly exposed children compared with those of the less-exposed children (69.4% versus 32.7%, respectively). Congruently, a significantly higher frequency of non-smoker households was associated with the group with lower exposure compared with the group with higher exposure (67.3% versus 30.7%, respectively). Similar results were reported by Jain (2015a) in children aged 6–11 years of the NHANES cycle 2011–2012; there was a significant correlation between the number of smokers in the home and urinary CEMA levels in the children residing in the home. Jain (2015b) also studied exposure of the adult population within the NHANES 2011–2012 cycle and explored the effect of age, gender, race/ethnicity, and smoking status on urinary CEMA levels. Age, gender, and race did not affect internal exposure to acrylonitrile, and CEMA levels were affected only by smoking and urinary creatinine content.

The effect of smoking on individual exposure to acrylonitrile was also demonstrated using a long-term biomarker, the N-terminal haemoglobin adduct CEV (Bergmark, 1997; Pérez et al., 1999; Schettgen et al., 2010; Table 1.10). Bergmark (1997) investigated CEV levels in non-smoking and smoking employees of Stockholm University. Whereas the CEV concentration in non-smokers (n = 8) was consistently below the LOD of 2 pmol/g globin, the CEV concentration in smokers

(n = 10) ranged between 25 and 178 pmol/g globin. Bergmark also reported a significant and strong correlation between number of cigarettes per day and CEV level (r = 0.94). Pérez et al. (1999) explored CEV levels in non-smokers, smokers, and snuff users. In non-smokers without exposure to secondhand smoking, CEV concentrations (mean ± SD) were 0.76 ± 0.36 pmol/g globin, whereas in non-smokers who reported secondhand smoking exposure and in snuff users, concentrations were 1.1 ± 0.6 and 1.2 ± 0.5 pmol/g globin, respectively. Mean concentrations of CEV in smokers were 8.8, 8.7, 64, and 91 pmol/g globin in occasional smokers and in smokers consuming 1, 10–15, and 20 cigarettes per day, respectively. [The Working Group noted that the number of participants was low in both studies, which may affect the sensitivity of statistical analyses.] Schettgen et al. (2010) investigated CEV levels in 104 non-smoking individuals in the general population in Germany with or without secondhand smoking exposure. Median concentration and range for CEV in 92 non-smokers without exposure to secondhand smoking were < 0.5 and < 0.5–4.2 pmol/g globin, respectively, whereas these values were 1.0 and < 0.5–3.4 pmol/g globin in individuals with exposure to secondhand smoking.

(b) Additional determinants of exposure

The possible effects of various determinants of exposure to VOCs, including acrylonitrile, were explored in 488 pregnant women in the National Children's Study (NCS), a longitudinal cohort study in the USA (Boyle et al., 2016). For urinary CEMA concentrations, the median and 75th percentile were 1.33 and 2.22 µg/L, respectively, whereas the maximum reached 812 µg/L. In the questionnaires, 74.2% of the participants indicated no smoking exposure, 19.0% indicated some smoking exposure (< 10 cigarettes per day), and 6.8% identified themselves as smokers (≥ 10 cigarettes per day). Smokers had increased levels of acrylonitrile metabolite compared with levels in participants having no or minimal smoke exposure. In the regression model analyses, participants from rural areas tended to have lower exposure to acrylonitrile than did urban residents (P > 0.01). No impact of any consumer products, e.g. use of air freshener, aerosol, paint and varnish, contributed to individual exposure to acrylonitrile.

In an intervention study, Weinstein et al. (2020) investigated the effect of switching from open fire and solid-fuel stoves to low-emission liquefied petroleum gas stoves on exposure to polycyclic aromatic hydrocarbons (PAHs) and VOCs (including acrylonitrile) in pregnant, non-smoking women living in a rural area in Guatemala (n = 50). The median and interquartile range of CEMA concentrations in urine were 5.0 and 2.8–11.0 µg/g creatinine before the intervention. After installation of the liquefied petroleum gas stoves, these values decreased to 3.1 and 1.7–7.7 µg/g creatinine, respectively, which corresponded to a reduction of 51% after adjusting for seasonal effects. In a previous study of pregnant women living in rural areas in Guatemala (n = 23), Weinstein et al. (2017) revealed that a sauna visit resulted in distinctly higher urinary CEMA excretion than did any other situation (after fasting, lunch, or dinner). The median and interquartile range of urinary CEMA concentrations in sauna visitors (n = 7) were 48.1 and 28.2–81.2) µg/g creatinine, respectively, whereas these levels were 7.8 and 5.5–14.7 µg/g creatinine in all survey participants combined (n = 68).

In a study in the USA, Yeager et al. (2020) investigated the association between residential-area vegetation and exposure to VOCs, including acrylonitrile. A cohort of 213 non-smoking individuals was recruited and peak, cumulative, and contemporaneous greenery near the participant's home was estimated using satellite-derived normalized difference vegetation index (NDVI). Exposure of the participants was determined by the determination of CEMA in urine. Residents with a low NDVI showed significant higher

CEMA levels (11.4 ± 37.1 µg/g creatinine) than did residents with a high NDVI (2.7 ± 5.0 µg/g creatinine). The data demonstrated that CEMA levels were inversely associated (31% lower) with 0.1 unit higher peak NDVI values within a 100 m radius of the participants' home. These associations were significant at radii ranging from 25 to 300 m, and the strongest associations were observed within a 200 m radius. CEMA levels were also inversely associated with the percentage of tree canopy and street trees around the participant's home.

The risk of chemical migration of acrylonitrile in ABS plastic toys was explored by Wang et al. (2023) (see also Section 1.4.1(e)). The migration of acrylonitrile from 94 ABS toys was used to predict potential exposure of children via contact with ABS toys. Levels of daily average exposure to acrylonitrile were estimated for children of different age stages (stage 1, 3 months to 1 year; stage 2, 1–2 years; stage 3, 2–3 years). The daily average exposure level of acrylonitrile ranged from [5.7×10^{-5}] µg/kg body weight (bw) per day for children of the third stage to [2.9×10^{-3}] µg/kg bw for children of the first stage.

1.5 Regulations and guidelines

Exposure limits have been developed by governmental and nongovernmental agencies to protect against the adverse effects of exposure to acrylonitrile. [The Working Group noted that a mass-based recommended exposure limit for the general population exists for inhalation of acrylonitrile; however, guidelines or recommendations are limited for food, drinking-water, and consumer products.] The World Health Organization (WHO) has stated that the use of food-contact materials that contain acrylonitrile is provisionally accepted on condition that the amount of acrylonitrile that migrates into food is reduced to the lowest level that is technologically attainable (WHO, 1984).

1.5.1 Occupational exposure limits

As summarized in Table 1.11, several countries in Africa, Asia, Europe, North America, and Oceania have developed occupational exposure limits (OELs) for acrylonitrile. Full-shift TWA_{8h} exposure limits ranged from 0.1 mg/m³ (New Zealand) to [8.7] mg/m³ (South Africa). Short-term exposure limit (STEL) values range from 2 mg/m³ (China) to 32.5 mg/m³ (France). The end-point considered to define the exposure limits may include cancer and/or non-cancer outcomes. In Europe, the Risk Assessment Committee of the European Chemicals Agency has derived limit values for the inhalation route and the evaluation for dermal exposure. For an OEL (TWA_{8h}), the limit proposed was 1 mg/m³ (0.45 ppm), and for an OEL (STEL), the limit was 4 mg/m³ (1.8 ppm). Since dermal exposure is also possible, the committee also proposed a biological limit value for CEV of 60 µg/L blood (erythrocyte fraction of whole blood) and a skin notation (ECHA, 2018).

1.5.2 Environmental exposure limits

For non-occupational settings, the US EPA has derived an inhalation reference concentration (RfC) of 0.002 mg/m³ of air. This RfC is an estimated daily inhalation exposure level for the general population (including sensitive subgroups) that is not likely to present an appreciable risk of deleterious non-carcinogenic effects during a lifetime (US EPA, 1991). The US EPA has derived an inhalation exposure concentration of 0.001 mg/m³ that would cause 1 in 10 000 people to develop cancer (10^{-4} cancer risk). The US EPA does not have an exposure limit for acrylonitrile in water but estimated the 10^{-4} cancer risk as an exposure of 0.006 mg/L (US EPA, 2018). Additionally, the US EPA has developed health-based provisional advisory levels (PALs) for acrylonitrile in air and drinking-water for the public in the event of an emergency response.

Table 1.11 Occupational exposure limits for acrylonitrile in various countries

Country	8-hour TWA (mg/m^3)	TWA notation	STEL (15 min) (mg/m^3)	STEL notation
Australia	4.3			
Austria	4.5	TRK	18	
Belgium	4.4	Ca, Sk		
Canada				
Ontario	[4.3]			
Quebec	4.3	Sk		
China	1		2	
Denmark	4	Sk	8	Sk
European Union	1[a]	Sk, BOELV	4[a]	Sk, BOELV
Finland	4.4		8.8	
France	4.5		32.5	
Germany – MAK				Group 2, Sh, Sk
Germany – AGS	2.6	PTCR	20.8	PTCR
Germany – AGS	0.26	PPACR		
Hungary	4.3			
Ireland	4.5			
Israel	4.3			
Japan – MHLW	[4.3]			
Japan – JSOH	4.3			
Latvia	0.5			
New Zealand	0.1	Ototoxic		
Norway	4	Sk		
Poland	2	Sk	10	Sk
Republic of Korea	4.5			
Romania	5		10	
Singapore	4.3			
South Africa – RHCA	[8.7]	Sk		
South Africa – mining	4	Sk		
Spain	4.4	Sk		
Sweden	4.5		13	
Switzerland	4.5		4.5	
United Kingdom	4.4	Sk		
USA – ACGIH (TLV)	4.3	A3, Sk		
USA – NIOSH (REL)	[2.2]	Ca, Sk	21.7 C	Ca, Sk
USA – OSHA (PEL)	[4.3]	Ca, Irr	21.7 C	Ca, Irr

A3, confirmed animal carcinogen with unknown relevance to humans; ACGIH, American Conference of Governmental Industrial Hygienists; AGS, Ausschuss für Gefahrstoffe; BOELV, binding occupational exposure limit value; C, ceiling limit (15 minutes); Ca, carcinogenic, mutagenic, and reproductive toxin; Group 2, considered to be carcinogenic for man (based on animal studies); Irr, skin irritation; JSOH, Japan Society for Occupational Health; MAK, Maximale Arbeitsplatz-Konzentration; MHLW, Ministry of Health, Labour and Welfare; min, minute(s); NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; PTCR, concentration corresponding to the proposed tolerable cancer risk; PPACR, concentration corresponding to the proposed preliminary acceptable cancer risk; REL, recommended exposure limit; RHCA, Regulations for Hazardous Chemical Agents (general industry); Sh, skin sensitizer; Sk, skin absorption; STEL, short-term exposure limit; TLV, threshold limit value; TRK, value based on technical feasibility; TWA, time-weighted average; USA, United States of America.

[a] Effective 5 April 2026.

From NIOSH (2020), ACGIH (2021), DFG (2023), ECHA (2023b), IFA (2023).

PALs are temporary limits developed for exposures of 24 hours, 30 days, 90 days, and 2 years. For acrylonitrile, the oral 30-day and 90-day Level 1 PAL is 0.35 mg/L in drinking-water and the inhalation 30-day and 90-day Level 1 PAL is [0.33] mg/m^3 (Goldhaber et al., 2009).

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

The Working Group undertook a critical appraisal of the exposure assessment methods used in 11 cohort studies and four case–control studies of cancer in humans.

For each key study on cancer in humans, the reviews and critiques undertaken in relation to different aspects of exposure assessment are tabulated in Table S1.12 (Annex 1, Supplementary material for Section 1, online only, available from: https://publications.iarc.who.int/646) and are summarized in the following sections. A brief summary of the exposure assessment critique and methods can also be found in the tables in Section 2 (Tables 2.1, 2.3, and 2.5).

In the workplace, the main route of exposure to acrylonitrile is inhalation, although some workers also are likely to have dermal exposure. All except one of the studies evaluated here covered occupational settings. The one study that covered a non-occupational setting was of the general population and measured urinary biomarkers in users and non-users of tobacco. None of the studies evaluated ingestion exposure.

(a) General limitations and strengths

The quality of the exposure assessment is one important criterion for informativeness of these studies. There are some notable limitations of these studies evaluated here. First, dermal exposure was generally not considered. This exposure route is primarily a concern in the occupational studies, as the workplace is the major source of dermal hazards, particularly for fibre operations (especially affecting the studies by Benn and Osborne, 1998, and Symons et al., 2008), but also for other operations to a lesser extent; however, it was unclear to what extent dermal exposure contributes to total exposure in the occupational setting. In addition, although there may be some correlation between inhalation and dermal exposure in some industrial processes, it was not known how strong that correlation would be, and the correlation is likely to vary according to process and job. Similarly, peak exposures were generally not assessed in these studies. Some workers may have a low annual TWA_{8h} exposure, but a high peak exposure (e.g. maintenance workers). This specific limitation could have affected the studies by Benn and Osborne (1998) and Czeizel et al. (2004). If cancer were related to dermal or peak exposures, nondifferential exposure misclassification could occur, but it was unclear how much weight to place on these limitations. Only in the assessments by Kauppinen et al. (1995) and Koutros et al. (2019) was dermal exposure considered, and only in those by Swaen et al. (2004) and Koutros et al. (2019) were peak exposures considered.

Second, many of these studies did not consider how changes in the workplace, including the use of protective equipment (although the effectiveness of the latter may be limited), could have had an impact on exposure levels over time. Most countries did not reduce allowable occupational exposure to acrylonitrile until around the late 1970s to early 1980s, when, generally, the allowable limit was lowered to [2.2–4.3 mg/m^3] (see Section 1.5). Few changes may have been made before this time, so that this limitation would be of greater concern for those studies that had substantial follow-up time both before and after this period (i.e. Kauppinen et al., 1995;

Scélo et al., 2004; Symons et al., 2008; Budroni et al., 2010; Marsh and Zimmerman, 2015; and Koutros et al., 2019). Ignoring the impact of such changes could have resulted in earlier exposures being underestimated. The only studies in which the authors considered how changes in the workplace influenced exposures over time were those by Ott et al. (1980), Swaen et al. (2004), Symons et al. (2008), Marsh and Zimmerman (2015), and Koutros et al. (2019).

Third, information on measurements was generally limited. Measurements taken before the mid to late 1970s were likely to be short-term area samples taken to evaluate engineering controls, which may not reflect personal exposures. The number of measurements taken, the years in which they were taken, the duration of the measurements, the jobs measured, and the reason for the sampling were typically missing in these studies, even if a summary measure of exposure levels was provided. Thus, it was difficult to have a clear understanding of past exposures or to rank studies by exposure levels. The area samples, more frequently collected before the late 1970s for the purpose of evaluating engineering controls, tended to show higher exposures than personal samples. If such samples were taken at face value to estimate exposure levels, overestimation of exposure levels may have occurred for earlier time periods. This limitation would have particularly affected the studies by Kauppinen et al. (1995), Scélo et al. (2004), Symons et al. (2008), Budroni et al. (2010), Marsh and Zimmerman (2015), and Koutros et al. (2019).

Fourth, no study indicated that the exposure occurred and that the assessment was conducted before outcome determination, but for most of the studies, this was a minor limitation. As follow-up time was generally long (e.g. see duration categories), it can be assumed that exposure occurred before outcome determination. Regarding the timing of the exposure assessment, exposure assessment must have occurred after outcome determination in case–control studies (Thomas et al., 1987; Kauppinen et al., 1995; Scélo et al., 2004; Karami et al., 2011), because study participants with a disease must be identified before they can be interviewed for their exposure history. In these studies, the authors stated that the assessors were blinded to case–control status, so the timing of the assessment in relation to determination of disease status was not an issue. This limitation is also minor for follow-ups of earlier cohort studies, because those earlier studies had typically assessed at least some of the exposures before follow-up. Finally, in the cohort studies by Symons et al. (2008), Swaen et al. (2004), and Blair et al. (1998) (affecting the study by Koutros et al., 2019) it was also indicated that the exposure assessment was done blind to disease status. In the remaining studies, it was not clear that the assessors were blind to disease status, which meant that the assessors could have biased their evaluations on the basis of disease status.

Fifth, most of the cohort studies identified here were likely to have well-defined, somewhat homogenous exposure groups (some based on job title or function, others based on estimated exposure levels). In some studies, only a single exposure group was identified (Thiess et al., 1980; Geĭko et al., 1996; Budroni et al., 2010), which probably resulted in heterogeneous exposure levels. The exposure groups in case–control studies are also likely to be heterogeneously exposed, because of the inherent nature of the settings in these studies (community-based) and the limited information typically available to the exposure assessor. This is likely to cause nondifferential misclassification. Differential misclassification can occur from greater recall by case participants than by control participants.

Sixth, respirators and gloves, if provided as part of an effective and comprehensive programme, may reduce exposure levels (e.g. see regulations such as OSHA, 2024). However, for most of the time during which exposures were assessed in the studies in the present monograph,

respirators and gloves were probably less than effective. Only Swaen et al. (2004) and Symons et al. (2008) indicated that respirators were considered. No study mentioned glove use.

One strength of these studies was that when the reference population is the general population, that population is unlikely to have been occupationally exposed because of the low prevalence of occupational acrylonitrile exposure. Internal reference populations were also probably not exposed to acrylonitrile, but some may have been exposed to other carcinogens. All participants (referent or otherwise) could have had additional exposure to acrylonitrile and other carcinogens from smoking tobacco (Etemadi et al., 2024).

Ideally, one would use cumulative exposure (generally considered to be the best predictor of cancer risk) to rank studies by exposure levels in order to understand exposure contrasts among the studies; however, this metric was estimated only in a few studies (Scélo et al., 2004; Swaen et al., 2004; Symons et al., 2008; Marsh and Zimmerman, 2015; Koutros et al., 2019). The Working Group considered several other factors to inform a ranking of studies by exposure level: (i) the acrylonitrile production process: monomer production, because it is a closed and continuous process, would generally result in lower exposure levels than would polymer and copolymer operations, which may be partially open or batch processes; the aforementioned processes are likely to result in lower exposure levels than those of fibre operations, which are open processes. It was unclear whether exposures in resin operations were more similar to those in polymer/copolymer or in fibre operations; (ii) date of exposure: levels before approximately 1980 were likely to be substantially higher than after 1980, when exposure levels started decreasing; (iii) reported measured levels: knowing the type of measurement (area, personal; short-term, full-shift) and the date of the measurement(s) can provide comparison of exposure levels across studies for the same approximate time period and possibly insight into historical levels; and (iv) reported analytical exposure categories: the range of estimated exposures can provide the expected range of exposures, similar to measured levels, if specification is made as to what metric they are representing (i.e. current or historical average intensity or cumulative exposure). Given the uncertainty of the data, the Working Group ranked the studies in terms of exposure levels as follows: (i) higher levels: Thiess et al. (1980), Symons et al. (2008), Marsh and Zimmerman (2015), Koutros et al. (2019), and Benn and Osborne (1998); (ii) moderate levels: Ott et al. (1980), Swaen et al. (2004), Delzell and Monson (1982), Kauppinen et al. (1995) and Geĭko et al. (1996); (iii) lower levels: Scélo et al. (2004), Czeizel et al. (2004), and Etemadi et al. (2024). The exposure levels reported in Budroni et al. (2010) and Thomas et al. (1987) could not be estimated.

(b) Cohort studies

Symons et al. (2008) recruited 2548 workers employed for ≥ 6 months between 1966 and 1970 at two acrylic fibre plants in the USA. First exposure ranged from 1947 through 1991, when acrylonitrile operations ended. Company work histories, a variety of exposure-related records, including both area and (after 1975) personal measurements (Wood et al., 1998), changes in the operations, use of protective equipment, and information from interviewed workers were used to develop a job/workplace/time period exposure matrix. The exposure groups were based on mean intensity estimates of acrylonitrile exposure: [< 4.3, 4.3–43.2, and > 43.4 mg/m^3]. Cumulative exposure categories were: [< 21.7, 21.7–108.3, 108.5–216.8, and > 217 mg/m^3-years]. [There was no information on measurements, and they were likely to be limited before the 1970s. Although there was no information on confounding exposures in this report, an earlier study (Chen et al., 1987) indicated that dimethylformamide, an

agent classified by IARC in Group 2B, *possibly carcinogenic to humans*, was present. The exposure assessment was well done, including developing exposure groups based on estimated exposure levels, reviewing a variety of exposure documents, and evaluating disease risk by several metrics. Although the methodology was strong, exposure levels may have been over- or underestimated in the early exposure periods. The exposure assessment was of good quality, although it was likely to have some nondifferential misclassification, which would tend to bias the results towards the null.]

Ott et al. (1980) used employer work history records to obtain jobs and start and stop dates for 100 workers who worked for ≥ 1 year and were exposed to acrylonitrile in the USA. [It was unclear to the Working Group as to the number of plants and operations, although the study appeared to cover ABS and SAN rubber processes.] The operation started in 1952, and follow-up was through 1975. Using job descriptions and industrial hygiene surveys, acrylonitrile-exposed jobs were grouped into six exposure groups, all of which had concomitant exposure to styrene and ethylbenzene. [The Working Group noted that no information was provided on actual jobs or exposure dates.] Changes in the workplace that had an impact on worker exposures were considered. The groups were assigned 8-hour time-weighted intensity estimates of [2–9 mg/m^3] (vapours 3A) and [11–20 mg/m^3] (vapours 3B). The authors noted that exposures were considered to be well controlled for the standards of the day. [The Working Group noted that before 1978, the standard for acrylonitrile was [43 mg/m^3] in the USA; in 1978, it was reduced to [4.3 mg/m^3].] The authors also noted that in 1954 the air concentrations were presumed to be <[2 mg/m^3] TWA for the developmental and pilot plant workers. The analytical metric was the exposure group by exposure duration (< 1, 1–4, ≥ 5 years). Other substances present in the plant included ethyltoluene, isopropylbenzene, and benzene. Colourants included cadmium, chromium, arsenic, and azo dyes. Included in the exposure categories were research and development workers, who may have had lower frequency and/or duration of exposure than did the production workers and thus lower cumulative exposures than did workers in the same assigned exposure group. Duration came from company records, and exposure intensity came from industrial hygiene measurements of styrene and ethyl benzene that were area samples, which, although limited, were available from the late 1940s. There were other possible co-exposures that were not accounted for, including benzene, heavy metals, and azo dyes. Workers were evaluated for the combined exposure of acrylonitrile/styrene/ethyl benzene, all "present in approximately equal concentrations". [The study had an exposure assessment of moderate quality, and the limitations would primarily cause nondifferential misclassification and tend to bias the results towards the null.]

Thiess et al. (1980) studied 1469 workers employed for ≥ 6 months in 12 companies making SAN and ABS polymers, organic intermediates, polymer dispersions, and polymer solutions from the early 1950s through 1978 in Germany. The single exposure group appeared to be defined as employment at any of the companies. No measurements were available historically, but manual handling operations, which occurred until about 1976, resulted in the authors speculating that exposures may have been as high as [43 mg/m^3] or, for short periods of time, even higher. Since 1976, processes were closed, protective measures were implemented, and measurements were less than the exposure limit of [6.5 mg/m^3]. [The Working Group presumed that "protective measures" meant the use of respirators and gloves] The analytical exposure groups were analysed by duration of employment categories (0–4, 5–9, and ≥ 10 years). Co-exposures varied by company but included cadmium (classified by IARC in Group 1, *carcinogenic to humans*), phenol, β-naphthylamine (also IARC Group 1),

dimethylsulfate, epichlorohydrin (IARC Group 2A, *probably carcinogenic to humans*), and vinyl chloride (IARC Group 1). The authors noted that a large proportion of the study participants had been employed in other chemical plants, probably resulting in increased exposure to these chemicals or to other confounding co-exposures. [The exposure group was likely to be heterogeneously exposed. No exposure assessment was conducted, and no measurements were available before 1976. The only metric evaluated was duration of exposure. Participants were exposed to a variety of carcinogenic co-exposures for which adjustment was not made. Two strengths were that exposure levels may have been high and that co-exposures probably varied by company. The study exposure assessment was of minimal quality and, because of the expected heterogeneity of exposures of the studied population, nondifferential misclassification was also likely, which would tend to bias the results towards the null.]

Benn and Osborne (1998) used work histories collected in an earlier study (Werner and Carter, 1981) of 1111 men employed between 1950 and 1968 in the polymerization of acrylonitrile and spinning of acrylic fibres at six factories in the UK and added 1904 men identified from employer records as polymerization or spinning workers employed between 1969 and 1978 in those same factories. Although follow-up was to 1991, exposure after 1980 was considered negligible and not evaluated. The exposure assessment consisted of the company personnel assigning workers to one of six groups that were then merged into three groups for the analysis. These qualitative analytical exposure groups required ≥ 1 year of exposure in the group: (i) a combined group of polymer workers and spinners (high exposure); (ii) maintenance, laboratory workers, and other (not identified) possibly exposed jobs ("other possible exposure"); and (iii) other (not identified) jobs with no possible exposure (and, in factory 5, "end of line worker"), in addition to workers from groups 1 and 2 with < 1 year in the group ("little or no acrylonitrile exposure"). Measurements became available in the late 1970s, when TWA_{8h} levels of [0.9–5.9 mg/m^3] were measured for the polymer workers and spinners. [The Working Group noted that although the language suggested that these measurements were personal samples, this conclusion was not clear.] Analytical metrics were by job group and by the job group with the highest exposure, duration of exposure (< 5, 5–10, 10–15, > 15 years), and time since first exposure (< 5, 5–10, 10–15, > 15 years). [Records for 785 workers first exposed between 1969 and 1978 who had not been included in the original 1969–78 workforce in factory 5 were later found. The reason for the missing records was not known. As a result of the additional workers, that factory contributed > 60% of the cohort, but only 13% of that factory's population was in the high-exposure category compared with almost 100% in that category for the other factories. No explanation was provided for this difference. This raised the issue of the validity and quality of the exposure assessment. It was unclear what percentage of workers had adequate latency, particularly in factory 5 where > 75% of that plant's population was employed after 1968 compared with 25% of the other companies (follow-up was to 1991). The end-of-line workers in factory 5 were originally coded as having high exposure; no reason was provided as to why this had been done. The exposure group was composed of both polymer operators and spinners. Exposure levels for these workers probably varied across plants and within the plants over time. Solvents, dyes, and pigments are probably found in these operations. In summary, this exposure assessment had severe limitations that may have resulted primarily in nondifferential exposure misclassification because of anomalies in cohort recruitment and exposure assessment, possible insufficient latency, lack of estimation of exposure levels, differences over time, and no information on possible confounders.

Nondifferential misclassification would tend to bias the results towards the null.]

The study by Delzell and Monson (1982) comprised 327 male workers employed for ≥ 2 years between 1940 and 1971 at a rubber-manufacturing plant in the USA (the exposure start date for acrylonitrile was not identified). The exposure groups were all workers in two (combined) departments in the chemical division of the plant that made nitrile rubber. The analytical metric was duration of employment (< 5, 5–14, > 15 years) and latency (< 5, 5–14, > 15 years) for this combined group. Butadiene (classified in IARC Group 1), styrene (IARC Group 1), and vinyl pyridine, and probably dyes and pigments, were co-exposures. There were 81 workers in the department with the higher acrylonitrile exposures, but all analyses combined the departments with both the higher and lower exposures. [The Working Group noted that it was not clear that all people in these departments were exposed, and exposures were likely to be heterogeneous. No quantitative exposure assessment was conducted. The outcome analysis was based on duration of employment. Adjustment for co-exposures to styrene and butadiene was not done. The exposure assessment was of minimal quality. The primary type of misclassification would be nondifferential misclassification, which would tend to bias the results towards the null.]

In the study by Geĭko et al. (1996), only 239 people were exposed in a company producing acrylonitrile monomer in the Russian Federation. The authors required that all participants be exposed for ≥ 3 years between 1938 and 1985. The only exposure group was that of ever exposed. The analysis was performed by duration of exposure (10–15, 20–24 years only). [The exposure group was likely to be heterogeneously exposed. No exposure assessment was conducted. Only levels of duration of exposure were evaluated. No co-exposures were reported. Sufficient latency and duration (10–15 and 20–24 years of exposure) were probably experienced by many workers. The exposure assessment was of minimal quality. Although misclassification could be present because only duration was evaluated, the exposure misclassification would be primarily nondifferential, which would tend to bias the results towards the null.]

Koutros et al. (2019) identified 25 460 workers (16 889 exposed) employed for ≥ 1 day at eight facilities (four acrylic monomer, three acrylic fibre, and one acrylic resin) in the USA. This was a 21-year extension of mortality follow-up from previous reports on this cohort through 2011. Detailed work histories were collected from the beginning of acrylonitrile operations, ranging from 1952 to 1965 through record abstraction in 1983. A detailed description of the exposure assessment was provided by Stewart et al. (1998). Briefly, the exposure assessment team visited each facility three to four times, during which they conducted walkthrough surveys of exposed areas [> 0.02 mg/m^3] and interviewed long-term workers. With the assistance of the companies and unions, the investigators interviewed two to five experienced workers from each exposed department to identify exposed job, job tasks, changes over time and other exposure-related information. Historical records were also obtained. Companies provided approximately 18 000 measurements. Approximately 13 000 were full-shift personal measurements from seven or eight companies covering approximately 300 jobs. Only one company had measurements from before 1977, and these were primarily short-term area samples to evaluated engineering controls. In addition, in 1986 the investigators collected measurements on 10 jobs at each plant to evaluate consistency of plant measurements across facilities. Exposure was assessed for 3700 similarly exposed groups on the basis of job, department, plant, and time period. Work history records, plant records, and monitoring data were used to estimate TWA exposures. As part of the exposure assessment, information relevant to dermal exposure was also collected, although it was not

clear whether it was used in the exposure assessment. Metrics were developed for cumulative, average, and duration of exposure, and a 10-year lag was used to address latency. [The Working Group noted that this was a very extensive, high-quality quantitative exposure assessment that had several key strengths related to acrylonitrile exposure assessment. First, this was a very large cohort with workers from eight facilities with a wide range of exposures and exposure circumstances. Second, exposures were assessed from the beginning of acrylonitrile operations at each of the plants. Third, the authors went to extensive efforts to quantitatively assess exposure using data collected from plant visits, plant records, and monitoring data from the companies and collected by the investigators. The primary limitation of this study was the lack of exposure data before 1977, which could contribute to exposure misclassification during earlier periods of the study. Another major limitation was that the exposure assessment was not extended to cover the additional 21 years of follow-up. Differential misclassification was unlikely given that the assessors were blind to disease status. Some nondifferential misclassification may have occurred, which would tend to bias the results towards the null. The exposure assessment was of high quality.] The study by Marsh and Kruchten (2023) was a reanalysis of the data by Koutros et al. (2019) is not described in the present section.

The study by Swaen et al. (2004) included 2842 men employed for ≥ 6 months in eight chemical plants in the Netherlands that made acrylonitrile, ABS products, acrylate, fibre, and resin, in addition to a catalyst experimental plant. The dates of exposure varied across the plants between 1959 to 1973 through 1978, and participants were assigned zero exposure in the first 6 months of employment and for exposure after 1980 to 2001. Company personnel records identified the work histories. A job-exposure matrix (JEM) of job, workplace, and time period was developed for exposure groups and used to derive estimates of intensity. The authors indicated that measurement data were used to estimate exposure. The average exposure varied by plant, ranging from [0 to 10.9 mg/m^3]. [The Working Group noted that it was not clear whether this was most recent or across all years of exposure.] Five categories of exposure intensity were developed and then multiplied by duration for cumulative exposure categories of [< 2.2, 2.2–21.7, and > 21.7 mg/m^3-years]. Peak exposures were estimated by exposure categories and qualitative (yes/no) evaluations of respirator use (not defined), and exposure to other IARC carcinogens (not specified in the publication) was analysed. [Exposure levels may be underestimated because exposures in the first 6 months or after 1980 were not counted. The lack of measurements in the early years and the presence of possible co-exposures in both the population at risk and the reference population were additional limitations. If measurements were limited before the later 1970s (as was likely), historical estimates may have been under- or overestimated. No mention was made of co-exposures. Sufficient latency was likely to have occurred. The authors used company records and measurements to assess exposures for a job/work area/time period exposure matrix for estimates of intensity. Several metrics were evaluated. Differential misclassification was unlikely given that the assessors were blind to case–control status. The exposure assessment was of good quality. Some nondifferential misclassification was likely, which would tend to bias the results towards the null.]

The study by Marsh and Zimmerman (2015) was an update of mortality for 2096 workers from two earlier studies (Marsh et al., 1999 and Marsh et al., 2001) in a plant that produced and used acrylonitrile to make acrylamide or resin in the USA (Blair et al., 1998). Originally, four industrial hygienists independent of the company used area measurements (from 1960 or later) and personal measurements (from 1978 or later) to classify job titles from 1955 through 1996. [The

paper suggested that the exposure estimates for the 1955–1959 period were those developed for the study by the National Cancer Institute (Blair et al., 1998), but Stewart et al. (1998) described exposure levels for this plant and did not identify any estimates for this period.] For the present study, work histories for workers employed at earlier periods and more recent hirees were collected through 2011. Company industrial hygienists and human resources representatives assigned the work history jobs to one of 20 job groups based on work performed and job type and estimated daily TWA arithmetic mean exposure levels using monitoring data from 1999–2011. Calculated from these means and exposure duration, the analytical metrics were [0–17.1, 17.4–238.5, and ≥ 238.7 mg/m^3] for cumulative exposure and [> 0–10.6, 10.9–25.8, and > 26 mg/m^3] for exposure intensity. Estimates of duration, intensity and latency were also used in analyses. Asbestos, butadiene, and a depleted uranium catalyst were identified as co-exposures and were adjusted for in the regression analysis. [The assessment was apparently done by three different groups, including company-independent industrial hygienists in 1960–1996 and company industrial hygienists in 1999–2011. Different approaches and different scales could have been used. Only area measurement data were available until 1978. It was unclear whether exposure estimates were developed for 1997–1998. Asbestos and butadiene (both classified in IARC Group 1) were present in the operation. The exposure assessment methodology was good and accessed personal measurements after 1978. The basis for the exposure estimation may have varied according to the various assessors. The exposure assessment was of good quality; however, nondifferential misclassification was likely, which would tend to bias the results towards the null.]

A petrochemical complex of seven factories in Italy that produced or used acrylonitrile and employed 2336 employees exposed to acrylonitrile since 1960 was studied by Budroni et al. (2010). Information on workers (employment start and end date and factory) employed for ≥ 6 months between 1990 and 2006 was collected from the National Social Security Institute. The only exposure group was qualitative (ever worked). For each factory operation, exposure to agents classified by IARC in Groups 1, 2A, and 2B (e.g. styrene, butadiene, benzene, toluene, xylene, dichloromethane, and asbestos) was identified. [No information was provided on the start date of exposure. No quantitative exposure assessment was conducted. Of the 2336 employees who worked in plants producing or using acrylonitrile, about 75% worked in companies producing or using styrene, whereas about 65% worked in companies producing or using butadiene, asbestos, benzene/toluene/xylene, and dichloromethane. The exposure assessment was of minimal quality. Misclassification in this exposure assessment was likely to be nondifferential because of the heterogeneity of the population and, as a result, would tend to bias the results towards the null.]

In the study by Etemadi et al. (2024), the only study of non-occupational exposure, 205 cases of oesophageal cancer and 226 controls were recruited from a previous cohort study in the general population of the Islamic Republic of Iran. Data collection involved a questionnaire on use of tobacco (cigarette, waterpipe/hookah, chewed tobacco) at least once per week, and urine samples. The exposure groups were current tobacco users (use within the year before enrolment); former users (cessation 1 year before the study started) and never users. [The Working Group inferred that the former users were combined with never users to comprise the group "do not currently use tobacco".] The urine samples were collected at cohort enrolment, before the cases were recruited for the nested study. Samples were analysed for acrylonitrile metabolites, and geometric means and 95% confidence intervals (CIs) of creatinine-corrected concentrations were

reported for both tobacco users and non-users. [The exposures of the participants who were assigned to the current users group were likely to be heterogeneous. Furthermore, the group, "do not currently use tobacco" was heterogeneous, as it comprised both former and never smokers. The biological markers represented only recent exposures, not lifetime exposures. The study did not adjust for other smoke components that are carcinogenic. Two of the metabolites are specific for acrylonitrile. This exposure assessment was well conducted, and any misclassification was likely to be nondifferential, which would tend to bias the results towards the null.]

(c) Case–control studies

Thomas et al. (1987) conducted a death certificate-based case–control study of case participants with astrocytic brain tumours (n = 300) and referents who died of other causes (n = 386) in three regions of the USA with a high prevalence of petrochemical refining or chemical manufacturing. Detailed occupational histories including all jobs were collected from next-of-kin interviews. The industrial hygienist, blinded to case/referent status, coded each job for potential exposure to acrylonitrile and other exposures of a priori interest. Jobs with potential exposure to acrylonitrile included those in the production of plastic and rubber, but "primarily" included jobs in farming, where acrylonitrile may have been used as a pesticide. Analyses were conducted for those whose jobs may have involved exposure and the duration of potential exposure. [The primary strength of the exposure assessment by Thomas et al. (1987) was the blind expert assessment of each job from the work histories. The primary weakness, which was inherent in the death certificate-based design, was the use of proxy respondents to provide lifetime work histories. Differential misclassification is possible if the next of kin differed in recall by case/control status. Another limitation was the inclusion of agricultural workers in the acrylonitrile-exposed category when the probability of exposure may have been low. The impact of this limitation would probably be nondifferential, which would tend to bias the results towards the null. The quality of the exposure assessment was moderate.]

Kauppinen et al. (1995) sent questionnaires by mail to the next of kin of 1419 deceased case participants in Finland who were diagnosed with pancreatic cancer between 1984 and 1987 and 3510 deceased participants with other cancers. Lifetime information on employers, facilities, jobs and start/stop years was asked for. The pertinent assessment for acrylonitrile was based on a UK JEM that had semiquantitative estimates of probability and level of exposure without measurements units (i.e. each metric: none, low, and high) (Pannett et al., 1985) through 1974. A second assessment was done for some chemicals [including, it appeared, acrylonitrile] on a case-by-case basis by industrial hygienists. Dermal exposure was evaluated. The exposure groups were based on probability and intensity of exposure. The reported analysis for acrylonitrile, however, was based on ever exposure including all exposed participants who reported having worked in the rubber industry. [Only 11 participants were evaluated as having acrylonitrile exposure and for none was that exposure considered to be probable. The questionnaires were mailed to the next of kin. The authors used the UK JEM for an initial assessment. It was unclear whether the exposure profiles in the UK were the same as those in Finland. Only the results for ever exposed are reported, probably because of small numbers. Eleven of the participants worked in the rubber-manufacturing industry, which was likely to have confounding exposures, possibly to styrene and butadiene. Differential misclassification was possible because of the use of next of kin as the questionnaire responders, although responders for case and control participants identified an average of 2.16 and 2.13 jobs held, respectively, suggesting little bias in reporting. The exposure assessment was of good

quality. Nondifferential misclassification was also possible, which would tend to bias the results towards the null.]

The study by Scélo et al. (2004) included 2861 case participants and 3118 control participants recruited between 1998 and 2002 from 15 centres in central and eastern Europe and the UK. Standardized questionnaires and, for specific jobs and industries, 18 specialized occupational questionnaires [although it was not known how detailed these specialized questionnaires were for acrylonitrile] were administered. Exposure assessors in each centre were trained and evaluated for validity. The confidence in the assessment (estimated as proportion, 1–5%, 5–30%, and > 30%), frequency (no categories identified), and intensity [< 2, 2–10, and > 10 mg/m^3] of exposure for each job were estimated. The midpoints of frequency were 3%, 17.5%, and 65%). [The Working Group noted that frequency was undefined, but the values representing the midpoints of each interval were the same as the midpoints of the confidence intervals.] A reliability evaluation of unidentified exposures was made by the assessors, and comparability was found among the experts but, although misclassification varied among agents, because of the low prevalence of acrylonitrile, the reliability was judged adequate. [The Working Group noted that it was indicated that validation exercises were regularly conducted, but no information was provided on the results of these exercises, which actually may be referring to the reliability study.] Analytical categories were developed for duration, weighted duration (duration × frequency) and cumulative exposure (weighted duration × intensity, using the midpoints of the intensity ranges (the latter, [< 1.1, 5, 20 mg/m^3]). Manufacture of rubber and plastics (23% of exposed jobs) and footwear (22%) were the primary industries employing the acrylonitrile-exposed participants. Co-exposures included styrene and vinyl chloride. [No mention of accounting for historical changes was indicated. The definitions of confidence in the assessment and frequency was not clear: they seemed to have the same values. To calculate cumulative exposure, the authors used frequency. If frequency represents probability of occurrence, rather than frequency (hours) in a work week, this was an untraditional definition of cumulative exposure and would not be comparable to the other cumulative exposures described in the present monograph. There was a large overlap of exposures between acrylonitrile and vinyl chloride and styrene: correlations with acrylonitrile were 0.84 and 0.90 for vinyl chloride and styrene, respectively. In addition, there may have been other carcinogens present at the participants' workplace. The number of jobs reported by cases participants and by control participants was approximately the same (3.7 versus 3.6, respectively), suggesting that reporting was not likely to have been a source of substantial bias. Differential misclassification was unlikely given that that the assessors were blind to case/control status. Nondifferential misclassification was likely, which would tend to bias the results towards the null. The exposure assessment was of good quality.]

Karami et al. (2011) conducted a hospital-based case–control study including case participants with renal cancer (n = 1097) and control participants who were patients with unrelated diagnoses (n = 1476) from Czechia, Poland, Romania, and the Russian Federation. Detailed lifetime work histories, with additional specialized questionnaires for some specific jobs and industries including the chemical and rubber industries, for jobs held for ≥ 12 months were collected by trained interviewers. Possible exposures to acrylonitrile and other exposures of interest were assessed by trained local experts, who also evaluated the frequency (< 5%, 5–30%, > 30%), intensity (low, medium, high), and confidence (possible, probable, definite) of exposure. Ever exposure and cumulative exposure (the product of duration, frequency, and intensity) were used in the analysis. [The Working Group

noted that the study had several strengths related to the exposure assessment. Data were collected by trained interviewers using standardized questionnaires with detailed work histories and specialized exposure assessment questionnaires for key industries or occupations. Frequency, intensity, and confidence of exposure to acrylonitrile and other exposures of interest were assessed by local experts trained by the study's lead exposure assessor, but with knowledge of exposure conditions within their country. The major limitation was the low prevalence of exposure in this population (only 10 cases and 6 controls), which limited the ability to explore exposure–response relations. Differential misclassification was unlikely given that the assessors were blind to case–control status. Nondifferential misclassification was likely, which would tend to bias the results towards the null. The exposure assessment was of good quality.]

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

The Working Group reviewed the exposure assessment for acrylonitrile in 19 mechanistic studies in exposed humans. The reviews and critiques undertaken in relation to different aspects of exposure assessment are reported in Table S1.13 (Annex 1, Supplementary material for Section 1, online only, available from: https://publications.iarc.who.int/646). All studies were of a cross-sectional design focusing on one of two groups: either occupationally exposed populations (10 studies) or the general population (nine studies).

Several studies had some characteristics in common. First, for both groups, inhalation was the primary route of exposure. The inhalation exposure levels of workers were likely to have been substantially higher than those in the general population (see Sections 1.4.2 and 1.4.3). It was possible, however, that some younger non-smoking participants in the occupational studies had lower cumulative exposures than did older long-time smoking participants in the general population. Second, dermal exposure was generally not considered in the working populations studied (the study by Cave et al. (2011) being the exception), and such exposure would not have occurred in the general population. Third, no evaluation was made of exposure homogeneity to determine whether the participants within each analytical group were similarly exposed, which was of particular concern for studies that assessed the outcome for a single group comprising, for example, all exposed workers at a company. This lack of homogeneity is referred to as "appropriateness" in the text below and could result in nondifferential misclassification. Fourth, exposure–response relations or latency were not investigated in any of the occupational studies. Fifth, there was substantial variability among the studies in identifying duration of exposure, level of exposure, or workplace operations of the exposed population, all of which are important to understanding the exposure situation. The outcomes assessed in these studies varied (e.g. testosterone levels, levels of pro-inflammatory cytokines or genetic markers), so it is likely that the exposure duration and intensity needed to detect exposure–response relations varied between studies. Sixth, none of the studies analysed the outcome by duration, cumulative exposure, or latency. Only in the study by Cave et al. (2011) was a unitless estimate of cumulative exposure developed. Seventh, typically, the workers in these studies were likely to have had exposures to other chemicals, including carcinogens, whereas studies in the general population have assessed metabolites of several carcinogens, such as those found in tobacco smoke, but no adjustments were made for these other exposures in any study. Lastly, in many studies, exposure was assessed only qualitatively, for example, by assigning exposure to workers involved in a process using acrylonitrile or to members of the general population who

were smokers. Although it was likely that some exposure to acrylonitrile had occurred in these cases, the exposure assessments in these studies were judged by the Working Group as being of minimal quality because no quantitative exposure assessment was performed and hence some or even substantial nondifferential exposure misclassification was likely.

Nondifferential misclassification generally can be expected to shift estimated risk results and odds ratios towards the null.

(a) Occupational exposure assessment

For the most part, the methodology for assessing exposures among working populations was limited (the study by Cave et al. (2011) being the exception). Identification of exposed groups was generally done categorically (i.e. qualitatively, for example, people who worked in an acrylonitrile-using plant, and air measurement data were not considered). Although dermal exposure was likely to have occurred among at least some of the participants of the working population, in only one study (Cave et al., 2011) was it considered. Additionally, none of the studies reporting air measurements described the sampling analytical methods, so it was unclear whether validated methods were used.

The study by Cave et al. (2011) had the best exposure assessment methodology of all the mechanistic studies. This study compared healthy workers with workers with toxicant-associated steatohepatitis and with other suspected liver disease. These authors estimated levels of acrylonitrile exposure of 82 workers in two ABS elastomer/polymer companies in the USA for whom exposure was categorized to evaluate the participants' levels of cytokeratin 18, a marker of liver disease. A ranking of acrylonitrile exposure level was assigned on a scale of 0 to 6 for each job held by one of the study participants, as identified from employer records. The occurrence of dermal exposure was assigned the highest ranking (6). The mean duration of employment [and presumably exposure] of the cohort was 21.57 ± 9.17 years. Cumulative exposure was estimated, although interpretation of the actual exposure (in mg/m^3) was not provided, and no exposure levels were reported. The statistical analyses also presented the mean employment duration. Exposures to two other carcinogens (1,3-butadiene and styrene) were also estimated, although the study did not adjust for them. [The Working Group noted that it was not clear whether the rankings were based on recent air measurements of acrylonitrile or whether changes in exposure levels over time were considered. Too much weight may have been placed on dermal exposure, since its occurrence was assigned the same ranked score (6) as the highest level of inhalation exposure, and the frequency of dermal exposure, the acrylonitrile concentration in the liquid, and glove use probably varied across the jobs with dermal exposure. The Working Group also noted that the correlation of acrylonitrile with 1,3-butadiene and styrene may have been high. Other chemicals may have been present, particularly pigments and dyes. Exposures may have been over- or underestimated in the early years of exposure because of the limited measurement data at that time. Moreover, exposures in the highest exposure category may have been overestimated because of the assignment of dermal exposure to the highest exposure category. This study had the strongest exposure assessment methods of the mechanistic studies. Nondifferential misclassification, which would tend to bias the results towards the null, was likely to be the lowest of all the mechanistic studies, because the investigators actually considered exposure levels. However, these exposure levels were not used in multivariable regression models with the inflammatory cytokines (see Section 4).]

Ivănescu et al. (1990) investigated the relation between acrylonitrile and blood testosterone levels as measured at three time points in a total of 297 acrylonitrile-exposed men who had been

working at an unspecified type of chemical plant for 6 months to 10 years. [On the basis of the affiliation of the authors, the Working Group assumed that the study took place in Romania.] An additional 65 people were evaluated longitudinally over 2 years. The authors stated that the major exposure being evaluated was acrylonitrile, but no information was provided on exposure levels or co-exposures. [Although duration of exposure was assessed, participants were not grouped by exposure duration but rather by date of blood collection. The workplace- and exposure-related information was limited, only exposure duration but not intensity was assessed. It was unclear whether carcinogens were present in the workplace. The exposure assessment was of minimal quality and as a result probably had nondifferential misclassification, which would tend to bias the results towards the null.]

Two job groups (continuous-polymerization workers, n = 7; and maintenance mechanics, n = 9) at an acrylonitrile polymerization plant were selected to measure blood CEV levels (see Sections 1.3.4 and 1.4.2) by job group by Tavares et al. (1996). [On the basis of the affiliation of the authors, the Working Group assumed that the study took place in Portugal.] The workers were identified as having co-exposure to dimethylformamide. [The workplace and exposure-related information was very limited, and no assessment of dermal or air exposure other than the outcome measurement (CEV levels) was conducted. It was unclear whether carcinogens were present in the workplace, although pigments and dyes may have been used in the process. The exposure assessment was of minimal quality and as a result probably had nondifferential misclassification, which would tend to bias the results towards the null.]

Two groups of workers (13 fibre producers; 13 maintainers) in a viscose rayon plant with 3–10 years of exposure (to acrylonitrile and/or dimethylformamide) were evaluated for lymphocytes, liver function, and genetic markers (Major et al., 1998). [On the basis of the affiliation of the authors, the Working Group assumed that the study took place in Hungary.] The range of peak area measurements was 0–17.6 mg/m^3 at the start of the study and 0.3–5.1 mg/m^3 7 months later, but these measurements were not used to estimate exposure levels. [The Working Group noted that these results were the highest measurements reported among the mechanistic studies on acrylonitrile, although they were area sample results and so may not be indicative of personal exposures.] No adjustment to the acrylonitrile outcome was made for dimethylformamide. There was no mention of any other co-exposures. [It was not clear that all participants were actually exposed to acrylonitrile: "with 3–10 years previous occupational exposure to undefined CAN [acrylonitrile] and/or DMF [dimethylformamide] concentrations". It was also unclear whether the decrease in the two sets of (probably recent) measurements reflected an actual decrease in exposure levels or different sampling locations, but it was more likely to be caused by random variation. The workplace and exposure-related information provided was very limited, and no quantitative exposure assessment was conducted. The Working Group noted that it was possible that the correlation with dimethylformamide was high and that other co-exposures such as pigments and dyes may have been present. Although the study had somewhat more information than did many of the other mechanistic studies of occupational exposures, it was still likely to be affected by nondifferential misclassification, which would tend to bias the results towards the null.]

In a study by Rössner et al. (2002), blood levels of p53 and p21^{WAF1} proteins were assessed in a single group of acrylonitrile workers in Czechia (n = 49). Although the authors indicated that the workers were employed in a "petrochemical plant" for ≥ 3 months before blood collection, no further information on the exposure was provided. Area samples for acrylonitrile in the

air were reported as 0.05–0.3 mg/m^3 but were not used in the exposure assessment. [The Working Group noted that there was only one exposure group comprising all employed workers. The workplace and exposure-related information was very limited, and no quantitative exposure assessment was conducted. It was unclear whether carcinogens were present in the workplace. The exposure assessment was of minimal quality and was likely to have nondifferential misclassification, which would tend to bias the results towards the null.]

Xu et al. (2003) studied DNA strand breakage and sex chromosome aneuploidy in the sperm of 30 workers exposed to acrylonitrile at a chemical plant. [On the basis of the affiliation of the authors, the Working Group assumed that the study took place in China.] The authors stated that the company had been in production for 2.8 years, so the maximum duration of exposure was 2.8 years. No exposure-related information on exposures or the workplace was provided, other than that the mean air concentration at the site was 0.8 ± 0.25 mg/m^3. The analysis was based on a single exposed group. [The Working Group noted that the measurements were not used in the exposure assessment, no information was provided as to the type of operation or type of job being performed, and no quantitative exposure assessment was conducted. It was unclear whether co-exposures were present in the workplace. The exposure assessment was of minimal quality and was likely to have nondifferential misclassification, which would tend to bias the results towards the null.]

Sram et al. (2004) reported on 45 polymerization workers making India rubber in Czechia. The range of area measurement results was 0.05–0.3 mg/m^3. [The Working Group noted that it was unclear whether the workers and procedures were the same as in the study by Sram et al. (2007). The workplace and exposure-related information was very limited, and no quantitative exposure assessment was conducted. It was unclear whether carcinogens were present in the workplace, although pigments and dyes may have been used in the process. The exposure assessment was of minimal quality and was likely to have nondifferential misclassification, which would tend to bias the results towards the null.]

Beskid et al. (2006) studied chromosomal aberrations among 61 polymerization workers making India rubber in Czechia. Area measurements results were provided: 0.05–0.3 mg/m^3 in 2000 and 0.05–0.7 mg/m^3 in 2003. Exposures occurred for ≥ 3 months before the biological sampling. [The Working Group noted that the two sets of measurement results were likely to reflect random variation, rather than a change in exposure. Little workplace or exposure information was provided, and no quantitative exposure assessment was conducted. Other carcinogens may have been present. The exposure assessment was of minimum quality and was likely to have nondifferential misclassification, which would tend to bias the results towards the null.]

Caciari et al. (2014) investigated peripheral blood counts in 218 workers in a polyacrylonitrile-fibre operation who were exposed to workplace levels of acrylonitrile of [< 4.3 mg/m^3] in 2011 in Italy. Employment duration was between 5 and 8 years (average, 6.5 years). No information was provided on the appropriateness of the single exposure group. The authors noted that the use of sulfuric acid (IARC Group 1), methacrylate, and acrylonitrile resulted in levels "below the TLV-TWA" in the workplace. Other chemicals used included various additives, including sodium metabisulfite, sodium nitrite, soda, and dimethylacetamide. [The Working Group noted that little exposure-related information was provided. It was unclear whether the statement that all participants "worked regularly in places where the levels … were below the threshold limit values ([< 4.3 mg/m^3] for 2011)" referred to current exposures or current and historical exposures. Other carcinogens such as sulfuric acid were present. The methacrylate used was

probably methyl methacrylate. The exposure assessment was of minimal quality and was likely to have nondifferential misclassification, which would tend to bias the results towards the null.]

(b) *Exposure assessment in the general population*

The mechanistic studies in the general population did not use airborne acrylonitrile levels as a metric of exposure or provide results for air levels. Rather, the studies measured two metabolites of acrylonitrile, CEMA and HEMA, and the haemoglobin adduct CEV as markers of exposure (see Section 1.3.4). A strength of these studies was that, in contrast to the occupational studies that presented air levels, all routes of exposure were considered, although dermal exposure and ingestion were likely to be minimal to non-existent among this population. Especially in general population studies, biomonitoring may be more reliable than measuring airborne acrylonitrile. CEMA levels reflect exposure shortly before sampling (hours), whereas CEV levels reflect exposure approximately 4 months before sampling. In addition, both CEMA and CEV are highly specific to acrylonitrile. However, biomarkers are of limited utility when efforts to understand sources of exposure are of interest. Information on latency was not provided, and duration of exposure was often missing, which could lead to exposure misclassification. Urinary metabolites of VOCs, including acrylonitrile, from either tobacco smoke or ambient air, were often assessed, but the other VOCs measured were not used as adjustment factors in the analyses for acrylonitrile metabolites. How the timing of sample collection for measurement of the metabolite or adduct related to the sample collection for the outcome measurement was often unclear in the report.

In a study in the general population in Germany, Schettgen et al. (2004) measured CEV levels in blood collected from the 29 participants and carried out an analysis by smoking status (smokers and non-smokers) using data obtained by questionnaire. CEV concentrations (mean, median, and range) were presented, as well as measurements of acrylamide and glycidamide adducts. In a larger study by Schettgen et al. (2010), also in Germany, CEV concentrations (median and range) and passive smoking status (yes/no) were reported for 104 participants who had no documented workplace exposures to ethylene oxide, propylene oxide, acrylonitrile, or acrylamide. No information was presented on the duration of exposure or the presence of co-exposures other than the ones identified. Qualitative exposure groups were based on passive smoking categories (yes/no), but no information was provided on the duration or frequency of passive smoking. Information on other smoke components was not provided, and no adjustments were made for other exposures. [The Working Group noted that questionnaire responses on smoking are subject to misreporting. Smoking was the likely source of exposure for both studies, and the most likely co-exposures were the components of tobacco smoke. The exposure assessment was of minimal quality and as a result it was likely to have nondifferential misclassification, which would tend to bias the results towards the null.]

De Smedt et al. (2014) conducted a biomonitoring survey of blood CEV levels among residents living near a train carrying acrylonitrile that was derailed and caught fire in Belgium. Three exposure groups of volunteers were identified via questionnaire: (i) those who evacuated within hours of the event because they lived within a 250 m perimeter of the derailment (n = 40); (ii) those who evacuated days after the event and who used emergency health services (n = 99); and (iii) a 10% sample of those who evacuated days after the event and who did not use emergency health services (n = 219). A suspected secondary source of acrylonitrile exposure was a sewage system near the residences of some of these participants to which water used to extinguish the fire had travelled. A variety of

pollutants was emitted, including the products of the decomposition of acrylonitrile, hydrogen cyanide, and nitrogen oxides. In the analysis, mean and median CEV levels were presented by exposure group. [The Working Group noted that air measurements were not collected. Questionnaires are subject to misreporting by study participants. No information was available on other combustion products, including possible carcinogens, that may have been present. Blood samples from the first group were taken within hours of the accident, which may have not allowed enough time for adduct formation. Because air levels were not presented, it was not known whether the controls were exposed, either from the event or because they were smokers. Some differential exposure misclassification may have occurred because of secondary exposure via the sewage system. Self-selection differential bias may have occurred from the voluntary nature of the recruitment. Although the study did provide more information than did most of the studies evaluated in the present section, it was still likely to result in nondifferential misclassification, which would tend to bias the results towards the null.]

Weinstein et al. (2017) investigated 23 women who were exposed to smoke from wood-fired cooking stoves in Guatemala. PAHs, particulate matter (2.5 μm in size, $PM_{2.5}$), and urinary metabolites of acrylonitrile and eight other VOCs from the smoke were analysed. The correlation between CEMA levels and 48-hour levels of particulate matter was 0.59. Only one exposure group was analysed for mean and median levels of CEMA obtained from three urine samples. No adjustment was made for other components of smoke, some of which are carcinogenic. [The Working Group noted that no information was available on exposure duration. The Working Group also noted the lack of exposure information, and the high correlation between CEMA levels and particulate matter levels. The exposure assessment was of moderate quality and as a result was likely to have nondifferential misclassification, which would tend to bias the results towards the null.]

Lin et al. (2018b) recruited 853 participants from the general population in Taiwan, China, to examine oxidative stress in relation to acrylonitrile exposure, as indicated by CEMA levels in spot urine samples. Two types of analyses were performed: (i) the participants' values were assigned into one of four categories of CEMA levels; and (ii) the actual (continuous) CEMA levels were used in regression analyses. The authors indicated that smoking is the most important source of exposure in the general population but did not speculate on the source of exposure for non-smokers. [The Working Group noted as a strength the use of the continuous CEMA levels in regression analysis. No information, however, was provided on duration of exposure or other co-exposures. The exposure assessment was of moderate quality. The exposure assessment relying on the results of a single urine sample was likely to result in nondifferential misclassification, which would tend to bias the results towards the null.]

Kuang et al. (2022) conducted two studies in the general population. In the first study, they measured urinary levels of VOC metabolites, including CEMA, and compared them with levels of a marker for oxidative stress in urine collected from adults ($n = 7$) and children ($n = 6$) before and after exposure to passive smoking in a closed room over 2 days. [On the basis of the affiliation of the authors, the Working Group assumed that the study took place in China.] In the second study, questionnaires were administered to and spot urine samples collected from 259 other children exposed to passive smoking. The analysis for the first study was based only on age (i.e. adult or child). For the second study, exposure groups were developed using information obtained from questionnaires regarding number of paternal cigarettes smoked per day (0, 1–10, and >10) and frequency of paternal

smoking (never, sometimes, often). Metabolites of 26 VOCs, including CEMA, were measured and reported. [The Working Group noted that no information was available on duration of exposure for either study. Information from questionnaires can be incorrectly reported. No adjustment for other smoking components was made, although measurements were available and many of the components were probably carcinogens. The exposure assessment was of moderate quality and was likely to have nondifferential misclassification, which would tend to bias the results towards the null.]

Wahlang et al. (2022, 2023) studied the associations between residential exposure to VOCs and markers of liver injury among 663 participants in the Health, Environment, and Action Study, a cross-sectional study in six communities in the USA. Blood and urine samples were collected, and smoking status was determined by questionnaire. Exposure to CEMA and 15 metabolites from 11 other VOCs, many of which are considered to be carcinogenic, was measured. [The authors referred to this metabolite as CYMA, but for consistency, it is referred to here as CEMA.] CEMA levels were used as a continuous variable in regression analyses. Exposure was assumed to be through inhalation from residential exposure, and 39.8% of CEMA measurements were below the LOD. [The Working Group noted that the use of the continuous levels of CEMA in a regression analysis is a strength. There was no information, however, on duration of exposure. Other than tobacco smoke, no other sources of exposure were discussed. No adjustment was made for the other co-exposures. The exposure assessment was of moderate quality and was likely to have nondifferential misclassification, which would tend to bias the results towards the null.]

Riggs et al. (2022) compared urinary levels of 16 metabolites, including CEMA, of 12 VOCs, using circulating angiogenic cell levels as a marker for cardiovascular disease in 603 participants selected from the same population as that studied by Wahlang et al. (2022, 2023). Ambient levels of $PM_{2.5}$ and ozone were measured. The primary analyses were based on the population as a single exposure group. Regression analyses were based on continuous CEMA values. [The Working Group noted that potential sources of VOC exposure were not identified, although it appeared that the authors considered the most likely source of exposure to be from cigarette smoking, as the comparison group was non-smokers. No information was available on the duration of exposure to acrylonitrile. No adjustment was made for the co-exposures. The exposure assessment was of moderate quality and was likely to have nondifferential misclassification, which would tend to bias the results towards the null.]

2. Cancer in Humans

Introduction

Acrylonitrile has been evaluated several times by the *IARC Monographs*. Most recently, in 1998 (Volume 71; IARC, 1999), acrylonitrile was classified in Group 2B, *possibly carcinogenic to humans*, with *inadequate* evidence for cancer in humans.

A systematic search was conducted in the PubMed database to identify relevant studies (see General Remarks in the present volume). The search terms and the lists of retrieved studies are available from: https://hawcproject.iarc.who.int/assessment/645/. Since the previous evaluation of the carcinogenicity of acrylonitrile in Volume 71, six studies have been published on acrylonitrile and cancer incidence and mortality.

For the evaluation of cancer in humans exposed to acrylonitrile, the Working Group considered a total of 19 studies, plus reanalyses. Most were retrospective cohort studies ($n = 13$) of workers occupationally exposed to acrylonitrile. Of the retrospective cohort studies, most were on

cancer mortality (n = 12), and one was on cancer incidence. A case–control study nested within a retrospective occupational cohort study reported on occupational exposures to acrylonitrile and cancer mortality, and a case–control study nested within a population-based prospective cohort study also reported on biomarkers of acrylonitrile exposure and cancer incidence. In addition, four hospital- or population-based case–control studies reported on occupational exposure to acrylonitrile and either cancer incidence (n = 2) or mortality (n = 2). Acrylonitrile exposure has been most commonly evaluated with respect to cancers of the respiratory system, gastrointestinal system, genitourinary system, and lymphohaematopoietic system, as well as with cancers of the brain, prostate gland, breast, and thyroid gland.

Cohort, nested case–control, and case–control studies were included. Case reports, ecological studies, and studies without cancer end-points were not considered informative for the evaluation and are not described here. Results reporting on "all cancers combined" were not considered informative for the evaluation, since determination is done at the cancer site or tissue level, and hence were not discussed further.

The studies included are described below in Section 2.1 (cohort studies first, in chronological order, followed by the meta-analyses), and the study results are subsequently presented by cancer type in the following sections. Exposure quality considerations for these studies are described in Section 1.6.1.

2.1 Description of the studies (cohorts and meta-analyses)

See Table 2.1.

2.1.1 *Cohort of workers in a factory in North Rhine-Westphalia, Germany*

Kiesselbach et al. (1979) conducted a company-sponsored retrospective cancer mortality study in a cohort of 884 male employees in production or processing who were exposed to acrylonitrile for ≥ 1 year between 1950 and 1 August 1977 in a plant in the city of Leverkusen, North Rhine-Westphalia, Germany. Follow-up was through 1 August 1977. Age- and calendar-specific rates from North Rhine-Westphalia were used to estimate the expected number of deaths (Kiesselbach et al., 1980). All workers in the plant were considered exposed to acrylonitrile, and their exposure was measured as duration in years; in addition, an unspecified number of workers had co-exposures to butadiene and styrene. [The Working Group considered that this study was minimally informative, since the number of exposed cases was small, and important details in the publication, such as the number of people co-exposed to butadiene–styrene, were not provided. Because acrylonitrile is an irritant, healthier workers may have stayed employed longer and accrued longer exposure, leading to healthy-worker survivor bias (HWSB).]

2.1.2 *Cohort of workers in two facilities manufacturing acrylic fibre, USA*

Symons et al. (2008) carried out a company-sponsored cancer mortality study in a cohort of workers in two facilities in Waynesboro, Virginia, and Camden, South Carolina, in the USA, where acrylonitrile was used during fibre production; the data on cancer mortality were extended relative to those in a previous report (Wood et al., 1998). The cohort included 2548 male workers with ≥ 6 months of acrylonitrile exposure from 1947 through 1991 and was followed through 2002. To ascertain mortality, the authors used a company registry that had been maintained for all active and retired employees since 1957.

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

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Reporting of results	Comments
Kiesselbach et al. (1979) Leverkusen, North Rhine-Westphalia, Germany 1950 to 1 August 1977 Cohort	884 male employees of a plant in Leverkusen who were exposed to AN (handled AN for ≥ 1 yr in production or processing) between 1950 and 1 August 1977. Expected deaths from North Rhine-Westphalia rates. Exposure assessment method: Duration of exposure in years.	Respiratory tract, mortality Stomach cancer, mortality	Reported in Table 2.3 Reported in Table 2.4	*Limitations*: The number of exposed cases was small. Co-exposure to butadiene and/or styrene was not reported or adjusted for. HWSB was likely. The study was considered to be minimally informative.
Symons et al. (2008) Waynesboro (VA) and Camden (SC), USA Enrolment, 1947–1991/follow-up, through 2002 Cohort	2548 male workers who were exposed to AN for ≥ 6 mo in two company facilities (Waynesboro, VA, and Camden, SC) manufacturing acrylic fibre, in 1947–1991. Exposure assessment method: A variety of company records, including measurements, were used to develop a JEM to estimate semiquantitative exposure intensity levels.	Lung, mortality Colon and rectum, mortality Prostate, mortality Bladder and kidney, mortality Brain and CNS, mortality Lymphatic and haematopoietic, mortality	Reported in Table 2.3 Reported in Table 2.4 Reported in Table 2.4 Reported in Table 2.4 Reported in Table 2.4 Reported in Table 2.5	*Exposure assessment critique*: The quality of the exposure assessment was considered to be good because of the use of a variety of company records, including measurements, to develop a JEM for job and work area over time. An important limitation was that information on co-exposure to dimethylformamide was not provided. *Other strengths*: Well-defined, moderate-sized cohort with long follow-up period. Used HR models and lagged analyses with sufficient latency. Cumulative and intensity of exposure were analysed; two reference populations, including the general population, which was probably unexposed. Vital status was verified for all employees by the National Death Index. *Other limitations*: Consideration of cross-plant differences was unclear; no adjustment for smoking; internal referents may have had additional co-exposures; HWSB was likely since workers with < 6 mo of employment were excluded. *Other comments*: This study was conducted by the company.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Reporting of results	Comments
Ott et al. (1980) USA Enrolment, 1937–1970/ follow-up, 1940–1975 Cohort	2904 (2740 without exposure to arsenicals, asbestos, or vinyl chloride); employed for ≥ 1 yr in the manufacture of styrene-based products between 1937 and 1970. Exposure assessment method: Used company work history records, job descriptions, workplace information, and industrial hygiene measurements to estimate intensity to six semiquantitative groups. Also analysed for duration.	Respiratory tract, mortality Digestive organs and peritoneum, mortality Genital organs, mortality Urinary organs, mortality Lymphatic and haematopoietic, excluding leukaemia, mortality Leukaemia, mortality	Reported in Table 2.3 Reported in Table 2.4 Reported in Table 2.4 Reported in Table 2.4 Reported in Table 2.5 Reported in Table 2.5	*Exposure assessment critique*: Strengths included the moderate exposure assessment quality using a variety of company records including measurement data to form semiquantitative exposure intensity and duration categories. Historical changes in job roles were considered. The external reference population was likely to be unexposed. A limitation was that some nondifferential misclassification was possible and that all AN-exposed workers were also exposed to styrene. *Other limitations*: Small proportion of exposed cases with insufficient duration and latency; no consideration of cross-plant differences; interpretation of exposure categories results unclear; measurements were limited before the late 1970s; no information on the reference population; and other co-exposures were not accounted for, in particular, styrene and ethylbenzene. The study was deemed to be minimally informative.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Reporting of results	Comments
Thiess et al. (1980) Germany Enrolment, varies/ follow-up, through 15 May 1978 Cohort	1469 workers employed for ≥ 6 mo at 12 AN-using plants from 1954 through 15 May 1978. Exposure assessment method: All workers in 12 companies. No exposure assessment. Evaluated exposure duration.	Lung, mortality	Reported in Table 2.3	*Exposure assessment critique*: A strength was that high exposures were likely before 1976 in exposed groups, whereas the reference population was likely to be unexposed. Limitations included the minimal assessment of exposure level differences between workers. The source of worker data was not identified. Historical changes in exposure, or differences across plants, were not considered. *Other limitations*: Small study size with short follow-up period. A lack of internal analyses, and several other carcinogenic co-exposures present without adjustment. HWSB was likely.
		Oesophagus, mortality	Reported in Table 2.4	
		Stomach, mortality	Reported in Table 2.4	
		Colon, mortality	Reported in Table 2.4	
		Rectum, mortality	Reported in Table 2.4	
		Liver, mortality	Reported in Table 2.4	
		Urinary bladder, mortality	Reported in Table 2.4	
		Hodgkin lymphoma, mortality	Reported in Table 2.5	
Waxweiler et al. (1981) USA Enrolment, 1942–1973/ follow-up, through 1973 Cohort	4806 men (98% White) employed at a VCM plant between 1942 and 1973. Exposure assessment method: Details were not provided.	Respiratory tract, mortality	Reported in Table 2.3	*Exposure assessment critique*: Limitations were that details on the exposure assessment for AN and the other chemicals were not provided. High co-exposures to other chemicals classified by IARC in Group 1, including butadiene and vinyl chloride, were noted. *Strengths*: The serially additive expected dose analysis is supposed to assess the individual contribution of each chemical, although this analysis is intricate. *Other limitations*: No adjustment for co-exposures. The study was deemed to be minimally informative.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Reporting of results	Comments
Benn and Osborne (1998) England, Wales, Scotland, Northern Ireland Enrolment, phase I (1950–1968); phase II (1969–1978)/follow-up, through 1991 Cohort	2763 male workers employed for ≥ 1 yr and exposed to polymerization of AN or the spinning of acrylic fibre in six factories in the UK identified within a study conducted by the HSE and the Chemical Industries Association. Exposure assessment method: Job groups identified from company records; duration and time since first exposure by job group.	Lung, mortality Stomach, mortality Large intestine, mortality Rectum, mortality Genitourinary organs, mortality Lymphatic and haematopoietic, mortality	Reported in Table 2.3 Reported in Table 2.4 Reported in Table 2.4 Reported in Table 2.4 Reported in Table 2.4 Reported in Table 2.5	*Exposure assessment critique*: A strength was that the reference population was likely to be unexposed. Limitations included that levels of duration were only semiquantitative. Nondifferential misclassification was expected, in part because of anomalies in identification and assessment of workers. Exposure levels probably varied by job and plant across time. *Other strengths*: Large cohort involving multiple plants. *Other limitations*: Short follow-up period, and latency may have been insufficient for much of cohort. No information on confounders. No internal analyses were conducted. Comparisons of stratified SMRs are not appropriate if their confounder distributions differ. Unclear reference group for Northern Ireland. Several other carcinogenic co-exposures were probably present but not adjusted for.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Reporting of results	Comments
Delzell and Monson (1982) Akron (OH), USA Enrolment, 1940–1971/ follow-up, 1940 through 1 July 1978 Cohort	327 White male production workers employed ≥ 2 yr between 1940–1971 at a rubber-manufacturing plant in Akron (OH) who had worked in the chemicals division in Departments 5578 or 5579 with potential AN exposure. Exposure assessment method: Workers in two departments using AN analysed by duration of employment.	Lung, mortality Digestive organs and peritoneum, mortality Urinary bladder, mortality Lymphatic and haematopoietic (ICD-7, 200–205), mortality	Reported in Table 2.3 Reported in Table 2.4 Reported in Table 2.4 Reported in Table 2.5	*Exposure assessment critique*: Limitations included that the exposure assessment was of minimal quality with no information on exposure levels or measurements. Only semiquantitative levels of duration were investigated. Heterogeneity in exposure between departments and over time was possible. *Strengths*: AN-specific results were reported. *Other limitations*: Small study size and short follow-up period. Internal analyses were conducted but not reported. Minimum of 2 yr employment required, which may induce HWSB. Several other carcinogenic co-exposures were present but not adjusted for. *Other comments*: Study was funded by the company.
Ott et al. (1989) USA 1940–1978 Nested case–control	Cases: 52 NHL, 20 multiple myeloma, 39 non-lymphocytic leukaemia, 18 lymphocytic leukaemia; mortality from the Social Security Administration, motor vehicle registry, and listings of war deaths. Death certificates obtained (cases). Controls: NR; selected using group-matched incidence density sampling and frequency-matched 5:1 to cases on decade of first employment for each disease category. Exposure assessment method: two retired senior manufacturing personnel reviewed available records and prepared chronological listings of substances present in each chemical processing area; Ott et al. (1989) exposure paper.	NHL, mortality Multiple myeloma, mortality Leukaemia (non-lymphocytic), mortality Leukaemia (lymphocytic), mortality	Reported in Table 2.5 Reported in Table 2.5 Reported in Table 2.5 Reported in Table 2.5	*Strengths*: Well-defined cohort. *Limitations*: Co-exposures likely since large numbers of chemicals were produced at these plants; small number of exposed deaths and small sample size.

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Reporting of results	Comments
Swaen et al. (2004) Netherlands Enrolment, varies by plant starting 1959–1973/follow-up, through 1 January 2001 Cohort	2842 men, citizens of the Netherlands, working in eight chemical companies with AN exposure for > 6 mo. Exposure assessment method: Used work histories, workplace information, and measurement data for a job/work area/time period exposure matrix. Semiquantitative estimates of cumulative exposure. Evaluated peak, respirator use, and important co-exposures.	Lung, mortality	Reported in Table 2.3	*Exposure assessment critique*: Strengths were the good-quality exposure assessment; that the exposure assessment used company records and measurements; historical changes were considered; intensity estimates were semiquantitative; there were multiple metrics with measurement units; and peaks were evaluated, and respirator use was considered. A limitation was that exposure was assumed to be zero after 1980. *Other strengths*: Expert assessment of quantitative exposure metrics for partial period. Sufficient latency. Analysed cohort with and without certain co-exposures. *Other limitations*: Only external comparisons were reported. Small sample size; no information on number of participants in high-exposure category. Did not start person-time until the 6-mo exposure minimum was met.
		Larynx, mortality	Reported in Table 2.3	
		Oesophagus, mortality	Reported in Table 2.4	
		Stomach and small intestine, mortality	Reported in Table 2.4	
		Large intestine, mortality	Reported in Table 2.4	
		Rectum, mortality	Reported in Table 2.4	
		Liver and bile ducts, mortality	Reported in Table 2.4	
		Pancreas, mortality	Reported in Table 2.4	
		Kidney, mortality	Reported in Table 2.4	
		Prostate, mortality	Reported in Table 2.4	
		Urinary bladder, mortality	Reported in Table 2.4	
		Male genital organs, mortality	Reported in Table 2.4	
		Brain, mortality	Reported in Table 2.4	
		Multiple myeloma, mortality	Reported in Table 2.5	
		Leukaemia, mortality	Reported in Table 2.5	

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Reporting of results	Comments
Mastrangelo et al. (1993) Italy Enrolment, 1959–1988/ follow-up, 1959–1990 Cohort	671 men employed for ≥ 12 mo at any time since the opening of the factory in 1959. Clerks and some workers with past exposure to VCM or to benzidine and its compounds were excluded, as were workers employed after 31 December 1988. Exposure assessment method: Exposure groups based on jobs noted in company records.	Lung, mortality	Reported in Table 2.3	*Limitations*: The Working Group considered this study to be minimally informative because of the low number of outcomes and imprecise estimates, the lack of individual-level cumulative assessment of exposure to AN, and the potential for healthy-worker biases.
		Stomach, mortality	Reported in Table 2.4	
		Intestine and colon, mortality	Reported in Table 2.4	
		Rectum, mortality	Reported in Table 2.4	
		Testis, mortality	Reported in Table 2.4	
		Brain, mortality	Reported in Table 2.4	
		Leukaemia, mortality	Reported in Table 2.5	
Geĭko et al. (1996) Russian Federation Enrolment, 1938–1985/ follow-up, through 1985 Cohort	239; workers exposed to AN for ≥ 3 yr between 1938–1985. Exposure assessment method: No date sources identified or described. Analysed by duration of exposure.	Lung, mortality	Reported in Table 2.3	*Exposure assessment critique*: A strength was that the reference population was probably unexposed. Limitations included the minimal exposure assessment quality. No exposure assessment described. Only semiquantitative levels of duration. High heterogeneity in exposure was likely. *Other strengths*: Some analyses provided by duration. Sufficient latency between exposure and outcome. *Other limitations*: Small sample size without numbers of cases reported. HWSB was likely.
		Gastrointestinal cancers combined, mortality	Reported in Table 2.4	
		Stomach, mortality	Reported in Table 2.4	
		Liver, mortality	Reported in Table 2.4	
		Pancreas, mortality	Reported in Table 2.4	
		Brain, mortality	Reported in Table 2.4	

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Reporting of results	Comments
Koutros et al. (2019) USA Enrolment, 1952–1983/ follow-up, through 2011 Cohort	25 460 (16 889 AN-exposed); All workers employed ≥ 1 day at any of the eight participating plants after the start of AN operations (1952–1965). Work histories covered the date of hire (i.e. 1942–1983) through date of record abstraction at the plant (1983). Exposure assessment method: Work histories from employers in combination with expert assessment of cumulative exposure based on plant visits and measurement data.	Lung, mortality	Reported in Table 2.3	*Exposure assessment critique*: Strengths were the high-quality exposure assessment, and that the quantitative exposure assessment was based on both expert assessment and exposure measurements. Limitations were that the exposure assessment ended in 1983 whereas cancer follow-up ended in 2011, probably causing nondifferential underestimation of exposure, but sensitivity analyses to determine the impact of this were conducted; and that the exposure circumstances were heterogeneous. *Other strengths*: Largest cohort of AN-exposed workers with a long follow-up. Robust analyses with different exposure metrics and sensitivity analyses for lung cancer. Controlled for smoking in lung cancer analyses. *Other limitations*: HWSB was possible, although bias analyses were conducted by Keil et al. (2024).
		Mesothelioma, mortality	Reported in Table 2.3	
		Digestive system, mortality	Reported in Table 2.4	
		Oesophagus, mortality	Reported in Table 2.4	
		Stomach, mortality	Reported in Table 2.4	
		Colon and rectum, mortality	Reported in Table 2.4	
		Liver, mortality	Reported in Table 2.4	
		Pancreas, mortality	Reported in Table 2.4	
		Urinary system, mortality	Reported in Table 2.4	
		Kidney and renal pelvis, mortality	Reported in Table 2.4	
		Urinary bladder, mortality	Reported in Table 2.4	
		Prostate, mortality	Reported in Table 2.4	

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Reporting of results	Comments
Koutros et al. (2019) USA Enrolment, 1952–1983/ follow-up, through 2011 Cohort (cont.)		Breast, mortality	Reported in Table 2.4	
		Brain, mortality	Reported in Table 2.4	
		Lymphoma (type not specified, includes Hodgkin lymphoma and and NHL), mortality	Reported in Table 2.5	
		NHL, mortality	Reported in Table 2.5	
		Leukaemia, mortality	Reported in Table 2.5	
		Multiple myeloma, mortality	Reported in Table 2.5	
		NHL (CLL), mortality	Reported in Table 2.5	
		Leukaemia (AML), mortality	Reported in Table 2.5	
Keil et al. (2024) NCI Acrylonitrile Cohort Study, USA Enrolment, 1952–1983/ follow-up, through 2011 Cohort	25 460; see Koutros et al. (2019). Here, the impact of HWSB was assessed. Expected lung cancer mortality was estimated adjusting for HWSB using the parametric g-formula under different hypothetical occupational AN exposure limits. Exposure assessment method: See Koutros et al. (2019).	Lung: (HWSB adjustment analysis), mortality	Reported in Table 2.3	*Strengths*: See Koutros et al. (2019). Data were re-analysed with adjustment for HWSB using the g-method. *Limitations*: See Koutros et al. (2019).

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Reporting of results	Comments
Marsh and Zimmerman (2015) Lima (OH, USA) Enrolment, starting in 1955/follow-up, 1955–2011 Cohort	2096 (789 with AN exposure); Employees (any time length) in Lima (OH) factory between 1955–2011. Exposure assessment method: Three different groups assessed exposures at three different times, considering work histories, measurements and workplace information. Developed 20 job groups. Estimated arithmetic means of measurements and used to develop semiquantitative categories of cumulative and average exposure. Also analysed for duration.	Lung, mortality	Reported in Table 2.3	*Exposure assessment critique*: Strengths were the good-quality exposure assessment; the quantitative exposure measurement based on exposure levels; the availability of work histories and personal measurements (after 1978); the use of daily TWA arithmetic means used to develop average intensity and cumulative exposure; and that the external reference population was unlikely to have had substantial co-exposures. Limitations were that job grouping methods were not specified; only area measurement data were available before 1978; the semiquantitative categories of cumulative and average exposure; and the partial overlap with Koutros et al. (2019). *Other strengths*: Long follow-up period. *Other limitations*: Only a small number and proportion of exposed deaths; all newly accrued lung cancer deaths were considered AN-unexposed in extended follow-up. Smoking data not used in internal analyses. Unclear which reference population was used for the female component. HWSB was likely. The study was deemed to be minimally informative.
		Larynx, mortality	Reported in Table 2.3	
		Oesophagus, mortality	Reported in Table 2.4	
		Stomach, mortality	Reported in Table 2.4	
		Large intestine, mortality	Reported in Table 2.4	
		Rectum, mortality	Reported in Table 2.4	
		Liver and bile ducts, mortality	Reported in Table 2.4	
		Pancreas, mortality	Reported in Table 2.4	
		Kidney, mortality	Reported in Table 2.4	
		Urinary bladder, mortality	Reported in Table 2.4	
		Prostate, mortality	Reported in Table 2.4	
		Testis and other male genital organs, mortality	Reported in Table 2.4	
		Hodgkin lymphoma, mortality	Reported in Table 2.5	
		NHL, mortality	Reported in Table 2.5	
		Leukaemia and aleukaemia, mortality	Reported in Table 2.5	

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Reporting of results	Comments
Budroni et al. (2010) Porto Torres, Sardinia, Italy 1990–2006 Cohort	5350 (2336 with AN exposure); male workers with ≥ 6 mo of employment in one or more chemical plants of the Porto Torres industrial area (43 facilities identified), working in the plants and alive as of 1 January 1990. Workers were identified using the records of the National Italian Pension funds. Workers that joined between 1 January 1990 and 31 December 2001 were also included. Outcome data: Sassari province tumour registry, cause of death registry of the Sassari health authority. For participants not found in the two registries, vital status (as alive) was confirmed by each of the towns of residency. Exposure assessment method: Company raw materials used to identify ever "exposure".	Lung, incidence	Reported in Table 2.3	*Exposure assessment critique*: Strengths were that the average employment duration was sufficient; and the reference population was likely to be unexposed. Limitations to the minimal-quality exposure assessment were that it covered only working at the plant at the start of follow-up; the insufficient details on the start of actual exposure, source of worker data, or exposure levels; and the lack of consideration of historical levels or cross-plant differences. *Other strengths*: Cancer linkage for incidence with histopathological validation (only 9 participants were not traceable). *Other limitations*: Co-exposure to other carcinogens was noted in 65–75% of population, without adjustment. HWSB was likely. The study was deemed to be minimally informative.
		Larynx, incidence	Reported in Table 2.3	
		Pleura, incidence	Reported in Table 2.3	
		Stomach, incidence	Reported in Table 2.4	
		Colon and rectum, incidence	Reported in Table 2.4	
		Liver and bile ducts, incidence	Reported in Table 2.4	
		Urinary bladder, incidence	Reported in Table 2.4	
		Prostate, incidence	Reported in Table 2.4	
		Kidney, incidence	Reported in Table 2.4	
		Hodgkin lymphoma, incidence	Reported in Table 2.5	
		NHL, incidence	Reported in Table 2.5	
		Leukaemia (lymphoid), incidence	Reported in Table 2.5	
		Leukaemia (myeloid), incidence	Reported in Table 2.5	
		Myeloma, incidence	Reported in Table 2.5	

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Reporting of results	Comments
Etemadi et al. (2024) Golestan, Islamic Republic of Iran Enrolment, January 2004 to June 2008/ follow-up, through 1 January 2018 Nested case–control	Cases: 205 oesophageal cancer cases identified through linkage with cancer registry and with available urine sample at baseline. Controls: 226 controls matched on age, sex, residence, time of enrolment in cohort, and tobacco use at baseline. Alive and free from cancer of the upper gastrointestinal tract (oesophageal and gastric) at the time of case diagnosis. Selected from a cohort of 50 045 residents aged 40–75 yr at enrolment from the general population in Golestan, Islamic Republic of Iran. Exposure assessment method: Baseline spot urine samples and questionnaires. Geometric means of biomarker concentrations in spot urine samples, by current or former tobacco use (collected via questionnaire)	Oesophagus (squamous cell carcinoma), incidence	Reported in Table 2.4	*Exposure assessment critique*: Strengths were the good exposure assessment quality using biomarkers specific to AN exposure; the use of recognized methods for urine collection before the start of the study; and that that the population was unlikely to have occupational exposure. A limitation was the use of short-term urinary biomarkers of exposure *Other strengths*: Prospectively collected urine, clearly defined cases and selected controls nested in a cohort, and detailed available data for multivariable adjustment (including opium use). *Other limitations*: No assessment of exposure in non-tobacco users. Many carcinogens in smoke were not adjusted for.

AML, acute myeloid leukaemia; AN, acrylonitrile; CI, confidence interval; CLL, chronic lymphocytic leukaemia; CNS, central nervous system; HR, hazard ratio; HSE, Health and Safety Executive; HWSB, healthy-worker survivor bias; ICD, International Classification of Diseases; JEM, job-exposure matrix; NCI, National Cancer Institute; NHL, non-Hodgkin lymphoma; mo, month(s); NR, not reported; OH, Ohio; SC, South Carolina; SMR, standardized mortality ratio; TWA, time-weighted average; UK, United Kingdom; US, United States; USA, United States of America; VA, Virginia; VCM, vinyl chloride monomer; yr, year(s).

In addition, vital status was verified through the National Death Index using social security number as an identifier. An estimate of cumulative exposure to acrylonitrile was calculated for each worker by adding the products of each worker's mean exposure intensity multiplied by duration of time for all job assignments and work areas (see Section 1.6.1). Standardized mortality ratio (SMR) estimates used as reference populations both the USA population and the company's list of regional employees (which included all White male employees in Alabama, Florida, Georgia, Mississippi, North Carolina, South Carolina, Tennessee, and Virginia, but excluded workers in the acrylonitrile cohort). Hazard ratio (HR) estimates for an increase of 100 ppm-year in cumulative acrylonitrile exposure assumed a log-linear model form. Cox proportional hazards models used age as the timescale and adjusted for birth period (six decades from 1900 to 1960), and employment in the South Carolina start-up group (a binary indicator of employment in the first phase of fibre production, meant to account for differences in occupational exposures at the onset of the implementation of the production process). Regression models of cumulative acrylonitrile exposures were also lagged by 5, 10, and 15 years. [The Working Group noted that the cohort had a long follow-up period (95 657 person-years of observation). Strengths included the development of quantitative cumulative exposure estimates using a JEM that was based on personal protective equipment use, personal and area monitoring data, and information on work conditions and practices. Another strength was the use of internal proportional hazards models and lagged analyses. Adjustment by start-up group probably controls for some differences within the cohort, but is unlikely to control for co-exposure to dimethylformamide, a solvent that is used together with acrylonitrile in the manufacture of acrylic fibre (Chen et al., 1987) and classified by IARC as Group 2A, *probably carcinogenic to humans* (with *limited* evidence for testicular cancer in humans) (IARC, 2018). Although the authors noted that information on race was available only for 42% of the cohort, the participants can be assumed to be predominantly White. No information on smoking was available. HWSB was likely, because workers with < 6 months of employment were excluded.]

2.1.3 Cohort of workers in a chemical company, USA

In a study conducted by the company's medical department, Ott et al. (1980) evaluated mortality in a cohort of 2904 predominantly White men employed for ≥ 1 year in the manufacture of styrene-based products from 1937 to 1970 in several plants belonging to the same company in the USA. Follow-up was from 1940 through 1975. US age- and calendar year-specific mortality rates were used to estimate expected numbers of death. Since workers were exposed concurrently to multiple chemical agents, including acrylonitrile, the authors developed categories according to chemical presence and relative exposure intensities. Exposure duration came from company records, and intensity of exposure came from industrial hygiene measurements. Acrylonitrile-exposed jobs were grouped into several exposure groups, all of which also had co-exposures to styrene and ethylbenzene, and some that were also exposed to extrusion vapours, polymer dusts (from cutting), and colourants. The groups were assigned semi-quantitative 8-hour time-weighted intensity estimates of 1–4 ppm (vapours A) and 5–9 ppm (B). Acrylonitrile was included in a category together with styrene and ethylbenzene. The authors assessed mortality for this and other exposure categories by duration of exposure. [The Working Group noted that the study had sample size limitations (e.g. only a maximum of 100 people had been exposed to acrylonitrile at > 1 ppm, just 23 of these had ≥ 5 years of exposure duration, and most (69) had < 15 years of

latency). In addition, all acrylonitrile-exposed workers were also exposed to styrene (classed by IARC in Group 2A; IARC, 2019a). Furthermore, several possible confounders were not accounted for, in particular, exposures to styrene and ethylbenzene, and possibly benzene, if workers switched jobs. HWSB was likely. The Working Group also observed that the study had some limitations relating to the exposure assessment for acrylonitrile. The Working Group considered this study to be minimally informative.]

2.1.4 Cohort of workers in 12 production plants, Germany

Thiess et al. (1980) conducted a retrospective mortality study among 1469 employees employed for ≥ 6 months in 12 production plants in Germany that used various methods to process acrylonitrile. Start of follow-up varied by plant, but the earliest started in 1954 and all plants ended follow-up on 15 May 1978. Ten plants were still operating at the end of follow-up. SMRs were calculated using the populations of the Rheinhessen-Pfalz administrative district and the city of Ludwigshafen in Germany as referents. Smoking habits and concurrent exposures were reported for each death. [The Working Group noted that imprecise estimates were reported, and that there was a short follow-up period (15 350 person-years at risk), a lack of internal analyses, limited exposure assessment for acrylonitrile, and multiple co-exposures. HWSB was likely.]

2.1.5 Cohort of workers in a chemical plant using vinyl chloride monomer, USA

Waxweiler et al. (1981) evaluated mortality in a cohort of 4806 men ever employed at a synthetic chemicals plant in the USA where they were exposed to vinyl chloride monomer (VCM) and other chemicals, including acrylonitrile, from 1942 to 1973. Retrospective mortality analyses, including use of serially additive expected dose (SAED) models, were conducted to evaluate lung cancer risk from exposure to VCM and to 19 additional chemicals (including acrylonitrile). SMR estimates were based on US White male death rates and were standardized by age and calendar interval. The SAED model was used to compare the observed exposure of each case with the exposures of other plant workers who were close in year of birth and in age at start of work at the company, so that each case was considered as part of a subcohort of workers with approximately the same year of birth and age at start of work in the plant. Exposure was then compared with that of the other members of this subcohort without lung cancer who were working in that year. Cumulative dose differences per case were evaluated to test whether one or more chemicals used at the plant, including acrylonitrile, were responsible for the excess risk of lung cancer that was observed. [The Working Group considered that this study was minimally informative, because it was mainly focused on the effect of VCM exposure. Details on how the exposure assessment for acrylonitrile and the other chemicals was conducted were not provided. There were co-exposures to other chemicals that have been classified by IARC (such as certain acrylates in Group 2B, IARC, 2019a; butadiene in Group 1, vinyl chloride in Group 1, and vinylidene chloride in Group 2B, IARC, 2012); and even though the SAED analysis was meant to assess the individual contribution of each chemical, it can be complex to adequately separate the individual contributions.]

2.1.6 Cohort of workers in acrylonitrile factories, UK

Benn and Osborne (1998) evaluated mortality in a cohort of 2763 men employed between 1950 and 1978 for ≥ 1 year in six facilities involved in the polymerization of acrylonitrile and spinning of acrylic fibre in the UK. Follow-up was through

1991. On the basis of expert judgement, workers were classified by their job titles as exposed to acrylonitrile, possibly exposed to acrylonitrile, and little or not exposed to acrylonitrile. Expected deaths were calculated using national death rates for England and Wales. For a factory in Scotland, Scottish death rates were used. Since factory 5 showed a deficit in overall mortality, some analyses were conducted excluding workers from this factory. Additional analyses were conducted to explore mortality by exposure group and among workers exposed to high levels of acrylonitrile, by age group, year of first exposure, time since first exposure, and duration of exposure. [The Working Group noted that the cohort involved multiple plants (63 058 person-years at risk); however, the study had several limitations, including the lack of reference data for the factory in Northern Ireland and a short follow-up period. The exposure assessment was limited to three exposure categories based on job title, and the analysis based on length of exposure was inadequate because the date of departure from the job was unknown for almost half (49.9%) of the workers. The Working Group also noted that HWSB was likely, that no internal analyses were conducted, and that comparisons of stratified SMRs are not appropriate if their confounder distributions differ.]

2.1.7 Cohort of workers in a rubber-manufacturing plant in Ohio, USA

Delzell and Monson (1982) conducted a company-sponsored analysis of mortality among 327 male employees of a rubber-manufacturing plant in the USA who had potential exposure to acrylonitrile for ≥ 2 years from 1940 to 1971. Mortality follow-up was assessed from 1 January 1940 to 1 July 1978. SMRs were calculated using US White men as referent. SMRs were also estimated comparing workers potentially exposed to acrylonitrile in two departments with workers not employed in the chemicals division and thus considered not exposed, but these SMRs were not reported. [The Working Group considered that although the study provided acrylonitrile-specific results, it was limited by the imprecise effect estimates, short follow-up period (no person-years at risk were provided), and inadequate assessment of exposure. No internal analyses were reported, and HWSB was likely.]

2.1.8 Cohort of workers involved in the manufacture of styrene-based products, USA

Ott et al. (1989) reported on a set of industry-conducted case–control studies of mortality (1940–1978), including cases who died from non-Hodgkin lymphoma (NHL) (52 cases), multiple myeloma (20 cases), non-lymphocytic leukaemia (39 cases), and lymphocytic leukaemia (18 cases) nested in a cohort assembled by the National Institute for Occupational Safety and Health (NIOSH) and that comprised 29 139 male employees ever employed at two chemical-manufacturing facilities and/or one research and development centre in the USA (Rinsky et al., 1988). Controls were randomly selected (with replacement) in a 5:1 ratio to cases from among those with the same decade of first employment and who survived to at least the start of the same 5-year survival period as the cases. Ever exposure and duration of exposure to acrylonitrile were assessed using job records and the assessment of two retired senior manufacturing personnel who were contracted to review available records and prepare chronological listings of substances present in each chemical processing area. The numbers of exposed cases were very low for each of the studied outcomes (NHL, 6 cases; multiple myeloma, 3 cases; non-lymphocytic leukaemia, 1 case; and lymphocytic leukaemia, 1 case). [The Working Group noted the low number of exposed cases for each outcome and that HWSB was likely in the duration-based analysis. This, together with the multiple correlated exposures to several

chemicals, precluded meaningful assessment of the association between acrylonitrile exposure and death from the noted outcomes.]

2.1.9 Cohort of workers in an acrylic fibre factory, Italy

Mastrangelo et al. (1993) conducted a company-sponsored retrospective cohort study in 671 male workers with ≥ 12 months of exposure to acrylonitrile between 1959 and 1988 in an acrylic fibre factory in Italy. Clerks and some workers with past exposure to vinyl chloride or benzidine were excluded. Information on the smoking habits of workers was extracted from work records. The cohort was followed for mortality between 1959 and 1990, and a total of 32 deaths were reported from all causes. SMRs were calculated for the total cohort, adjusting for age and calendar period and based on regional death rates. [The Working Group noted that the lack of individual-level cumulative assessment of exposure to acrylonitrile may have resulted in nondifferential exposure misclassification. The mortality comparisons using regional death rates may have also introduced bias associated with the healthy-worker hire effect (HWE), and the 12-month exposure criterion may have introduced further downward bias resulting from the HWSB. The Working Group considered that this study was minimally informative.]

2.1.10 Occupational cohort in the Russian Federation

Geĭko et al. (1996) reported on a retrospective cohort study of workers exposed to acrylonitrile (n = 239) for ≥ 3 years between 1938 and 1985 in the Russian Federation. These workers were considered exposed on the basis of the primary product they were working with. Work hygiene studies and measurements were also conducted for exposure to methyl methacrylate, methyl acrylate and butyl acrylate, and methacrylic acid. Workers were followed for mortality through 1985 using regional oncology registries; workers who left employment were also located for mortality follow-up (10.8% could not be located). SMRs were calculated comparing the cancer mortality of exposed workers with that in the city of Dzerzhinsk. With 5382 person-years of follow-up through 1985, 49% of the observed mortality was from cancer (no total numbers were provided). [The Working Group noted that the unknown numbers of deaths and the limited characterization of cumulative exposure to acrylonitrile make this study minimally informative. HWSB was likely.]

2.1.11 Cohort of workers involved in acrylic fibre, monomer, and resin production, USA

In a study by the National Cancer Institute (NCI), Koutros et al. (2019) updated the mortality follow-up of a cohort of workers employed at eight acrylonitrile-producing facilities in the USA that had been previously investigated by Blair et al. (1998). The characteristics of each facility are reported in Table 2.2. The cohort included 25 460 workers (20 270 men and 5190 women) who worked ≥ 1 day from 1952–1965 through 1983. Follow-up was through 2011. Quantitative estimates of acrylonitrile exposure were developed using historical exposure estimates for each job, department, and plant combination by period based on work histories, more than 18 000 historical measurements (including more than 12 000 personal samples) from plant production records in 1960–1989, and 400 measurements from personal monitoring in 1986. Time-dependent exposure variables included cumulative exposure (ppm-years), average intensity (ppm), and duration of exposure (years). Overall and exposure-specific expected cases were calculated using 5-year age- and calendar time-, race-, and sex-specific mortality rates from the US population. SMRs were calculated for the whole cohort,

Table 2.2 Characteristics of the plants selected for the study by Koutros et al. (2019) in the USA

Plant	Location	Acrylonitrile-related process	Year first used acrylonitrile	Other exposures	Overlap with other studies
1	Pensacola, Florida	Fibres	1958	Methyl methacrylate, sodium thiocyanate, DMF	Complete overlap with Collins et al. (1989)
2	Chocolate Bayou, Texas	Monomer	1965	NH_3, propylene, HCN	None
3	Lima, Ohio	Monomer, resins, acrylamide	1960	NH_3, propylene, HCN, methyl acrylate, butadiene	Partial overlap with the Lima cohort separate publications (Marsh and Zimmerman, 2015) (see Section 2.1.13)
4	Williamsburg, Virginia	Fibres	1958	Vinyl bromide, methyl acrylate, zinc chloride	None
5	Decatur, Alabama	Fibres, adiponitrile	1952	Vinyl acetate, vinyl bromide, HMDA	None
6	Fortier, Louisiana	Monomer, acrylamide	1964	NH_3, HCN, acetylene, propylene, H_2S0_4	Complete overlap with Collins et al. (1989)
7	Ohio	Resins	1959	Butadiene, styrene	None
8	Texas City, Texas	Monomer	1953	NH_3, HCN, acetylene, propylene	None

DMF, dimethylformamide; NH_3, ammonia; HCN, hydrogen cyanide; HMDA, hexamethylenediamine; H_2SO_4, sulfuric acid; USA, United States of America.

and for workers considered to be exposed and unexposed to acrylonitrile. HRs were calculated for acrylonitrile exposure (cumulative, average, and duration) related to each outcome for which there were at least 10 acrylonitrile-exposed cases, using Cox proportional hazard models with age as the time metric and adjusting for race, sex, birth year, and salary–wage classification. Cumulative and average exposures were lagged by 10 years; sensitivity analyses considered 5- and 15-year lag periods. The authors also evaluated adjustments according to operation type (fibre versus non-fibre), plant, and possible co-exposures. They also evaluated work-related metrics, including age at first exposure, year of first exposure, and years since last exposure. Sensitivity analyses were also conducted to explore the absence of exposure information beyond 31 December 1983. The effect of potential confounding by cigarette smoking on the association between acrylonitrile and lung cancer was assessed using smoking information collected from a subgroup of participants (case–cohort analysis). Using the same data from the cohort reported by Koutros et al. (2019), Keil et al. (2024) evaluated the potential for HWSB. HRs to quantify associations between employment and lung cancer mortality and between exposure and leaving employment were evaluated. Using the parametric g-formula, HWSB was accounted for and estimates of cumulative lung cancer mortality at hypothetical acrylonitrile exposure limits were presented. [The Working Group considered this study to be informative. It had important strengths, including being the largest cohort of acrylonitrile-exposed workers across multiple facilities with a wide range of exposures and exposure circumstances and a long follow-up (1 023 922 person-years at risk), and with internal comparisons of exposed and unexposed workers. Other strengths included the exposure assessment metrics, and a measure of potential co-exposure to additional substances, including asbestos. Exposures were assessed

from the beginning of acrylonitrile operations at each of the plants. Exposure was assessed quantitatively using data collected from plant visits, plant records, and monitoring data from the companies, including data collected by the investigators themselves. The authors conducted robust analyses and explored different sensitivity analyses for lung cancer, including calendar period analyses. The major limitation of this study was that only the mortality follow-up was extended to 2011; the exposure assessment ended in 1983, when approximately 30% of the study population was still employed. The probable impact of this would be underestimation of exposure that was nondifferential with respect to case status. The authors investigated the impact through sensitivity analyses either excluding workers still active in 1983 or truncating their follow-up in 1983. In addition, they controlled for smoking in lung cancer analyses. Although smoking was not adjusted for in analyses for cancer of the urinary bladder, results were probably not confounded since no confounding was evident for lung cancer. HWSB was also accounted for.]

Marsh and Kruchten (2023) conducted an industry-sponsored reanalysis of the study by Koutros et al. (2019), evaluating acrylonitrile exposure and mortality for lung and bladder cancer, mesothelioma, and pneumonitis. The death and exposure information were identical to what is reported in Koutros et al. (2019). The primary aims included: (i) evaluating the relation between cumulative acrylonitrile exposure and mortality for lung cancer, bladder cancer, mesothelioma, and pneumonitis using external comparisons (SMRs); and (ii) indirect adjustment for potential confounding by smoking (lung and bladder cancer) and asbestos. [The Working Group considered these analyses to be uninformative because the analysis by Koutros et al. (2019) previously presented SMRs and was able to directly (rather than indirectly) adjust for confounding by smoking and asbestos, concluding that these were not important confounders of the acrylonitrile–lung cancer relation.]

2.1.12 Cohort of workers in eight chemical and fibre plants, the Netherlands

Swaen et al. (2004) reported on an industry-conducted retrospective cohort study of 2842 men employed by one of eight chemical plants that produced or used acrylonitrile in the Netherlands. To be eligible for the study, workers had to be male, citizens of the Netherlands, and either exposed to acrylonitrile for > 6 months or employed in the comparison plant for > 6 months. Quantitative assessment of exposure to acrylonitrile between 1959 and 1979 was conducted using job records and expert assessment for each combination of job, workplace, and time period; exposure was presumed to cease after 1 January 1980 because of efforts to improve engineering controls and personal protective equipment, although situations where exposure was known to have occurred (based on odour threshold) were documented after 1 January 1980. The total cohort was followed for all-cause mortality through 1 January 2001 using national registries. SMRs were calculated for the cohort overall, by category of cumulative exposure level categories (< 1 ppm-year, 1–10 ppm-years, and > 10 ppm-years), peak exposures having occurred on a regular basis at least once per week (< 10, 10–20, and > 20 ppm), respirator use (yes, no), and exposure to other carcinogenic agents (yes, no). SMRs were also calculated for a ninth plant, a nitrogen-fixation plant of 3961 workers, considered "unexposed" to acrylonitrile, but direct comparison of the exposed and unexposed group was not reported. [The Working Group noted that the quantitative exposure assessment for the period between 1959 and 1979 reflected cumulative exposure, which is a strength of the study. Exposure information for 1980–2000 was missing, which may have

introduced nondifferential exposure misclassification (underestimation of exposure). The study presented comparisons only with external mortality rates for different acrylonitrile exposure groupings, meaning that the results were probably prone to the HWE. The 6-month employment criterion led to concern about HWSB. Additional analyses for lung cancer mortality also did not account for possible confounding from smoking or co-exposures. For these reasons, the Working Group considered that this study was somewhat informative.]

2.1.13 Cohort of workers in a chemical-manufacturing plant in Lima, Ohio, USA

Marsh and Zimmerman (2015) reported on an industry-conducted retrospective cohort study in an acrylonitrile-manufacturing plant in Lima, Ohio, USA. The cohort was expanded three times (Marsh et al., 1999, 2002; Marsh and Zimmerman, 2015) to include a final total of 2096 workers who had been employed at the facility between 1955 and 2011, 789 of whom were exposed to acrylonitrile. In the original study, a panel of four company-independent industrial hygienists conducted an exposure assessment (using location monitoring measurements and personal monitoring data) to provide quantitative, time-dependent historical estimates of acrylonitrile exposure. In the 2015 update, industrial hygienists and human resources representatives classified every job into one of 20 job groups on the basis of work performed and job type. For each job group, the arithmetic mean of available acrylonitrile measurements (gathered from acrylonitrile monitoring data from 1999 through 2011) was used to represent the daily time-weighted exposure estimate. The cohort was observed for all-cause mortality through 31 December 2011, using company records and the US National Death Index. Smoking data (ever/never) were collected but were characterized by the authors as "misclassified" and not used for internal comparisons. SMRs and 95% confidence intervals were computed using death rates for White men from the USA and the local county. Relative risks were calculated to compare exposed and unexposed workers, but only for lung cancer mortality. [The Working Group noted the quantitative exposure assessment as a strength but also noted several limitations, including the absence of internal comparison analyses for some cancer outcomes. Although the follow-up period was long (> 50 years), the expansion of the cohort from 992 to 2096 participants yielded only 6 additional acrylonitrile-exposed deaths for cancers of the trachea, bronchus, and lung and included a maximum of 15 acrylonitrile-exposed deaths. The assessment of exposure in the period between 1997 and 1998 appeared to be missing, and the authors used the arithmetic mean of the available acrylonitrile measurement by job group; thus, exposure may have been underestimated during this period. Also, there was no additional attempt to account for HWSB. The study by Marsh and Zimmerman (2015) included an overlap of 894/2096 (~43%) participants from Koutros et al. (2019). Because of this overlap and the few additional exposed participants, the Working Group considered that this study was minimally informative.]

2.1.14 Cohort of workers in an acrylonitrile-using factory, Hungary

Czeizel et al. (2004) reported on 783 workers in a factory using acrylonitrile in Hungary. Eligible participants were all workers employed at the factory in June 2000. Workers in the factory were divided into three groups: Group A included workers who used acrylonitrile (considered to be direct and continuous exposure, $n = 452$); Group B included workers who helped in the transport of materials, care, and maintenance of the working process (considered to be some/direct occasional exposure); and Group C included management

personnel, administrators, and other workers without direct exposure to acrylonitrile. A total of 12 prevalent cancers and 12 deaths from any cancer were reported based on self-report (questionnaire) or medical records from the occupational medical service. The authors reported in text that the observed age- and sex-specific total rates (living and fatal) of cancer did not exceed the expected figures based on Hungarian baseline rates. [The Working Group considered that this study was uninformative. The design was cross-sectional and primarily relied on self-reported cancer information from five cancers reported among workers with direct exposure. The assessment of exposure to acrylonitrile was based primarily on job title and not estimated at the individual level.]

2.1.15 Cohort of workers in a petrochemical plant in Porto Torres, Italy

Budroni et al. (2010) reported a retrospective cohort study of 5350 male petrochemical workers, who worked for ≥ 6 months in one or more chemical plants (43 facilities identified) of the Porto Torres industrial area in Sardinia, Italy, and were followed between 1990 and 2006. Workers were identified using the records of the National Institute for Social Security (INPS) and included men working in the plants and alive as of 1 January 1990 as well as workers who were hired between 1 January 1990 and 31 December 2001. Cancer incidence was ascertained from the provincial tumour registry and supplemented with death registry information from the Sassari regional health authority. Standardized incidence ratios (SIRs) were calculated, using rates in the Province of Sassari as the referents, for the cohort overall and for workers potentially exposed to acrylonitrile (2336 workers) and to several other chemical agents. An overall analysis by duration of employment (≤ 10 years, > 10 years) was performed but did not take acrylonitrile exposure into consideration. [The Working Group noted that the cancer data were of high quality and there was histopathological validation; however, the limitation in the assessment of exposure to acrylonitrile, based only on the plant (no individual-level cumulative exposure, or historical assessment before the follow-up period), the lack of accounting for possible co-exposure to other agents, and the absence of internal comparisons may have led to a dilution of any observed excesses in incidence. HWSB was likely. The Working Group considered that this study was minimally informative.]

2.1.16 Case–control study nested in the Golestan cohort, Islamic Republic of Iran

Etemadi et al. (2024) reported on an oesophageal cancer case–control study nested in the Golestan cohort, for which 50 045 study participants aged 40–75 years were recruited from the Golestan province of the Islamic Republic of Iran between January 2004 and June 2008. Follow-up of the cohort was from enrolment to 1 January 2018. Incident oesophageal cancer cases (n = 205) were identified by annual phone calls and confirmed by linkage with the provincial cancer registry. Controls (n = 226) were randomly selected from among cohort members who did not have cancer of the upper gastrointestinal tract and were alive at the time of case diagnosis. The controls were also matched to cases on age, sex, residence, time of enrolment in the cohort, and tobacco use. Urinary concentrations of 33 biomarkers of exposure to nicotine, including three metabolites of acrylonitrile, were measured in spot urine samples collected at study enrolment. Two acrylonitrile urinary metabolites are specific to acrylonitrile (1CyHEMA [referred to in the present monograph as CHEMA] and 2CyEMA [CEMA]) and the third can be formed from the metabolism of both acrylonitrile and ethylene oxide (2HEMA [referred to in the present monograph as HEMA]). Participants were stratified

on the basis of current tobacco use (57 cases and 63 controls), which was defined as the use of one of the three types of tobacco (cigarettes, waterpipe, and nass) at least once per week, including the year before enrolment in the study, and for current non-users (individuals who did not currently use tobacco at baseline, 148 cases and 163 controls). Geometric means and 95% confidence intervals for creatinine-corrected concentrations were calculated for tobacco users and current non-users. Odds ratios (ORs) and 95% confidence intervals for associations between urinary biomarkers and incident oesophageal squamous cell carcinoma were calculated for current tobacco users and current non-users, employing conditional logistic regression, conditioned on case–control matching. Models were adjusted for ethnicity, education, wealth, body mass index (BMI), tea temperature, tooth loss, opium use, and urinary cotinine. [The Working Group noted the high-quality design of the study, including the collection of urine at enrolment, clearly defined cases and selected controls, and detailed available data for multivariable adjustment (including opium use). The results for HEMA should be interpreted with caution because this metabolite is not specific to acrylonitrile. Another limitation was that these urinary biomarkers indicate recent exposure (short half-lives), which may not be relevant for long-latency diseases like oesophageal cancer. The detection of acrylonitrile metabolites in current non-users suggested that there may be another source of acrylonitrile exposure, which was not assessed in this study. The Working Group considered this study to be somewhat informative.]

2.1.17 Description of available meta-analyses

Several meta-analyses on the association between acrylonitrile and all cancers (Rothman, 1994), lung or respiratory cancer (Rothman, 1994; Collins and Acquavella, 1998; Sponsiello-Wang et al., 2006; Alexander et al., 2021), central nervous system tumours (Collins and Strother, 1999), pancreatic cancer (Ojajärvi et al., 2000), and prostate cancer (Krstev and Knutsson, 2019) have been published.

Among the meta-analyses on lung or respiratory cancer, only that by Alexander et al. (2021) included the most recently published data from Koutros et al. (2019), which was the largest study available. This industry-sponsored meta-analysis reported summary relative risk estimates that combined estimates of SMRs, ORs, RRs, and HRs for a range of heterogeneous studies. Several of the studies included had limited assessment of exposure to acrylonitrile. [The Working Group noted that the meta-analysis by Alexander et al. was not informative for several reasons, including the combining of studies with different or no assessments of exposure to acrylonitrile (cumulative exposure versus qualitative assessment of exposure), summary estimates that mixed external (SMRs) and internal (HRs) estimates, and no consideration of healthy-worker biases.] The previously reported lung or respiratory cancer meta-analyses by Rothman (1994), Collins and Acquavella (1998), and Sponsiello-Wang et al. (2006) did not include the study by Koutros et al. (2019). [The Working Group noted that the meta-analyses that did not include Koutros et al. (2019) were uninformative because they did not include recently published data. Because of the heterogeneity in the design and exposure assessment quality of the available studies on lung cancer, the Working Group noted that updating the meta-analysis would not be informative.]

The available meta-analyses for central nervous system tumours (Collins and Strother, 1999), pancreatic cancer (Ojajärvi et al., 2000), and prostate cancer (Krstev and Knutsson, 2019) did not consider the largest, highest-quality study by Koutros et al. (2019) in the estimates. [The Working Group considered the meta-analyses for sites other than lung to be uninformative,

because they did not include the most recently available data.]

2.2 Cancers of the respiratory system and mesothelium

See Table 2.3.

2.2.1 Cancers of the trachea, bronchus, and lung

The relation between acrylonitrile and cancer of the lung (with most studies including trachea and bronchus in this category) has been the most frequently evaluated association in the available studies. Thirteen retrospective cohort studies of workers and one large case–control study with detailed occupational data were deemed informative for the evaluation. Most of the cohort studies reported on incidence or mortality among workers with potential exposure to acrylonitrile compared with the general population, and generally did not have a detailed quantitative exposure assessment (Kiesselbach et al., 1979; Ott et al., 1980; Thiess et al., 1980; Waxweiler et al., 1981; Delzell and Monson, 1982; Mastrangelo et al., 1993; Geĭko et al., 1996; Benn and Osborne, 1998; Swaen et al., 2004; Budroni et al., 2010). [The Working Group noted that these studies compared the number of observed deaths with the number of expected deaths on the basis of external mortality rates, which may lead to an underestimation of the risk of cancer because of the HWE. For most studies, there was a 6- or 12-month minimum employment duration criterion for cohort entry, leading to concerns about HWSB.] Four studies were conducted to evaluate quantitative metrics of exposure to acrylonitrile in exposed and unexposed workers (for internal comparison) (Scélo et al., 2004; Symons et al., 2008; Marsh and Zimmerman, 2015; Koutros et al., 2019), and two of these (Scélo et al., 2004; Koutros et al., 2019) also presented smoking-adjusted models to assess confounding from tobacco use. [The Working Group prioritized the studies with internal comparisons and cumulative exposure characterizations in its evaluation of cancer of the trachea, bronchus, and lung.]

Kiesselbach et al. (1979) reported on a cohort of 884 male employees exposed to acrylonitrile in a plant in Germany (see Section 2.1.1). The SMR for respiratory tract cancer was [0.87] (95% CI, [0.32–1.88]; 6 observed deaths). [The Working Group noted that this study was minimally informative because of imprecise estimates and downward HWE and HWSB.]

Symons et al. (2008) reported on mortality in a cohort of 2548 workers in two facilities in the USA that used acrylonitrile during fibre production (see Section 2.1.2). The results reflected the results of the extended follow-up of the cohort previously reported in O'Berg (1980), O'Berg et al. (1985), Chen et al. (1987), and Wood et al. (1998). SMRs and HRs were calculated for a continuous increase in cumulative exposure of 100 ppm-years. Models of cumulative acrylonitrile exposure were also lagged by 5, 10, and 15 years. Adjusted HR estimates for an increase in cumulative exposure of 100 ppm-years indicated no increase in risk for lung cancer (International Classification of Diseases, ninth revision, ICD-9, 162) mortality (unlagged HR, 0.95; 95% CI, 0.73–1.23; 88 deaths; 5-year lag HR, 0.95; 95% CI, 0.70–1.28; 75 deaths; 10-year lag HR, 0.84; 95% CI, 0.59–1.19; 72 deaths; and 15-year lag HR, 0.80; 95% CI, 0.53–1.22; 63 deaths). [The Working Group noted that no smoking or co-exposure data were available for the assessment of confounding, and the reported hazards for lung cancer were imprecise in many subgroup analyses. No adjustment was made for HWSB.]

Ott et al. (1980) evaluated mortality in a cohort of men employed for ≥ 1 year in the manufacture of styrene-based products in facilities in the USA (see Section 2.1.3). The SMR for malignant diseases of the respiratory system was [0.56] (95% CI, [0.31–0.94]). [The Working Group noted that this study was minimally informative because of

Table 2.3 Epidemiological studies on exposure to acrylonitrile and cancers of the respiratory system and mesothelioma

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kiesselbach et al. (1979) Germany 1950 to 1 August 1977 Cohort	884 male employees of a plant in Leverkusen, who were exposed to AN (handled AN for ≥ 1 yr in production or processing) between 1950 and 1 August 1977. Some of these men handled butadiene–styrene. Expected deaths from North Rhine-Westphalia rates. Outcome data sources not reported. Exposure assessment method: See Table 2.1.	Respiratory tract, mortality	SMR (North Rhine-Westphalia referent): Overall cohort	6	[0.87 (0.32–1.88)]	Age, calendar period	*Strengths*: See Table 2.1. *Limitations*: See Table 2.1.
Symons et al. (2008) Waynesboro (VA) and Camden (SC), USA Enrolment, 1947–1991/follow-up, through 2002 Cohort	2548 male workers who were exposed to AN for ≥ 6 mo in two company facilities (Waynesboro, and Camden) manufacturing acrylic fibre, in 1947–1991. Exposure assessment method: Quantitative measurements; see Table 2.1.	Lung, mortality	SMR (US referent): Total cohort	88	[0.74 (0.6–0.91)]	Age, calendar period Birth period, employment in SC start-up group, age	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations:* See Table 2.1.
		Lung, mortality	Cumulative AN exposure (HR): Continuous (per 100 ppm-yr), no lag	88	0.95 (0.73–1.23)		
			Continuous (per 100 ppm-yr), 5-yr lag	75	0.95 (0.7–1.28)		
			Continuous (per 100 ppm-yr), 10-yr lag	72	0.84 (0.59–1.19)		
			Continuous (per 100 ppm-yr), 15-yr lag	63	0.8 (0.53–1.22)		
		Lung, mortality	Mean AN exposure intensity, men with cumulative exposure > 10 ppm-yr (HR): < 10 ppm	25	1		
			≥ 10 ppm	55	1.09 (0.67–1.77)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ott et al. (1980) USA Enrolment, 1937–1970/follow-up, 1940–1975 Cohort	2904 (2740 without exposure to arsenicals, asbestos, or vinyl chloride); employed for ≥ 1 yr in the manufacture of styrene-based products between 1937 and 1970. Exposure assessment method: See Table 2.1.	Respiratory tract, mortality	SMR (US White male referent): Cohort, less workers with arsenicals, asbestos, or high vinyl chloride exposure	14	[0.56 (0.31–0.94)]	Age, calendar period	*Strengths*: See Table 2.1. *Limitations*: See Table 2.1.
Thiess et al. (1980) Germany Enrolment, varies/follow-up, through 15 May 1978 Cohort	1469 workers employed for ≥ 6 mo at 12 AN-using plants from 1954 through 15 May 1978. Exposure assessment method: See Table 2.1.	Lung, mortality Lung, mortality	SMR (Rheinhessen-Pfalz referent): Total cohort Duration of exposure, excluding plant 5 (SMR, Rheinhessen-Pfalz referent): 0–4 yr 5–9 yr ≥ 10 yr	11 2 4 3	[1.86 (0.93–3.33)] [2.16 (0.26–7.82)] [3.86 (1.05–9.89)] [2.23 (0.46–6.52)]	Age	*Exposure assessment critique*: See Table 2.1. *Other limitations*: Small study size with short follow-up period, lack of internal analyses, and multiple co-exposures. *Other comments*: Lung cancer described in the paper as "Bronchial-Karzinom" or "bronchial cancer".
Waxweiler et al. (1981) USA Enrolment, 1942–1973/follow-up, through 1973 Cohort	4806 men (98% White) employed at a VCM plant between 1942 and 1973. Exposure assessment method: See Table 2.1.	Respiratory tract, mortality	SMR (US White male referent): Total cohort	42	[1.49 (1.07–2.01)]	Age, calendar period	*Strengths*: See Table 2.1. *Limitations*: See Table 2.1.

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Benn and Osborne (1998) England, Wales, Scotland, Northern Ireland Enrolment, phase I (1950–1968); phase II (1969–1978)/follow-up, through 1991 Cohort	2763 male workers employed for ≥ 1 yr and exposed to polymerization of AN or the spinning of acrylic fibre in six factories in the UK identified in a study conducted by the HSE and the Chemical Industries Association. Exposure assessment method: See Table 2.1.	Lung, mortality	SMR (national referent):			Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Strengths*: See Table 2.1. *Other limitations*: SMRs were inadequately used for trend analyses. Additional limitation in Table 2.1. *Other comments*: Unclear reference group for Northern Ireland. Lung cancer was specified as "trachea, bronchus & lung".
			Total cohort	53	[1.028 (0.77–1.35)]		
		Lung, mortality	Highest AN exposure level (SMR, national referent):				
			No or little AN exposure	19	[0.995 (0.6–1.55)]		
			Other possible AN exposure	7	[0.526 (0.21–1.08)]		
			High	27	[1.411 (0.93–2.05)]		
			Trend-test *P* value, 0.201				
		Lung, mortality	Age group, workers exposed to high levels of AN (SMR, national referent):				
			15–44 yr	5	[6.098 (1.98–14.2)]		
			45–54 yr	3	[0.737 (0.15–2.15)]		
			55–64 yr	9	[1.151 (0.53–2.18)]		
			≥ 65 yr	10	[1.57 (0.75–2.89)]		
		Lung, mortality	Calendar year of first exposure, workers exposed to high AN levels (SMR, national referent):				
			Before 1690	10	[1.323 (0.63–2.43)]		
			1960–1968	10	[1.12 (0.54–2.06)]		
			1969 onwards	7	[2.703 (1.09–5.57)]		
		Lung, mortality	Time since first exposure, workers exposed to high AN levels (SMR, national referent):				
			< 5 yr	1	[1.01 (0.03–5.63)]		
			5–10 yr	4	[2.151 (0.59–5.51)]		
			10–15 yr	5	[1.608 (0.52–3.75)]		
			> 15 yr	17	[1.297 (0.76–2.08)]		
			Trend-test *P* value, 0.665				

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Benn and Osborne (1998) (cont.)		Lung, mortality	Duration of exposure, workers exposed to high AN levels (SMR, national referent):			Age, calendar period	
			< 5 yr	10	[1.422 (0.68–2.62)]		
			5–10 yr	7	[1.316 (0.53–2.71)]		
			10–15 yr	6	[1.274 (0.47–2.77)]		
			> 15 yr	4	[2.041 (0.56–5.23)]		
			Trend-test *P* value, 0.765				
Delzell and Monson (1982) Akron (OH), USA Enrolment, 1940–1971/follow-up, 1940 through 1 July 1978 Cohort	327 White male production workers employed for ≥ 2 yr between 1940 and 1971 at a rubber-manufacturing plant in Akron, who had worked in the chemicals division in Departments 5578 or 5579 with potential AN exposure. Exposure assessment method: See Table 2.1.	Lung, mortality	SMR (US referent):			Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
			Total cohort	9	1.5 (0.7–2.9)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Swaen et al. (2004) Netherlands Enrolment, varied by plant, starting 1959–1973/follow-up, through 1 January 2001 Cohort	2842 men, citizens of the Netherlands, working in eight chemical companies with AN exposure for > 6 mo. Exposure assessment method: See Table 2.1.	Lung, mortality	SMR (national referent):			Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1. *Other comments*: Lung cancer described in the paper as "trachea and lung". Latency was defined as time since the particular dose group was entered. SMR for high cumulative exposure was missing in the table, therefore it was calculated by the Working Group.
			Total cohort	67	[1.072 (0.831–1.361)]		
		Lung, mortality	Cumulative AN exposure (SMR, national referent):				
			Low (< 1 ppm-yr)	7	[0.921 (0.369–1.89)]		
			Moderate (1–10 ppm-yr)	36	[1.065 (0.746–1.474)]		
			High (> 10 ppm-yr)	24	[1.148 (0.736–1.709)]		
		Lung, mortality	Cumulative AN exposure by latency period (time since dose group entered) (SMR, national referent):				
			Low (< 1 ppm-yr), < 10 yr latency	0	[0 (0–5.3)]		
			Low (< 1 ppm-yr), 10–20 yr latency	3	[1.034 (0.208–2.968)]		
			Low (< 1 ppm-yr), > 20 yr latency	4	[1 (0.269–2.531)]		
			Moderate (1–10 ppm-yr), < 10 yr latency	1	[0.313 (0.004–1.579)]		
			Moderate (1–10 ppm-yr), 10–20 yr latency	16	[1.29 (0.737–2.094)]		
			Moderate (1–10 ppm-yr), > 20 yr latency	19	[1.044 (0.628–1.629)]		
			High (> 10 ppm-yr), < 10 yr latency	3	[1.2 (0.241–3.442)]		
			High (> 10 ppm-yr), 10–20 yr latency	12	[1.429 (0.737–2.492)]		
			High (> 10 ppm-yr), > 20 yr latency	9	[0.9 (0.411–1.704)]		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Swaen et al. (2004) (cont.)		Lung, mortality	Peak exposure occurring on a regular basis at least once per week (SMR, national referent):			Age, calendar period	
			None	19	[1.08 (0.65–1.685)]		
			< 10 ppm	26	[1.111 (0.726–1.628)]		
			10–20 ppm	18	[1.017 (0.602–1.606)]		
			> 20 ppm	4	[1.053 (0.283–2.664)]		
		Lung, mortality	Respirator use (SMR, national referent):				
			Yes	11	[0.815 (0.406–1.455)]		
			No	56	[1.143 (0.863–1.484)]		
		Lung, mortality	Exposure to other carcinogens (SMR, national referent):				
			Yes	27	[0.941 (0.62–1.368)]		
			No	40	[1.183 (0.845–1.611)]		
		Larynx, mortality	SMR (national referent):				
			Total cohort	4	[2.222 (0.598–5.625)]		
Mastrangelo et al. (1993) Italy Enrolment, 1959–1988/follow-up, 1959–1990 Cohort	671 men employed for ≥ 12 mo at any time since the opening of the factory in 1959. Clerks and some workers with past exposure to vinyl chloride monomer or to benzidine and its compounds were excluded, as were workers employed after 31 December 1988. Exposure assessment method: See Table 2.1.	Lung, mortality	SMR (regional referent):			Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
			Total cohort	2	[0.77 (0.09–2.77)]		
Geĭko et al. (1996) Russian Federation Enrolment, 1938–1985/follow-up, through 1985 Cohort	239 workers exposed to AN for ≥ 3 yr between 1938 and 1985. Exposure assessment method: See Table 2.1.	Lung, mortality	SMR (reference population: city of Dzerzhinsk)			Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
			Total cohort	NR	[2.273 (0.62–5.82)]		
			AN-exposed, 10–14 yr	NR	[12.82]		
			AN-exposed, 20–24 yr	NR	[5.747]		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2019) USA Enrolment, 1952–1983/follow-up, through 2011 Cohort	25 460 (16 889 AN-exposed); all workers employed for ≥ 1 day at any of the eight participating plants after the start of AN operations (1952–1965). Work histories covered the date of hire (i.e. 1942–1983) through date of record abstraction at the plant (1983). Exposure assessment method: See Table 2.1.	Lung, mortality	SMR (US referent): AN-exposed	559	0.88 (0.81–0.96)	Sex, race, age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths*: Robust analyses with different exposure metrics and sensitivity analyses for lung cancer. Controlled for smoking in lung cancer analyses. Additional strengths in Table 2.1. *Other limitations*: See Table 2.1. *Other comments*: Lung cancer described in the paper as "lung and bronchus".
		Lung, mortality	Cumulative AN exposure (through 1983), 10-yr lag (HR): Not exposed	263	1	Age, race, sex, birth year, salary–wage classification	
			Quintile 1 (0–0.09 ppm-yr)	109	1.15 (0.92–1.45)		
			Quintile 2 (> 0.09 to 0.64 ppm-yr)	109	0.96 (0.77–1.21)		
			Quintile 3 (> 0.64 to 2.30 ppm-yr)	109	1.03 (0.81–1.29)		
			Quintile 4 (> 2.30 to 12.08 ppm-yr)	109	1.06 (0.84–1.33)		
			Quintile 5 (> 12.08 ppm-yr)	109	1.43 (1.13–1.81)		
			Trend-test *P* value, 0.05				

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2019) (cont.)		Lung, mortality	Average AN exposure (through 1983), 10-yr lag (HR):			Age, race, sex, birth year, salary–wage classification	
			Not exposed	263	1		
			Quintile 1 (0–0.06 ppm)	110	1 (0.79–1.25)		
			Quintile 2 (> 0.06 to 0.14 ppm)	108	1.21 (0.96–1.53)		
			Quintile 3 (> 0.14 to 0.37 ppm)	109	1.07 (0.85–1.35)		
			Quintile 4 (> 0.37 to 1.46 ppm)	109	1.05 (0.84–1.32)		
			Quintile 5 (> 1.46 ppm)	109	1.22 (0.97–1.54)		
			Trend-test *P* value, 0.14				
		Lung, mortality	Duration of exposure (through 1983) (HR):				
			Not exposed	249	1		
			Quintile 1 (0–0.96 yr)	112	1.08 (0.85–1.36)		
			Quintile 2 (> 0.96 to 3.79 yr)	112	1 (0.79–1.25)		
			Quintile 3 (> 3.79 to 9.96 yr)	112	1.01 (0.8–1.27)		
			Quintile 4 (> 9.96 to 17.19 yr)	112	1.27 (1.01–1.6)		
			Quintile 5 (> 17.19 yr)	111	1.16 (0.92–1.46)		
			Trend-test *P* value, 0.10				

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2019) (cont.)		Lung, mortality	Cumulative AN exposure (through 1983), 10-yr lag, main analysis (no additional exposure after 1983) (HR):			Age, race, sex, birth year, salary–wage classification	
			Not exposed	263	1		
			0 to < 1 ppm-yr	257	1.06 (0.88–1.27)		
			1 to < 2 ppm-yr	59	1 (0.75–1.33)		
			2 to < 4 ppm-yr	59	1.13 (0.84–1.5)		
			4 to < 8 ppm-yr	41	0.99 (0.71–1.38)		
			8 to < 16 ppm-yr	43	1.22 (0.88–1.69)		
			16 to < 32 ppm-yr	38	1.27 (0.89–1.8)		
			≥ 32 ppm-yr	48	1.47 (1.07–2.02)		
			Trend-test *P* value, 0.02				
		Lung, mortality	Cumulative AN exposure (through 1983), 10-yr lag, sensitivity analysis (censoring all active workers at the end of 1983) (HR):				
			Not exposed	226	1		
			0 to < 1 ppm-yr	227	1.19 (0.98–1.44)		
			1 to < 2 ppm-yr	41	0.98 (0.7–1.38)		
			2 to < 4 ppm-yr	43	1.19 (0.86–1.66)		
			4 to < 8 ppm-yr	23	0.88 (0.57–1.36)		
			8 to < 16 ppm-yr	29	1.38 (0.93–2.04)		
			16 to < 32 ppm-yr	23	1.28 (0.83–1.98)		
			≥ 32 ppm-yr	30	1.64 (1.11–2.42)		
			Trend-test *P* value, 0.04				

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2019) (cont.)		Lung, mortality	Cumulative AN exposure (through 1983), 10-yr lag, sensitivity analysis (excluding workers still active in 1983) (HR):			Age, race, sex, birth year, salary–wage classification	
			Not exposed	226	1		
			0 to < 1 ppm-yr	227	1.2 (0.98–1.46)		
			1 to < 2 ppm-yr	41	1.01 (0.71–1.44)		
			2 to < 4 ppm-yr	43	1.24 (0.88–1.76)		
			4 to < 8 ppm-yr	23	0.92 (0.59–1.45)		
			8 to < 16 ppm-yr	29	1.49 (1–2.24)		
			16 to < 32 ppm-yr	23	1.44 (0.92–2.25)		
			≥ 32 ppm-yr	30	1.73 (1.15–2.61)		
			Trend-test *P* value, 0.009				
		Lung, mortality	Cumulative AN exposure (through 1983), 10-yr lag, workers with 0 score for probability of asbestos exposure (HR):				
			Not exposed	228	1		
			Quintile 1 (0–0.09 ppm-yr)	90	1.2 (0.93–1.54)		
			Quintile 2 (> 0.09 to 0.64 ppm-yr)	74	0.91 (0.7–1.19)		
			Quintile 3 (> 0.64 to 2.30 ppm-yr)	52	0.85 (0.63–1.16)		
			Quintile 4 (> 2.30 to 12.10 ppm-yr)	60	0.98 (0.73–1.31)		
			Quintile 5 (> 12.10 ppm-yr)	45	1.63 (1.17–2.26)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2019) (cont.)		Lung, mortality	Cumulative AN exposure (through 1983), 10-yr lag, subcohort with non-missing smoking information (HR):			Age, race, sex, birth year, salary–wage classification	
			Not exposed	NR	1		
			Quintile 5 (> 12.1 ppm-yr)	NR	1.36 (0.57–3.22)		
		Lung, mortality	Cumulative AN exposure (through 1983), 10-yr lag, subcohort with non-missing smoking information (HR):			Age, race, sex, birth year, salary–wage classification, smoking status	
			Not exposed	NR	1		
			Quintile 5 (> 12.1 ppm-yr)	NR	1.32 (0.55–3.17)		
		Lung, mortality	Cumulative AN exposure (through 1983), 10-yr lag, subcohort with non-missing smoking information (HR):			Age, race, sex, birth year, salary–wage classification, duration of smoking	
			Not exposed	NR	1		
			Quintile 5 (> 12.1 ppm-yr)	NR	1.46 (0.62–3.46)		
		Lung, mortality	Years since last AN exposure (HR):			Age, race, sex, birth year, salary–wage classification, cumulative AN exposure	
			< 15 yr	107	1		
			15–29 yr	155	0.77 (0.58–1.01)		
			> 30 yr	177	0.62 (0.46–0.84)		
			Unknown	120	NC		
			Trend-test *P* value, 0.02				
		Mesothelioma, mortality	SMR (US referent):			Sex, race, age, calendar period	
			AN-exposed	16	2.36 (1.35–3.83)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2019) (cont.)		Mesothelioma, mortality	Cumulative AN exposure (through 1983), 10-yr lag (HR):			Age, race, sex, birth year, salary–wage classification	
			Not exposed	5	1		
			≤ Median (0–1.33 ppm-yr)	8	1.27 (0.4–4.01)		
			> Median (> 1.33 ppm-yr)	8	1.15 (0.37–3.66)		
			Trend-test *P* value, 0.84				
		Mesothelioma, mortality	Average AN exposure (through 1983), 10-yr lag (HR):				
			Not exposed	5	1		
			≤ Median (0–0.16 ppm)	8	1.67 (0.53–5.23)		
			> Median (> 0.16 ppm)	8	0.94 (0.3–2.99)		
			Trend-test *P* value, 0.79				
		Mesothelioma, mortality	Duration of exposure (through 1983) (HR):				
			Not exposed	5	1		
			≤ Median (0–14.19 yr)	8	0.87 (0.27–2.74)		
			> Median (> 14.19 yr)	8	2.01 (0.63–6.44)		
			Trend-test *P* value, 0.20				
Keil et al. (2024) NCI Acrylonitrile Cohort Study, USA Enrolment, 1952–1983/follow-up, through 2011 Cohort	25 460 (see Koutros et al., 2019); the impact of HWSB was assessed; expected lung cancer mortality was estimated adjusting for HWSB using the parametric g-formula under different hypothetical occupational AN exposure limits.	Lung, mortality	No. of prevented or delayed lung cancer deaths (per 1000 workers) by age 90 yr			Sex, race, wage status, date of birth, fibre plant, time-varying employment status	*Strengths*: See Table 2.1. *Limitations*: See Table 2.1.
			Hypothetical scenario of AN exposure elimination	NR	7.21 (2.72–11.7)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Marsh and Zimmerman (2015) Lima (OH), USA Enrolment, starting in 1955/follow-up, 1955–2011 Cohort	2096 (789 with AN exposure); employees (any time length) in Lima factory between 1955 and 2011. The Lima site was included in Koutros et al. (2019), but the overlap between the two Lima cohorts was only partial. Exposure assessment method: See Table 2.1.	Lung, mortality	Exposure group (SMR, county referent):			Sex, race, age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1. *Other comments*: Unclear which reference population was used for the female component. Lung cancer described in the paper as "bronchus, trachea, lung" cancer.
			Unexposed	29	0.84 (0.56–1.2)		
			AN-exposed	15	0.73 (0.41–1.2)		
		Lung, mortality	Duration of AN exposure (RR):			Age, sex, race, year of birth	
			Unexposed	29	1		
			> 0 to 4.9 yr	5	0.76 (0.27–2.09)		
			5–14.9 yr	6	1.93 (0.77–4.84)		
			≥ 15 yr	4	0.84 (0.29–2.47)		
			Trend-test *P* value, 0.473				
		Lung, mortality	Cumulative AN exposure (RR):				
			Unexposed	29	1		
			> 0 to 8.91 ppm-yr	5	1.02 (0.38–2.75)		
			8.92–79.79 ppm-yr	5	1.12 (0.41–3)		
			≥ 79.80 ppm-yr	5	1.02 (0.38–2.74)		
			Trend-test *P* value, 0.997				
		Lung, mortality	Average intensity of exposure to AN (RR):				
			Unexposed	29	1		
			> 0 to 3.37 ppm	5	0.89 (0.34–2.36)		
			3.38–9.87 ppm	5	1.44 (0.53–3.9)		
			≥ 9.88 ppm	5	0.96 (0.35–2.62)		
			Trend-test *P* value, 0.889				
		Larynx, mortality	Exposure group (SMR, county referent):			Sex, race, age, calendar period	
			Unexposed	2	1.87 (0.23–6.76)		
			AN-exposed	0	0 (0–4.59)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Budroni et al. (2010) Porto Torres, Sardinia, Italy 1990–2006 Cohort	5350 (2336 with AN exposure); male workers with ≥ 6 mo of employment in one or more chemical plants in the Porto Torres industrial area, working in the plants and alive as of 1 January 1990. Workers that joined between 1 January 1990 and 31 December 2001 were also included. Exposure assessment method: See Table 2.1.	Lung, incidence	SIR (local referent): AN-exposed	41	1.03 (0.76–1.4)	Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
		Larynx, incidence	SIR (local referent): AN-exposed	5	0.65 (0.27–1.57)		
		Pleura, incidence	No. of deaths AN-exposed	0	NC	None	

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Scélo et al. (2004) Liverpool (UK), Romania (Bucharest), Poland (Lodz, Poland), the Russian Federation (Moscow), Czechia (Brno, Olomuc, Prague), Hungary (Budapest, Borsod, Heves, Szabolcs, Szolnok), Slovakia (Banska Bystica, Bratislava, Nitra) 1998–2002 Case–control	Cases: 2861 newly diagnosed primary lung cancers (1998–2002) in people living in the study area for ≥ 1 yr. Cases were recruited in hospitals that treated all lung cancer in the area. In the Russian Federation, cases were also recruited in a dispensary. Controls: 3118 hospital controls in all centres except for two (one in Poland and one in the UK) in which population controls were selected. Hospital controls selected from hospitals covering the same area as cases, and patients admitted for cancer or smoking-related diseases were excluded. Frequency-matched on age and sex. Exposure assessment method: Structured questionnaires administered to participants; evaluated on a case-by-case basis for frequency and intensity.	Lung, incidence	Exposure to AN (OR):			Centre, sex, age, tobacco consumption, vinyl chloride, styrene, carbon black, plastics pyrolysis products	*Exposure assessment critique*: Strengths were that exposure groups were based on exposure levels, and industrial hygienists reviewed training and reliability study; semiquantitative estimates had measurement units; the referent probably had no exposure to AN; and the potential for differential misclassification was unlikely. Limitations were that exposure was not well defined because cumulative exposure may not in fact have been cumulative exposure; multiple exposure assessors were used; and it was unclear whether historical changes were considered.
			Never	2822	1		
			Ever	39	2.2 (1.11–4.36)		
		Lung, incidence	Duration of AN exposure (OR):				
			Not exposed	2822	1		
			1–10 yr	17	4.76 (1.45–15.65)		
			11–18 yr	7	1.91 (0.44–8.31)		
			> 18 yr	15	1.65 (0.63–4.34)		
			Trend-test *P* value, 0.20				
		Lung, incidence	Weighted duration of AN exposure (based on the relative duration and frequency of exposure in each job) (OR):				
			Unexposed	2822	1		
			0.01–1.00 yr	13	2.03 (0.72–5.73)		
			1.01–2.25 yr	9	2.73 (0.73–10.2)		
			> 2.25 yr	17	2.91 (0.87–9.79)		
			Trend-test *P* value, 0.05				

Table 2.3 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Scélo et al. (2004) (cont.)		Lung, incidence	Cumulative AN exposure (OR):			Centre, sex, age, tobacco consumption, vinyl chloride, styrene, carbon black, plastics pyrolysis products	*Other strengths*: Large sample size. Sensitivity analyses to address possible selection bias (hospital vs population-based controls) which showed similar results to those presented. *Other limitations*: Co-exposures to styrene and vinyl chloride were high and not adjusted for; however, the Working Group noted that these two agents are not established lung carcinogens.
			Unexposed	2822	1		
			0.01–0.46 ppm-yr	13	2.03 (0.72–5.73)		
			0.47–1.61 ppm-yr	10	2.76 (0.68–11.22)		
			> 1.61 ppm-yr	16	2.87 (0.85–9.66)		
			Trend-test *P* value, 0.06				

AN, acrylonitrile; CI, confidence interval; HR, hazard ratio; HSE, Health and Safety Executive; HWSB, healthy-worker survivor bias; mo, month(s); NCI, National Cancer Institute; NC, not calculated; NR, not reported; OH, Ohio; OR, odds ratio; ppm, parts per million; RR, rate ratio; SC, South Carolina; SIR, standardized incidence ratio; SMR, standardized mortality ratio; UK, United Kingdom; US, United States; USA, United States of America; VA, Virginia; VCM, vinyl chloride monomer; vs, versus; yr, year(s).

downward biases due to HWE and HWSB and lack of quantitative exposure estimates.]

Thiess et al. (1980) conducted a mortality study among 1469 employees employed at 12 companies that processed acrylonitrile between 1954 and 1978 in Germany (see Section 2.1.4). SMRs were calculated using the population of the Rheinhessen-Pfalz as the referent. Some excesses in the SMRs were observed for lung cancer (SMR, [1.86]; 95% CI, [0.93–3.33]), including with increasing duration of exposure, but these were based on a total of 9 deaths. [The Working Group noted that no cumulative assessment of exposure to acrylonitrile was evaluated. Excess mortality estimates were probably affected by downward HWE and HWSB; however, several additional co-exposures were evident among workers, which may introduce bias away from the null.]

Waxweiler et al. (1981) evaluated mortality in a cohort of men ever employed at a synthetic chemicals plant in the USA who had been exposed to VCM. There was additional exposure to 19 other chemicals, including acrylonitrile, in the facility (see Section 2.1.5). The SMR for malignant diseases of the respiratory system was [1.49] (95% CI, [1.07–2.01]). [The Working Group noted that this study was minimally informative because of the limited assessment of exposure to acrylonitrile and the presence of co-exposures. Therefore, the excess mortality could not be confidently attributed to acrylonitrile.]

Benn and Osborne (1998), in an update to the study by Werner and Carter (1981), reported SMRs for trachea, bronchus, and lung cancer (53 total deaths) that were similar to the expected rates for workers involved in the polymerization of acrylonitrile and spinning of acrylic fibre in six facilities in the UK (SMR, [1.028]) (see Section 2.1.6). When workers were grouped into three exposure categories (high, possible, little or none), mortality from trachea, bronchus, and lung cancer was (SMR for high exposure, [1.411]; 95% CI, [0.93–2.05]; SMR for possible exposure, [0.526]; 95% CI, [0.21–1.08]; SMR for little exposure or none, [0.995]; 95% CI, [0.60–1.55]). Among workers exposed to high levels of acrylonitrile, some excesses were observed for workers in the age group 15–44 years (SMR, [6.098]; 95% CI, [1.98–14.2]; *P* for heterogeneity, 0.029) and for workers first exposed in 1969 or later (SMR, [2.703]; 95% CI, [1.09–5.57]; *P* for heterogeneity, 0.22), whereas other metrics related to time since first exposure and duration of potential exposure showed no consistent increases in mortality (*P* for trend, > 0.05). [The Working Group noted the greater excess mortality among workers with high acrylonitrile exposure compared with workers with none or possible exposure; however, the estimates were imprecise.]

Delzell and Monson (1982) analysed mortality among 327 male employees of a rubber-manufacturing plant in the USA who had potential exposure to acrylonitrile between 1940 and 1971 (see Section 2.1.7). Mortality rates among men working in specific departments (the "chemicals division") in the plant with exposure to acrylonitrile were compared with mortality rates in the USA and with mortality rates among other production workers not employed in the chemicals division. The SMR for lung cancer (ICD-7, 162, 163) among men with potential exposure was 1.5 (95% CI, 0.7–2.9; 9 observed deaths), compared with the US population. [The Working Group noted the imprecise effect estimates and probably downward biases due to HWSB and HWE.]

Swaen et al. (2004) (an update of the reports by Swaen et al., 1998 and Swaen et al., 1992), conducted a retrospective cohort study of 2842 men employed in one of eight chemical plants with exposure to acrylonitrile in the Netherlands (see Section 2.1.12). SMRs were calculated for the cohort overall, by category of cumulative exposure level categories (low, < 1 ppm-year; moderate, 1–10 ppm-years; and high, > 10 ppm-years), peak exposures occurring on a regular basis at least

once per week (< 10, 10–20, and > 20 ppm), respirator use (yes, no), and exposure to other carcinogenic agents (yes, no). SMRs were also calculated for a ninth plant considered "unexposed" to acrylonitrile, but direct comparison of the exposed and non-exposed groups was not conducted. A total of 67 deaths from lung cancer were observed; the SMRs for increasing cumulative exposure were: low, SMR, [0.921]; 95% CI, [0.369–1.890]; moderate, SMR, [1.065]; 95% CI, [0.746–1.474]; and high, SMR, [1.148]; 95% CI, [0.736–1.709]. [The Working Group noted that internal comparisons between the exposed and unexposed groups were not evaluated, despite the fact that the information was available to do so. The SMRs were probably biased downward, owing to the HWE and HWSB.]

Mastrangelo et al. (1993) conducted a retrospective cohort study in 671 workers with ≥ 12 months of exposure to acrylonitrile in an acrylic fibre factory in Italy (see Section 2.1.9). The reported SMR (regional referent) based on 2 deaths from lung cancer was [0.77] (95% CI, [0.09–2.77]). [The Working Group considered this study to be minimally informative because of the imprecise SMR estimates, lack of individual-level cumulative assessment of exposure to acrylonitrile (which may have resulted in nondifferential exposure misclassification), and downward biases due to HWE and HWSB.]

Geĭko et al. (1996) reported on a retrospective cohort of workers exposed to acrylonitrile (n = 239) for ≥ 3 years between 1938 and 1985 in the Russian Federation (see Section 2.1.10). These 239 workers were considered to be exposed on the basis of the primary product they were working with. Significant excess mortality was observed for lung cancer among men (SMR, [12.82]; $P < 0.05$), although the number of deaths was not provided. [The Working Group noted the unknown number of deaths and the limited characterization of cumulative exposure to acrylonitrile and considered that this study was minimally informative. Downward biases due to HWE and HWSB were considered likely.]

Koutros et al. (2019) (updated from Blair et al., 1998) reported on 808 observed bronchus and lung cancers, 559 of which were among workers exposed to acrylonitrile (see Section 2.1.11). Multivariable-adjusted HRs were significantly elevated in the highest quintile of cumulative exposure (quintile 5, > 12.08 ppm-years) – quintile 5 versus unexposed, lagged 10-years for the full cohort, HR, 1.43 (95% CI, 1.13–1.81; P for trend, 0.05) – but not for 10-year lagged average intensity or duration of exposure. There was a consistent increasing but non-monotonic trend in risk across the full range of cumulative exposure (for ≥ 32 ppm-years versus unexposed, HR, 1.47; 95% CI, 1.07–2.02; P for trend, 0.02). Sensitivity analyses for lung cancer in which active workers were censored after 1983 (the date of last job record) resulted in HRs that were higher in magnitude for the relation between cumulative acrylonitrile lagged 10 years and lung cancer mortality: with censoring, for ≥ 32 ppm-years versus unexposed, HR, 1.64 (95% CI, 1.11–2.42; P for trend, 0.04).

Among workers who had zero probability of being exposed to asbestos, Koutros et al. (2019) found that the HR for lung cancer in the top quintile of cumulative acrylonitrile exposure was 1.63 (95% CI, 1.17–2.26). The null exposure–response relation between acrylonitrile and mesothelioma also offered supportive evidence of limited confounding by asbestos. There was no correlation between acrylonitrile exposure levels and smoking information (correlation coefficient, 0.07), and smoking-adjusted HRs for acrylonitrile exposure and lung cancer were similar to the HRs that were not adjusted for smoking. The observed SMR for lung cancer (SMR, 0.88; 95% CI, 0.81–0.96) was higher than that for chronic obstructive pulmonary disease (SMR, 0.68), which also provides evidence against positive confounding by smoking.

Using the same data as those from the cohort reported by Koutros et al. (2019), Keil et al. (2024) evaluated the potential for HWSB and adjusted for this bias when assessing expected lung cancer mortality under different hypothetical occupational exposure limits. Employment was associated with lung cancer mortality (HR for leaving work in the previous 2 years, 1.59; 95% CI, 1.02–2.47), and acrylonitrile exposure was associated with leaving employment (HR per ppm, 1.03; 95% CI, 1.02–1.04), showing that conditions for HWSB were present, which could have led to underestimation of exposure–response coefficients. Using the parametric g-formula, which can adjust for HWSB, under a hypothetical intervention to eliminate exposure, lung cancer mortality per 1000 workers would be expected to drop from 71.7 to 64.4 deaths, leading to 7.21 (95% CI, 2.72–1.70) lung cancer deaths delayed or prevented by age 90 years by eliminating acrylonitrile exposure. [The Working Group noted that HWSB probably led to an underestimation of the true risk in the analysis by Koutros et al. (2019). After adjusting for HWSB, the findings supported higher mortality from lung cancer at higher acrylonitrile exposures, compared with lower (Keil et al., 2024).] [The Working Group regarded this study as being the most informative because of its large size and the fact that it included a high-quality, quantitative, retrospective exposure assessment component that was developed using historical exposure estimates for each job, department, and plant combination by time period. The positive exposure–response relations that were observed were robust and appeared not to be confounded by cigarette smoking or co-exposures, and they were strengthened after considering the impact of the HWSB.]

Marsh and Zimmerman (2015) reported on an industry-sponsored extended follow-up of a cohort of acrylonitrile-manufacturing workers in the USA (see Section 2.1.13). In the original study by Marsh et al. (1999), quantitative, time-dependent historical estimates of acrylonitrile exposure were calculated; this study included 894/992 participants who were also included in the studies by Koutros et al. (2019) and Blair et al. (1998). The cohort was expanded once in 2002 (Marsh et al., 2002) and then again in 2015, when industrial hygienists and human resources representatives classified every job into one of 20 job groups on the basis of work performed and job type to extend the work history information (1955–2011). External and internal comparisons for lung cancer included a total of 44 deaths (15 in acrylonitrile-exposed workers) and did not reveal any trends in risk by duration, intensity, or cumulative exposure to acrylonitrile (all $P > 0.05$). [The Working Group noted the changing assessment of exposure over time, and that missing exposure information for some periods may have introduced nondifferential exposure misclassification. In addition, risk estimates were imprecise, and the results were not independent of those in the study described by Koutros et al. (2019), as 38 of the 44 deaths were included in the study by Koutros et al.]

Budroni et al. (2010) reported on a retrospective cohort study of 5350 (2336 acrylonitrile-exposed) male petrochemical workers in the industrial area of Porto Torres, Sardinia, Italy, followed for cancer incidence between 1990 and 2006 (see Section 2.1.15). Among workers exposed to acrylonitrile, the SIR for lung cancer was 1.03 (95% CI, 0.76–1.14; 41 observed cases). [The Working Group considered this study to be minimally informative because of bias towards the null from nondifferential exposure misclassification, downward biases due to HWE and HWSB, and potential bias away from the null owing to lack of adjustment for carcinogenic co-exposures.]

Scélo et al. (2004) conducted a large case–control study of 2861 cases of incident lung cancer and 3118 controls from seven European countries. Except for two centres where population controls were used, the (hospital) controls were patients without cancer or tobacco-related diseases who

were recruited from public hospitals serving the same areas as those from which the cases were identified. Detailed occupational questionnaires were administered to living case participants, and local experts assessed exposure to several occupational agents, including acrylonitrile, for each job held. Lung cancer was found to be associated with exposure to acrylonitrile among 39 cases and 20 controls with ever exposure to acrylonitrile (OR, 2.20; 95% CI, 1.11–4.36). A positive linear trend in ORs was observed for weighted duration and cumulative exposure to acrylonitrile ($P = 0.05$ and $P = 0.06$, respectively). Using a 20-year lag did not appreciably change the results (results for the 20-year lag were not reported in the original publication). Results were adjusted for centre, sex, age, tobacco consumption, vinyl chloride, styrene, carbon black, plastics pyrolysis products, and were robust to sensitivity analyses (results not shown). [The Working Group noted that this study included a large, detailed analysis of exposed and unexposed participants and a semiquantitative assessment of exposure, and reported an exposure–response relation with lung cancer after adjustment for smoking and co-exposures. The strengths included a sensitivity analysis to address possible selection bias (hospital versus population-based controls); although not presented, the results were similar to those described. Co-exposures to styrene and vinyl chloride were high; however, the Working Group noted that these two agents have not been identified as lung carcinogens. Apart from the fact that acrylonitrile exposures occurred during the manufacture of rubber, plastics, and footwear, measurement data were not available to offer a direct contrast between levels in this study and those reported in other occupational cohorts exposed to acrylonitrile. The study had potential downward biases due to HWE and HWSB. The Working Group considered that this study was moderately informative.]

2.2.2 Cancer of the larynx

There were three studies on mortality (Swaen et al., 2004, 4 deaths; and Marsh and Zimmerman, 2015, 2 deaths) or incidence (Budroni et al., 2010, 5 observed cases) from cancer of the larynx in workers. The numbers of reported deaths or cases observed were compared with those in the respective general populations in the study regions and were found to be comparable in all instances reported. [The Working Group noted that the estimated SMRs or SIRs were imprecise, which precluded any meaningful assessment of the relation between cancer of the larynx and acrylonitrile exposure.]

2.2.3 Mesothelioma

The study by Koutros et al. (2019) was the only one to have reported on the relation between acrylonitrile and mesothelioma (see Section 2.1.11). An excess in deaths from mesothelioma among acrylonitrile-exposed workers (SMR, 2.36; 95% CI, 1.35–3.83) was reported, but internal analyses using quantitative estimates of acrylonitrile exposure showed no exposure–response relation for duration of exposure (P for trend, 0.20), 10-year lagged average exposure (P for trend, 0.79), or 10-year lagged cumulative exposure (P for trend, 0.84), with confidence intervals for all adjusted HRs including 1.0.

2.3 Cancers of the digestive system and genitourinary system, and other solid cancers

See Table 2.4.

Thirteen cohort or nested case–control studies and two case–control studies (Kauppinen et al., 1995; Karami et al., 2011) examined the association between acrylonitrile exposure and cancers of the digestive system and genitourinary system, and other solid cancers. Most of the cohort studies reported on mortality among

Table 2.4 Epidemiological studies on exposure to acrylonitrile and cancers of the digestive system and genitourinary system, and other solid cancers

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kiesselbach et al. (1979) Germany 1950 to 1 August 1977 Cohort	884 male employees of a plant in Leverkusen who were exposed to AN (handled AN for ≥ 1 yr in production or processing) between 1950 and 1 August 1977. Some of these men handled butadiene–styrene. Expected deaths from North Rhine-Westphalia rates. Outcome data sources were not reported. Exposure assessment method: See Table 2.1.	Stomach, mortality	SMR (North Rhine-Westphalia referent): Overall cohort	4	[1.18 (0.32–3.02)]	Age, calendar period	*Strengths*: See Table 2.1. *Limitations*: See Table 2.1.
Symons et al. (2008) Waynesboro (VA) and Camden (SC), USA Enrolment, 1947–1991/follow-up, through 2002 Cohort	2548 male workers who were exposed to AN for ≥ 6 mo in two company facilities (Waynesboro and Camden) manufacturing acrylic fibre, in 1947–1991. Exposure assessment method: See Table 2.1.	Colon and rectum, mortality	SMR (US referent): All exposed workers	28	[0.85 (0.57–1.24)]	Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
		Colon and rectum, mortality	SMR (other employees referent): All exposed workers	28	[1.11 (0.74–1.61)]		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Symons et al. (2008) (cont.)		Colon and rectum, mortality	Cumulative AN exposure (HR):			Birth period, employment in SC start-up group, age	
			Continuous (per 100 ppm-yr), unlagged	28	1.16 (0.75–1.81)		
			Continuous (per 100 ppm-yr), 5-yr lag	26	1.06 (0.64–1.76)		
			Continuous (per 100 ppm-yr), 10-yr lag	24	0.9 (0.49–1.66)		
			Continuous (per 100 ppm-yr), 15-yr lag	23	0.81 (0.4–1.66)		
		Colon and rectum, mortality	Mean AN exposure intensity, men with cumulative exposure > 10 ppm-yr (HR):				
			< 10 ppm	9	1		
			≥ 10 ppm	15	0.96 (0.41–2.28)		
		Bladder and kidney, mortality	SMR (US referent):			Age, calendar period	
			Total cohort	16	[1 (0.57–1.62)]		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Symons et al. (2008) (cont.)		Bladder and kidney, mortality	Cumulative AN exposure (HR):			Birth period, employment in SC start-up group, age	
			Continuous (per 100 ppm-yr), unlagged	16	0.98 (0.53–1.79)		
			Continuous (per 100 ppm-yr), 5-yr lag	13	1.27 (0.67–2.43)		
			Continuous (per 100 ppm-yr), 10-yr lag	13	1.29 (0.63–2.67)		
			Continuous (per 100 ppm-yr), 15-yr lag	13	1.1 (0.47–2.57)		
		Bladder and kidney, mortality	Mean AN exposure intensity, men with cumulative exposure > 10 ppm-yr (HR):				
			< 10 ppm	5	1		
			≥ 10 ppm	8	0.74 (0.23–2.39)		
		Prostate, mortality	SMR (US referent):			Age, calendar period	
			Total cohort	25	[0.91 (0.59–1.35)]		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Symons et al. (2008) (cont.)		Prostate, mortality	Cumulative AN exposure (HR):			Birth period, employment in SC start-up group, age	
			Continuous (per 100 ppm-yr), unlagged	25	0.78 (0.46–1.32)		
			Continuous (per 100 ppm-yr), 5-yr lag	22	0.86 (0.48–1.53)		
			Continuous (per 100 ppm-yr), 10-yr lag	19	0.98 (0.5–1.92)		
			Continuous (per 100 ppm-yr), 15-yr lag	15	0.95 (0.41–2.22)		
		Prostate, mortality	Mean AN exposure intensity, men with cumulative exposure > 10 ppm-yr (HR):				
			< 10 ppm	6	1		
			≥ 10 ppm	16	1.45 (0.56–3.81)		
		Brain: and CNS, mortality	SMR (US referent):			Age, calendar period	
			Total cohort	6	[0.71 (0.26–1.54)]		
		Brain: and CNS, mortality	Cumulative AN exposure, 5-yr lag (HR):			Birth period, employment in SC start-up group, age	
			Continuous (per 100 ppm-yr)	4	1.38 (0.43–4.47)		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ott et al. (1980) USA Enrolment, 1937–1970/follow-up, 1940–1975 Cohort	2904 (2740 without exposure to arsenicals, asbestos, or vinyl chloride); employed for ≥ 1 yr in the manufacture of styrene-based products between 1937 and 1970. Exposure assessment method: See Table 2.1.	Digestive organs and peritoneum, mortality	SMR (US White male referent): Cohort, less workers with arsenicals, asbestos, or high vinyl chloride exposure	17	[0.8 (0.46–1.28)]	Age, calendar period	*Strengths*: See Table 2.1. *Limitations*: See Table 2.1.
		Genital organs, mortality	SMR (US White male referent): Cohort, less workers with arsenicals, asbestos, or high vinyl chloride exposure	2	[0.44 (0.05–1.61)]		
		Urinary organs, mortality	SMR (US White male referent): Cohort, less workers with arsenicals, asbestos, or high vinyl chloride exposure	2	[0.43 (0.05–1.54)]		
Thiess et al. (1980) Germany Enrolment, varies/follow-up, through 15 May 1978 Cohort	1469; workers employed for ≥ 6 mo at 12 acrylonitrile-using plants from 1954 through 15 May 1978. Exposure assessment method: See Table 2.1.	Oesophagus, mortality	SMR (Rheinhessen-Pfalz referent): Total cohort	1	[1.79 (0.05–9.97)]	Age	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
			Excluding plant 5: exposure duration ≥ 10 yr	1	[7.94 (0.2–44.2)]		
		Stomach, mortality	SMR (Rheinhessen-Pfalz referent): Total cohort	3	[1.05 (0.22–3.08)]		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Thiess et al. (1980) (cont.)		Colon, mortality	SMR (Rheinhessen-Pfalz referent):			Age	
			Total cohort	1	[0.76 (0.02–4.25)]		
			Excluding plant 5: exposure duration 5–9 yr	1	[4.41 (0.11–24.5)]		
		Rectum, mortality	SMR (Rheinhessen-Pfalz referent):				
			Total cohort	2	[1.79 (0.22–6.47)]		
			Excluding plant 5: exposure duration 5–9 yr	1	[5.1 (0.13–28.4)]		
		Liver, mortality	SMR (Rheinhessen-Pfalz referent):				
			Total cohort	1	[2.38 (0.06–13.2)]		
			Excluding plant 5: exposure duration 5–9 yr	1	[13.9 (0.35–77.4)]		
		Urinary bladder, mortality	SMR (Rheinhessen-Pfalz referent):				
			Total cohort	2	[3.1 (0.38–11.2)]		
Benn and Osborne (1998) England, Wales, Scotland, Northern Ireland Enrolment, phase I (1950–1968); phase II (1969–1978)/follow-up, through 1991 Cohort	2763 male workers employed for ≥ 1 yr and exposed to polymerization of AN or the spinning of acrylic fibre in six factories in the UK identified within a study conducted by the HSE and the Chemical Industries Association. Exposure assessment method: See Table 2.1.	Stomach, mortality	SMR (national referent):			Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
			Total cohort	11	[0.962 (0.48–1.72)]		
			Excluding workers from factory 5	7	[1.772 (0.71–3.65)]		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Benn and Osborne (1998) (cont.)		Stomach, mortality	Highest AN exposure level (SMR, national referent):			Age, calendar period	
			No or little	1	[0.232 (0.01–1.29)]		
			Possible	3	[1.027 (0.21–3)]		
			High	7	[1.663 (0.67–3.43)]		
		Stomach, mortality	Duration of exposure, workers exposed to high AN levels (SMR, national referent):				
			< 5 yr	5	[3.165 (1.03–7.39)]		
			5–10 yr	0	[0 (0–3.07)]		
			10–15 yr	1	[1.01 (0.03–5.63)]		
			> 15 yr	1	[2.439 (0.06–13.6)]		
		Large intestine, mortality	SMR (national referent):				
			Total cohort	11	[1.257 (0.63–2.25)]		
		Rectum, mortality	SMR (national referent):				
			Total cohort	6	[1.002 (0.37–2.18)]		
		Genitourinary organs, mortality	SMR (national referent):				
			Total cohort	12	[0.808 (0.42–1.41)]		
Delzell and Monson (1982) Akron (OH), USA Enrolment, 1940–1971/follow-up, 1940 through 1 July 1978 Cohort	327 White male production workers employed for ≥ 2 yr between 1940–1971 at a rubber-manufacturing plant in Akron who had worked in the chemicals division in Departments 5578 or 5579 with potential AN exposure. Exposure assessment method: See Table 2.1.	Digestive organs and peritoneum, mortality	SMR (US referent):			Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
			Total cohort	4	0.8 (0.2–2)		
		Urinary bladder, mortality	SMR (US referent):				
			Total cohort	2	4 (0.5–14.5)		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Swaen et al. (2004) Netherlands Enrolment, varies by plant starting 1959–1973/follow-up, through 1 January 2001 Cohort	2842 men, citizens of the Netherlands, working in eight chemical companies with AN exposure for > 6 mo. Exposure assessment method: See Table 2.1.	Oesophagus, mortality	SMR (national referent): Total cohort	2	[0.377 (0.042–1.315)]	Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
		Stomach and small intestine, mortality	SMR (national referent): Total cohort	4	[0.37 (0.1–0.938)]		
		Large intestine, mortality	SMR (national referent): Total cohort	12	[1.053 (0.543–1.836)]		
		Rectum, mortality	SMR (national referent): Total cohort	5	[1.22 (0.393–2.824)]		
		Liver and bile ducts, mortality	SMR (national referent): Total cohort	4	[1.38 (0.371–3.491)]		
		Pancreas, mortality	SMR (national referent): Total cohort	3	[0.411 (0.083–1.179)]		
		Kidney, mortality	SMR (national referent): Total cohort	2	[0.392 (0.044–1.367)]		
		Urinary bladder, mortality	SMR (national referent): Total cohort	5	[1.087 (0.35–2.517)]		
		Prostate, mortality	SMR (national referent): Total cohort	8	[0.92 (0.396–1.806)]		
		Male genital organs, mortality	SMR (national referent): Total cohort	1	[1.111 (0.015–5.614)]		
		Brain, mortality	SMR (national referent): Total cohort	6	[1.25 (0.456–2.706)]		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mastrangelo et al. (1993) Italy Enrolment, 1959–1988/follow-up, 1959–1990 Cohort	671 men employed for ≥ 12 mo at any time since the opening of the factory in 1959. Clerks and some workers with past exposure to vinyl chloride monomer or to benzidine and its compounds were excluded, as were workers employed after 31 December 1988. Exposure assessment method: See Table 2.1.	Stomach, mortality	SMR (regional referent):			Age, calendar period	*Strengths*: See Table 2.1. *Limitations*: See Table 2.1.
			Total cohort	2	[3.4 (0.4–12)]		
		Intestine and colon, mortality	SMR (regional referent)				
			Total cohort	4	[10.5 (2.9–27)]		
		Rectum, mortality	SMR (regional referent):				
			Total cohort	1	[5.6 (0.1–31)]		
		Testis, mortality	SMR (regional referent):				
			Total cohort	1	[8.3 (0.2–46)]		
		Brain, mortality	SMR (regional referent):				
			Total cohort	1	[2.6 (0.1–15)]		
Geĭko et al. (1996) Russian Federation Enrolment, 1938–1985/follow-up, through 1985 Cohort	239; workers exposed to AN for ≥ 3 yr between 1938 and 1985. Exposure assessment method: See Table 2.1.	Gastrointestinal cancers combined, mortality	SMR (reference population: city of Dzerzhinsk):			Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1. *Other comments*: ICD-9 code 192 was reportedly used for brain.
			Total cohort	NR	[3.425 (1.64–6.3)]		
		Stomach, mortality	SMR (reference population: city of Dzerzhinsk):				
			Total cohort	NR	[3.704 (1.6–7.3)]		
			AN-exposed, 10–14 yr	NR	[8.065]		
		Liver, mortality	SMR (reference population: city of Dzerzhinsk):				
			Total cohort	NR	[10 (0.25–55.7)]		
		Pancreas, mortality	SMR (reference population: city of Dzerzhinsk):				
			Total cohort	NR	[5.556 (0.14–30.95)]		
		Brain, mortality	SMR (reference population: city of Dzerzhinsk):				
			Total cohort	NR	[5.556 (0.14–30.95)]		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2019) USA Enrolment, 1952–1983/follow-up, through 2011 Cohort	25 460 (16 889 AN-exposed); all workers employed ≥ 1 day at any of the eight participating plants after the start of AN operations (1952–1965). Work histories covered the date of hire (i.e. 1942–1983) through date of record abstraction at the plant (1983). Exposure assessment method: See Table 2.1.	Digestive system, mortality	SMR (US referent):			Sex, race, age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
			Total cohort	555	0.83 (0.76–0.90)		
			AN-exposed	369	0.81 (0.73–0.90)		
		Oesophagus, mortality	SMR (US referent):				
			Total cohort	81	0.95 (0.76–1.18)		
			AN-exposed	57	0.93 (0.71–1.21)		
		Oesophagus, mortality	Cumulative AN exposure (through 1983), 10-yr lag (HR):			Age, race, sex, birth year, salary–wage classification	
			Unexposed	NR	1		
			Tertile 1	NR	1.03 (0.56–1.90)		
			Tertile 2	NR	0.84 (0.46–1.53)		
			Tertile 3	NR	0.94 (0.51–1.75)		
			Trend-test *P* value, 0.68				
		Stomach, mortality	Cumulative AN exposure (through 1983), 10-yr lag (HR):				
			Unexposed	NR	1		
			≤ Median	NR	0.97 (0.47–2.02)		
			> Median	NR	0.84 (0.4–1.79)		
			Trend-test *P* value, 0.66				
		Colon and rectum, mortality	Cumulative AN exposure (through 1983), 10-yr lag (HR):				
			Unexposed	NR	1		
			Quintile 1	NR	1.12 (0.72–1.75)		
			Quintile 2	NR	0.98 (0.62–1.53)		
			Quintile 3	NR	1.08 (0.69–1.68)		
			Quintile 4	NR	0.76 (0.49–1.20)		
			Quintile 5	NR	0.71 (0.45–1.11)		
			Trend-test *P* value, 0.09				
		Liver, mortality	SMR (US referent):			Sex, race, age, calendar period	
			AN-exposed	36	0.7 (0.49–0.97)		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2019) (cont.)		Liver, mortality	Cumulative AN exposure (through 1983), 10-yr lag (HR):			Age, race, sex, birth year, salary–wage classification	
			Unexposed	NR	1		
			≤ Median	NR	0.98 (0.47–2.04)		
			> Median	NR	0.68 (0.32–1.43)		
			Trend-test *P* value, 0.30				
		Pancreas, mortality	Cumulative exposure lagged 10 yr (HR):				
			Unexposed	NR	1		
			Tertile 1	NR	1.04 (0.64–1.71)		
			Tertile 2	NR	0.85 (0.52–1.41)		
			Tertile 3	NR	1.23 (0.74–2.04)		
			Trend-test *P* value, 0.70				
		Urinary system, mortality	SMR (US referent):			Sex, race, age, calendar period	
			Total cohort	123	0.89 (0.74–1.06)		
			AN-exposed	85	0.88 (0.71–1.09)		
		Kidney and renal pelvis, mortality	SMR (US referent):				
			AN-exposed	45	0.92 (0.67–1.24)		
		Kidney and renal pelvis, mortality	Cumulative AN exposure (through 1983), 10-yr lag (HR):			Age, race, sex, birth year, salary–wage classification	
			Unexposed	NR	1		
			Tertile 1	NR	1 (0.50–2.00)		
			Tertile 2	NR	1.43 (0.71–2.88)		
			Tertile 3	NR	1.17 (0.57–2.41)		
			Trend-test *P* value, 0.47				
		Urinary bladder, mortality	SMR (US referent):			Sex, race, age, calendar period	
			AN-exposed	39	0.86 (0.61–1.17)		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2019) (cont.)		Urinary bladder, mortality	Cumulative AN exposure (through 1983), 10-yr lag (HR):			Age, race, sex, birth year, salary–wage classification	
			Unexposed (0 ppm-yr)	16	1		
			Tertile 1 (0–0.37 ppm-yr)	13	0.94 (0.45–1.99)		
			Tertile 2 (> 0.37 to 6.69 ppm-yr)	13	0.78 (0.37–1.65)		
			Tertile 3 (> 6.69 ppm-yr)	13	1.45 (0.69–3.08)		
			Trend-test *P* value, 0.56				
		Urinary bladder, mortality	Average AN exposure (through 1983), 10-yr lag (HR):				
			Unexposed	16	1		
			Tertile 1 (0–0.26 ppm)	13	0.63 (0.30–1.32)		
			Tertile 2 (> 0.26 to 2.56 ppm)	13	0.94 (0.45–1.99)		
			Tertile 3 (> 2.56 ppm)	13	2.96 (1.38–6.34)		
			Trend-test *P* value, 0.02				
		Urinary bladder (including both underlying and contributing causes of death), mortality	Average AN exposure (through 1983), 10-yr lag (HR):				
			Unexposed	NR	1		
			Tertile 3 (> 2.56 ppm)	NR	2.62 (1.33–5.15)		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2019) (cont.)		Urinary bladder, mortality	Duration of exposure (through 1983) (HR):			Age, race, sex, birth year, salary–wage classification	
			Unexposed	16	1		
			Tertile 1 (0–1.37 yr)	13	1.29 (0.61–2.74)		
			Tertile 2 (> 1.37 to 7.66 yr)	13	0.96 (0.46–2.02)		
			Tertile 3 (> 7.66 yr)	13	0.73 (0.35–1.54)		
			Trend-test *P* value, 0.33				
		Prostate, mortality	SMR (US referent):			Race, age, calendar period	
			AN-exposed	125	0.98 (0.81–1.16)		
		Prostate, mortality	Cumulative AN exposure (through 1983), 10-yr lag (HR):			Age, race, birth year, salary–wage classification	
			Unexposed	NR	1		
			Quintile 1	NR	1.43 (0.87–2.35)		
			Quintile 2	NR	0.9 (0.56–1.46)		
			Quintile 3	NR	0.76 (0.46–1.24)		
			Quintile 4	NR	1.64 (1.00–2.67)		
			Quintile 5	NR	0.94 (0.57–1.54)		
			Trend-test *P* value, 0.94				
		Brain, mortality	SMR (US referent):			Sex, race, age, calendar period	
			Total cohort	80	1.05 (0.83–1.31)		
			AN-exposed	48	0.94 (0.69–1.25)		
		Brain, mortality	Cumulative AN exposure (through 1983), 10-yr lag (HR):			Age, race, sex, birth year, salary–wage classification	
			Unexposed	NR	1		
			Tertile 1	NR	0.86 (0.46–1.60)		
			Tertile 2	NR	0.81 (0.43–1.50)		
			Tertile 3	NR	0.83 (0.44–1.56)		
			Trend-test *P* value, 0.50				

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2019) (cont.)		Breast, mortality	SMR (US referent):			Sex, race, age, calendar period	
			Total cohort	73	0.86 (0.68–1.08)		
			AN-exposed	16	0.59 (0.34–0.95)		
		Breast, mortality	Cumulative AN exposure (through 1983), 10-yr lag (HR):			Age, race, sex, birth year, salary–wage classification	
			Unexposed	NR	1		
			≤ Median	NR	0.63 (0.29–1.35)		
			> Median	NR	0.93 (0.44–1.98)		
			Trend-test *P* value, 0.52				
		Melanoma, mortality	SMR (US referent):			Sex, race, age, calendar period	
			AN-exposed	33	0.96 (0.66–1.34)		
		Melanoma, mortality	Cumulative AN exposure (through 1983), 10-yr lag (HR):			Age, race, sex, birth year, salary–wage classification	
			Unexposed	NR	1		
			≤ Median	NR	1.25 (0.62–2.52)		
			> Median	NR	0.97 (0.48–1.99)		
			Trend-test *P* value, 0.94				
Marsh and Zimmerman (2015) Lima (OH), USA Enrolment, starting in 1955/follow-up, 1955–2011 Cohort	2096 (789 with AN exposure) employees (any time length) in Lima factory between 1955 and 2011. The Lima site was included in the study by Koutros et al. (2019), but the overlap between the two Lima cohorts was only partial. Exposure assessment method: See Table 2.1.	Oesophagus, mortality	Exposure group (SMR, county referent):			Sex, race, age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths* and limitations: See Table 2.1. *Other comments*: It was unclear which reference population was used for the female component.
			Unexposed	3	0.9 (0.19–2.62)		
			AN-exposed	2	0.84 (0.1–3.02)		
		Stomach, mortality	Exposure group (SMR, county referent):				
			Unexposed	1	0.51 (0.01–2.84)		
			AN-exposed	2	1.54 (0.19–5.56)		
		Large intestine (ICD-9, 153), mortality	Exposure group (SMR, county referent):				
			Unexposed	7	0.71 (0.29–1.47)		
			AN-exposed	3	0.59 (0.12–1.71)		
		Rectum, mortality	Exposure group (SMR, county referent):				
			Unexposed	1	0.56 (0.01–3.12)		
			AN-exposed	1	1 (0.03–5.59)		
		Liver and bile ducts, mortality	Exposure group (SMR, county referent):				
			Unexposed	3	1.29 (0.27–3.76)		
			AN-exposed	2	1.41 (0.17–5.1)		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Marsh and Zimmerman (2015) (cont.)		Pancreas, mortality	Exposure group (SMR, county referent):			Sex, race, age, calendar period	
			Unexposed	11	1.96 (0.98–3.5)		
			AN-exposed	1	0.32 (0.01–1.77)		
		Kidney, mortality	Exposure group (SMR, county referent):				
			Unexposed	1	0.34 (0.01–1.89)		
			AN-exposed	1	0.57 (0.01–3.18)		
		Urinary bladder, mortality	Exposure group (SMR, county referent):				
			Unexposed	5	1.82 (0.59–4.24)		
			AN-exposed	4	2.27 (0.62–5.8)		
		Prostate, mortality	Exposure group (SMR, county referent):			Race, age, calendar period	
			Unexposed	4	0.79 (0.21–2.01)		
			AN-exposed	5	1.32 (0.43–3.09)		
		Testis: and other male genital organs, mortality	Exposure group (SMR, county referent):				
			Unexposed	0	0 (0–14.49)		
			AN-exposed	1	4.97 (0.12–27.7)		
Budroni et al. (2010) Porto Torres, Sardinia, Italy 1990–2006 Cohort	5350 (2336 with AN exposure) male workers with ≥ 6 mo of employment in one or more chemical plants in the Porto Torres industrial area, working in the plants and alive as of 1 January 1990. Workers that joined between 1 January 1990 and 31 December 2001 were also included. Exposure assessment method: See Table 2.1.	Stomach, incidence	SIR (local referent):			Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
			AN-exposed	7	0.83 (0.39–1.74)		
		Colon and rectum, incidence	SIR (local referent):				
			AN-exposed	32	1.29 (0.92–1.83)		
		Liver and bile ducts, incidence	SIR (local referent):				
			AN-exposed	14	1.24 (0.73–2.09)		
		Kidney, incidence	SIR (local referent):				
			AN-exposed	6	0.64 (0.29–1.42)		
		Urinary bladder, incidence	SIR (local referent):				
			AN-exposed	24	1.26 (0.84–1.87)		
		Prostate, incidence	SIR (local referent):				
			AN-exposed	24	0.92 (0.62–1.38)		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Etemadi et al. (2024) Golestan, Islamic Republic of Iran Enrolment, January 2004 to June 2008/ follow-up, through 1 January 2018 Nested case–control	Cases: 205 oesophageal cancer cases identified through linkage with cancer registry and with available urine sample at baseline. Controls: 226 controls matched on age, sex, residence, time of enrolment in cohort, and tobacco use at baseline. Alive and free from cancer of the upper gastrointestinal tract (oesophageal and gastric) at the time of case diagnosis. Exposure assessment method: See Table 2.1.	Oesophagus (squamous cell carcinoma), incidence	Metabolites in urine (90th vs 10th percentile), not currently using tobacco at baseline (OR):			Age, sex, place of residence, time of enrolment, ethnicity, education, wealth score, BMI, tea temperature, tooth loss, opium use, urinary cotinine	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
			1CyHEMA [CHEMA]	148	2.1 (0.9–4.9)		
			2CyEMA [CEMA]	148	4.3 (1.4–13.5)		
			2HEMA [HEMA]	148	2.3 (1.1–5.1)		
		Oesophagus (squamous cell carcinoma), incidence	Metabolites in urine (90th vs 10th percentile), currently using tobacco at baseline (OR):				
			1CyHEMA [CHEMA]	57	1.2 (0.1–12.9)		
			2CyEMA [CEMA]	57	0.6 (0.1–5.8)		
			2HEMA [HEMA]	57	0.4 (0.1–2.4)		

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Thomas et al. (1987) North New Jersey, Philadelphia, Gulf Coast of Louisiana, USA 1 January 1978 to 30 June 1980 (Louisiana), 1979–1981 (New Jersey, Pennsylvania) Case–control	Cases: 300 White men aged ≥ 30 yr identified through death certificates with a diagnosis of glioblastoma multiforme, astrocytoma, or mixed glioma with astrocytic cells. Interviews were done with the next of kin (response rate, 74%). Controls: 386 White men who died for causes other than brain tumour, epilepsy, cerebrovascular diseases, suicide, homicide. Frequency-matched on age at death, year of death, study area. Interviews were done with the next of kin (response rate, 63%). Exposure assessment method: Work histories from next-of-kin interviews assessed by experts.	Astrocytic tumour, mortality	AN exposure (OR): Never Ever	 NR 27	 1 0.9 (0.5–1.6)	Study area, age, year of death, education, other exposures	*Exposure assessment critique*: Limitations included that work histories were collected from next of kin and included all jobs potentially exposed to AN, including agriculture where the probability of exposure may be low.
Kauppinen et al. (1995) Finland Diagnoses, 1984–1987, deaths through 1 April 1990 Case–control	Cases: 595; all people deceased by 1 April 1990 with primary exocrine pancreatic cancer diagnosed at age 40–74 yr between 1984 and 1987 in Finland, identified through Finnish Cancer Registry. Postal questionnaire sent to the next of kin to collect occupational history (response rate, 47%).	Pancreas, mortality	AN exposure (OR): Never exposed Potentially exposed	 NR 6	 1 2.1 (0.9–4.7)	Age, sex, smoking, history of diabetes, alcohol intake	*Exposure assessment critique*: Strengths were that exposure groups were based on exposure levels; there were two assessors after JEM use, with a 10-year induction period; probability, intensity and dermal exposure were assessed.

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kauppinen et al. (1995) (cont.)	Controls: 1622 deceased participants with stomach cancer, colon cancer, or rectal cancer diagnosed at age 40–74 yr between 1984 and 1987 in Finland, identified through Finnish Cancer Registry. Postal questionnaire sent to the next of kin to collect occupational history (response rate, 50%). Exposure assessment method: Community-based study of lifetime exposures through 1974 assessed using a UK JEM and then by industrial hygienists.						Limitations were that differential misclassification of exposure was likely; questionnaire was mailed to the next of kin; a UK JEM was used in this Finnish study population; it was unclear whether historical changes were considered; exposure categories were not well defined; the semiquantitative estimates lacked measurement units; and no study participant was considered probably exposed to AN and the findings were based on 6 cases (1.0%) and 5 controls (0.3%) considered to be potentially exposed to AN. *Other limitations*: Small sample size; no latency information. No adjustment for confounding. Several other carcinogenic co-exposures were present but not adjusted for.

Table 2.4 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Karami et al. (2011) Romania (Bucharest), Poland (Lodz), the Russian Federation (Moscow), Czechia August 1999 to January 2003 Case–control	Cases: 1097 newly diagnosed histologically confirmed cases of renal cell carcinoma (August 1999 to January 2003) in people aged 20–88 yr living in the study area for ≥ 1 yr. Response rate ranged from 90% to 99% between study areas. Controls: 1476 selected from patients admitted to hospitals for diagnosis unrelated to smoking/urological diseases (August 1998 to March 2003). No single disease accounted for > 20% of the controls. Frequency-matched on age, sex, place of residence. Response rate ranged from 90% to 96% between study areas. Exposure assessment method: Work histories including detailed exposure questions collected by interviews assessed by local occupational health experts and industrial hygienists.	Kidney (renal cell carcinoma), incidence Kidney (renal cell carcinoma), incidence	AN exposure (OR): Unexposed Exposed Cumulative AN exposure (OR): Unexposed < Median ≥ Median Trend-test *P* value, 0.06	 816 10 816 NR NR	 1 2.5 (0.9–7.1) 1 1.6 (0.4–6.4) 4.3 (0.9–22.1)	Age, sex, study centre, BMI, hypertension, smoking status, family history of cancer	*Exposure assessment critique*: Strengths were the detailed work histories with specialized work questionnaires administered by trained interviewers with expert assessment of exposures; and that the potential for differential unlikely, given that experts were blind to case–control status. *Other limitations*: Small number of exposed participants, with imprecise estimates.

AN, acrylonitrile; BMI, body mass index; CI, confidence interval; CNS, central nervous system; CHEMA, 1-cyano-2-hydroxyethylmercapturic acid; CEMA, 2-cyanoethylmercapturic acid; HEMA, *N*-acetyl-*S*-(2-hydroxyethyl)cysteine; HR, hazard ratio; HSE, Health and Safety Executive; HWSB, healthy-worker survivor bias; ICD, International Classification of Diseases; JEM, job-exposure matrix; mo, month(s); NC, not calculated; NR, not reported; OH, Ohio; OR, odds ratio; ppm, parts per million; SC, South Carolina; SIR, standardized incidence ratio; SMR, standardized mortality ratio; UK, United Kingdom; US, United States; USA, United States of America; VA, Virginia; vs, versus; yr, year(s).

workers with potential exposure to acrylonitrile compared with that of the general population and were generally without a detailed quantitative exposure assessment (Kiesselbach et al., 1979; Ott et al., 1980; Thiess et al., 1980; Delzell and Monson, 1982; Mastrangelo et al., 1993; Geĭko et al., 1996; Benn and Osborne, 1998; Swaen et al., 2004; Marsh and Zimmerman, 2015). [The Working Group noted that these studies compared observed deaths with external mortality rates, which may lead to an underestimation of the risk of cancer because of the HWE. There was also concern about the HWSB for those studies that had a minimum employment duration requirement of ≥ 6 months.] One cohort study (Budroni et al., 2010) reported the incidence of cancer among workers compared with the general population. [The Working Group noted that this study identified only a single exposure group.] In two studies, internal comparisons were conducted to evaluate quantitative metrics of exposure to acrylonitrile in exposed and unexposed workers (Symons et al., 2008; Koutros et al., 2019), but only Koutros et al. (2019) and Etemadi et al. (2024) considered the impact of confounding from tobacco smoking. [The Working Group prioritized the studies with internal comparisons and exposure assessment in its evaluation of digestive system, genitourinary system, and other solid cancers. The Working Group also noted that HWSB was likely in all these occupational studies.]

2.3.1 Cancers of the digestive system

(a) Cohort studies

Kiesselbach et al. (1979) (see Section 2.1.1) reported stomach cancer findings in a cohort of male employees exposed to acrylonitrile in a plant in Germany (SMR, [1.18]; 95% CI, [0.32–3.02]). [The Working Group noted that the imprecise estimates were based on 4 deaths and were minimally informative.]

Symons et al. (2008) (see Section 2.1.2) reported findings for colorectal cancer mortality in a cohort of male workers exposed to acrylonitrile for ≥ 6 months in two acrylic fibre facilities, using as referent either the US population (SMR, [0.85]; 95% CI, [0.57–1.24]) or a regional employee population from the company (SMR, [1.11]; 95% CI, [0.74–1.61]). The authors also reported the adjusted HRs for colorectal cancer mortality per increase of 100 ppm-year in cumulative exposure among all workers with exposure to acrylonitrile (HR, 1.16; 95% CI, 0.75–1.81) and among workers with cumulative exposure of > 10 ppm-years for a mean acrylonitrile exposure intensity of ≥ 10 ppm (HR, 0.96; 95% CI, 0.41–2.28) compared with < 10 ppm.

Ott et al. (1980) (see Section 2.1.3) reported findings for malignant diseases of the digestive system and peritoneum in a cohort of men employed for ≥ 1 year in the manufacture of styrene-based products in facilities in the USA, excluding workers with exposure to arsenicals, asbestos, or high levels of vinyl chloride (SMR, [0.80]; 95% CI, [0.46–1.28]. [The Working Group noted that this study was minimally informative because of imprecise estimates and downward biases due to HWE and HWSB.]

Thiess et al. (1980) (see Section 2.1.4) reported SMRs for cancers of the stomach (SMR, [1.05]; 95% CI, [0.22, 3.08]), oesophagus (SMR, [1.79]; 95% CI, [0.05–9.97]), rectum (SMR, [1.79]; 95% CI, [0.22–6.47]), for colon cancer (SMR, [0.76]; 95% CI, [0.02–4.25]), among a cohort of workers at 12 companies that processed acrylonitrile in Germany, using the Rheinhessen-Pfalz region as the referent. In analyses that excluded plant 5, the authors reported SMRs for oesophageal cancer (SMR, [7.94]; 95% CI, [0.2–44.2]) among workers with ≥ 10 years of exposure, colon cancer (SMR, [4.41]; 95% CI, [0.11–24.5]), and rectal cancer (SMR, [5.10]; 95% CI, [0.13–28.4]) among workers with 5–9 years of exposure. [The Working Group considered that these findings were minimally informative since they were

based on only 3 deaths for stomach cancer, 2 deaths for rectal cancer, and 1 death for each of the other cancers. Furthermore, the study had limited exposure assessment of acrylonitrile exposure and multiple co-exposures. It lacked internal analyses and probably had downward biases due to HWE and HWSB.]

Benn and Osborne (1998) (see Section 2.1.6) reported SMRs for cancers of the stomach (SMR, [0.962]; 95% CI, [0.48–1.72]), rectum (SMR, [1.002]; 95% CI, [0.37–2.18]), and large intestine (SMR, [1.257]; 95% CI, [0.63–2.25]) in a cohort of men employed in six facilities involved in polymerization of acrylonitrile and spinning of acrylic fibre in the UK, using as referents the national death rates for the plants in England and Wales and Scottish death rates for the factory in Scotland. Workers from factory 5 had a deficit of all-cause mortality (SMR, [0.771]) compared with workers at the other factories (SMR, [0.979]); excluding workers from factory 5 showed a change in the SMR for stomach cancer (SMR, [1.772]; 95% CI, [0.71–3.65]). Among workers exposed to high levels of acrylonitrile, mortality from stomach cancer did not increase with duration of exposure [trend analyses were reported, but the Working Group did not consider them adequate, as they were based on SMRs, not direct internal comparisons.]

Delzell and Monson (1982) (see Section 2.1.7) reported findings for cancers of the digestive system and peritoneum among male workers employed for ≥ 2 years and ever employed in two departments that used acrylonitrile, taking the US population as the referent (SMR, 0.8; 95% CI, 0.2–2.0). [The Working Group noted the small study size and short follow-up period, as well as the inadequate assessment of exposure. The authors conducted internal analyses for White men employed in other company departments but did not report the results.]

Swaen et al. (2004) (see Section 2.1.12) reported mortality findings among workers considered to be exposed to acrylonitrile in eight chemical companies, using the population of the Netherlands as the referent. Analyses included cancers of the oesophagus (SMR, [0.377]; 95% CI, [0.042–1.315]), stomach and small intestine (SMR, [0.370]; 95% CI, [0.100–0.938]), large intestine (SMR, [1.053]; 95% CI, [0.543–1.836]), rectum (SMR, [1.220]; 95% CI, [0.393–2.824]), and pancreas (SMR, [0.411]; 95% CI, [0.083–1.179]). Stratified analyses by peak exposure, respirator use, and exposure to other carcinogens were not conducted for gastrointestinal cancers. [The Working Group noted that these estimates were based on small numbers of deaths. Although the assessment of acrylonitrile exposure was of good quality, it did not consider past exposures to known or potential human carcinogens. Downward biases from the HWE and HWSB were considered likely.]

Mastrangelo et al. (1993) (see Section 2.1.9) reported mortality findings in a cohort of men employed for ≥ 12 months in an acrylic fibre factory, using a regional referent. The results were shown for cancers of the stomach (SMR, [3.4]; 95% CI, [0.4–12]), intestine and colon (SMR, [10.5]; 95% CI, [2.9–27]), and rectum (SMR, [5.6]; 95% CI, [0.1–31]). [The Working Group noted as limitations that the results were based on 2 cases for stomach cancer, 4 cases for cancer of the intestine and colon, and 1 case for cancer of the rectum, there was no assessment of exposure to acrylonitrile; and downward biases from the HWE and HWSB were likely.]

Geĭko et al. (1996) (see Section 2.1.10) conducted a retrospective cohort study of workers exposed to acrylonitrile in the Russian Federation. Using the city of Dzerzhinsk as the referent, the authors reported excess mortality for total gastrointestinal cancer (SMR, [3.425]; 95% CI, [1.64–6.30]), primarily for stomach cancer (SMR, [3.704]; 95% CI, [1.60–7.30]). Among men with 10–14 years of exposure, excess mortality was observed for stomach cancer (SMR, [8.065]; $P < 0.05$). They also reported findings for pancreatic cancer, although the confidence intervals

were wide (SMR, [5.556]; 95% CI, [0.14–30.95]). [The Working Group noted that the number of deaths was not reported. In addition, the limited assessment of exposure to acrylonitrile rendered this study minimally informative.]

Koutros et al. (2019) (see Section 2.1.11) evaluated cancer mortality in a cohort of workers employed at eight acrylonitrile-producing facilities in the USA. The authors reported a deficit in oesophageal cancer mortality among workers with acrylonitrile exposure (SMR, 0.93; 95% CI, 0.71–1.21). In internal analyses using the Cox model with cumulative acrylonitrile exposure (through 1983) and a 10-year lag, no trends were observed for oesophageal cancer ($P = 0.68$), stomach cancer ($P = 0.66$), or pancreatic cancer ($P = 0.70$); and a downward trend with increasing exposure was observed for cancer of the colon and rectum ($P = 0.09$). [The Working Group noted that the strengths of the study included the large sample size and exposure assessment metrics. Since the exposure assessment was truncated to 1983, when approximately 30% of the study population was still employed, nondifferential exposure misclassification was possible. HWSB was likely for these outcomes, which were not examined using g-estimation.]

Marsh and Zimmerman (2015) (see Section 2.1.13) reported mortality findings for cancers of the oesophagus (SMR, 0.84; 95% CI, 0.10–3.02), large intestine (SMR, 0.59; 95% CI, 0.12–1.71), rectum (SMR, 1.00; 95% CI, 0.03–5.59), and pancreas (SMR, 0.32; 95% CI, 0.01–1.77) among workers considered to be exposed to acrylonitrile in an acrylonitrile-manufacturing plant in the USA, using local county rates as referents. [The Working Group noted the small number of exposed cancer deaths and considered that these findings were minimally informative.]

Budroni et al. (2010) (see Section 2.1.15) reported incidence findings for cancer of the colon and rectum (SIR, 1.29; 95% CI, 0.92–1.83) and for cancer of the stomach (SIR, 0.83; 95% CI, 0.39–1.74) among male petrochemical workers considered to be exposed to acrylonitrile in 43 facilities in Italy. [The Working Group noted that limitations in the exposure assessment constrained the informativeness of these findings.]

Etemadi et al. (2024) (see Section 2.1.16) reported on a case–control study on oesophageal cancer that was nested within a prospective population-based cohort in the Islamic Republic of Iran. Exposure was assessed using two urinary metabolites that are specific to acrylonitrile (CHEMA and CEMA) and a third metabolite (HEMA) that can be formed from the metabolism of both acrylonitrile and ethylene oxide. Among participants not using tobacco at baseline, the authors reported adjusted ORs, comparing the 90th versus the 10th percentile of metabolite concentration, for CHEMA (OR, 2.1; 95% CI, 0.9–4.9), CEMA (OR, 4.3; 95% CI, 1.4–13.5), and HEMA (OR, 2.3; 95% CI, 1.1–5.1). Corresponding ORs among participants using tobacco at baseline were also estimated for CHEMA (OR, 1.2; 95% CI, 0.1–12.9), CEMA (OR, 0.6; 95% CI, 0.1–5.8), and HEMA (OR, 0.4; 95% CI, 0.1–2.4). [The Working Group noted that the results for HEMA should be considered with caution because this metabolite is not specific to acrylonitrile. No information on an alternative source of acrylonitrile exposure was provided. Nonetheless, the Working Group noted the high-quality study design, although some risk estimates lacked precision.]

(b) Case–control studies

Kauppinen et al. (1995) reported the results of a population-based case–control study on pancreatic cancer and exposure to chemical and physical agents in Finland. The study included 595 deceased cases with primary exocrine pancreatic cancer diagnosed at age 40–74 years between 1984 and 1987 identified through the Finnish Cancer Registry. There were 1622 deceased controls with cancers of the stomach, colon, or rectum diagnosed at age 40–74 years

between 1984 and 1987, also identified through the Finnish Cancer Registry. Exposure was assessed through a questionnaire sent to the next of kin to obtain an occupational history. A JEM was used to code work histories into exposure indices for 50 agents, including acrylonitrile. The authors reported findings for pancreatic cancer among participants with potential acrylonitrile exposure (OR, 2.1; 95% CI, 0.9–4.7), after controlling for age, sex, smoking, history of diabetes, and alcohol intake. [The Working Group noted that no study participants were considered "probably exposed" to acrylonitrile and that the above findings were based on 6 cases (1.0%) and 5 controls (0.3%) considered to be potentially exposed to acrylonitrile while employed in the rubber industry. Differential misclassification of exposure was likely.]

2.3.2 Genitourinary cancers

There were nine cohort studies and one case–control study in which associations were reported between acrylonitrile exposure and genitourinary cancers.

(a) Cohort studies

Symons et al. (2008) (see Section 2.1.2) reported results for mortality from cancer of the urinary bladder and kidney in a cohort of male workers exposed to acrylonitrile for ≥ 6 months in two acrylic fibre facilities, using the US population as the referent (SMR, [1.00]; 95% CI, [0.57–1.62]). In addition, they reported internal analyses using the Cox model for bladder and kidney cancer mortality per 100 ppm-year increase in cumulative exposure among all workers using a 5-year lag (HR, 1.27; 95% CI, 0.67–2.43), and among workers with cumulative exposure of > 10 ppm-years for a mean acrylonitrile exposure intensity of ≥ 10 ppm (HR, 0.74; 95% CI, 0.23–2.39) compared with < 10 ppm. For prostate cancer, they reported an SMR of [0.91] (95% CI, [0.59–1.35]) compared with the US population. When assessing cumulative exposure within all workers, an HR of 1.45 (95% CI, 0.56–3.81) was observed for cumulative exposure of ≥ 10 ppm-year compared with < 10 ppm; and an HR of 0.86 (95% CI, 0.48–1.53) was observed per 100 ppm-year increase with a 5-year lag.

[The Working Group noted the same limitations as previously discussed for gastrointestinal cancer; in addition, bladder and kidney cancers were evaluated as one category, and estimates were imprecise since they were based on 16 deaths from both cancers. The Working Group considered that this study was minimally informative.]

Ott et al. (1980) (see Section 2.1.3) evaluated mortality in a cohort of men employed for ≥ 1 year in the manufacture of styrene-based products in facilities in the USA. The authors reported SMRs for malignant neoplasms of the genital organs, based on 2 deaths (SMR, [0.44]; 95% CI, [0.05–1.61]) and for urinary organs, based on 2 deaths (SMR, [0.43]; 95% CI, [0.05–1.54]). [The Working Group noted this study was minimally informative because of imprecise estimates.]

Thiess et al. (1980) (see Section 2.1.4) reported mortality findings for cancer of the bladder (SMR, [3.1]; 95% CI, [0.38–11.2]) among a cohort of workers employed for ≥ 6 months in 12 companies that used different methods to process acrylonitrile in Germany, using the Rheinhessen-Pfalz region as the referent. [The Working Group noted that this estimate was based on 2 deaths; additional limitations are described in Section 2.3.1(a).]

Benn and Osborne (1998) (see Section 2.1.6) reported an SMR for genitourinary cancer (SMR, [0.808]; 95% CI, [0.42–1.41]) in a cohort of men employed in six facilities involved in polymerization of acrylonitrile and spinning of acrylic fibre in the UK, using national death rates for England and Wales and Scottish death rates for the factory in Scotland. [The Working Group noted the previously addressed limitations of this study.]

Delzell and Monson (1982) (see Section 2.1.7) reported bladder cancer mortality results (SMR, 4.0; 95% CI, 0.5–14.5) among male workers employed for ≥ 2 years and ever employed in two departments that used acrylonitrile, using the US population as the referent. [The Working Group noted the imprecision of this estimate, which was based on 2 deaths, as well as additional limitations previously described (Section 2.3.1(a) and Section 2.1.7).]

Swaen et al. (2004) (see Section 2.1.12) reported mortality findings for cancers of the bladder (SMR, [1.087]; 95% CI, [0.350–2.517]), the male genital organs (SMR, [1.111]; 95% CI, [0.015–5.614]), kidney (SMR, [0.392]; 95% CI, [0.044–1.367]), and prostate (SMR, [0.920]; 95% CI, [0.396–1.806]) among workers considered to be exposed to acrylonitrile in eight chemical companies, using the population of the Netherlands as the referent. [The Working Group noted the imprecise estimates and other previously described limitations.]

Mastrangelo et al. (1993) (see Section 2.1.9) reported mortality findings for testicular cancer in a cohort of men employed for ≥ 12 months in an acrylic fibre factory, using a regional referent (SMR, [8.3]; 95% CI, [0.2–46)]). This result was based on one observation.

Koutros et al. (2019) (see Section 2.1.11) reported mortality findings for cancer of the urinary bladder among workers with acrylonitrile exposure (SMR, 0.86; 95% CI, 0.61–1.17) in a cohort of workers employed at eight acrylonitrile-producing facilities in the USA, using the US population as the referent. In internal analyses using the Cox model, a trend was reported between acrylonitrile average exposure lagged 10 years and bladder cancer (P = 0.02), with workers in the top tertile of average exposure (> 2.56 ppm, 10-year lag) having an elevated risk (HR, 2.96; 95% CI, 1.38–6.34), although this elevation was not observed in workers in the two lower tertiles compared with workers who were never exposed. Among workers with a cumulative exposure of > 6.69 ppm-years, lagged by 10 years, the HR estimate was imprecise (HR, 1.45; 95% CI, 0.69–3.08; P for trend, 0.56). In additional analyses that included 16 deaths for which bladder cancer was listed as a contributing cause, the HR was increased for workers with higher average exposure (tertile 3) versus unexposed workers (HR, 2.62; 95% CI, 1.33–5.15); results for cumulative and duration of exposure were non-statistically significant (results not shown). [The Working Group noted that the results from this study were not adjusted for smoking; however, the positive association between acrylonitrile and bladder cancer was credible, considering that smoking was not an important confounder of the relation between acrylonitrile exposure and lung cancer in the same study. The Working Group further noted that, because individuals with bladder cancer frequently die of other causes, mortality outcomes do not fully capture disease incidence.]

Koutros et al. (2019) also reported mortality findings (SMR, 0.92; 95% CI, 0.67–1.24) and internal analyses using the Cox model with cumulative acrylonitrile exposure and a 10-year lag (P = 0.47) for kidney and renal pelvis cancer. Likewise, mortality (SMR, 0.98; 95% CI, 0.81–1.16) and internal analyses using the Cox model (P = 0.94) were reported for prostate cancer.

Marsh and Zimmerman (2015) (see Section 2.1.13) reported mortality for cancers of the bladder (SMR, 2.27; 95% CI, 0.62–5.80), prostate (SMR, 1.32; 95% CI, 0.43–3.09), testis (SMR, 4.97; 95% CI, 0.12–27.70), and kidney cancer (SMR, 0.57; 95% CI, 0.01–3.18) among workers considered to be exposed to acrylonitrile in an acrylonitrile-manufacturing plant in the USA, using local county rates as referents. [The Working Group noted the small number of exposed cancer deaths, probable downward HWE, and absence of internal comparison analyses, and considered that this study was minimally informative.]

Budroni et al. (2010) (see Section 2.1.15) reported on the incidence of bladder cancer (SIR, 1.26; 95% CI, 0.84–1.87) and prostate cancer (SIR, 0.92; 95% CI, 0.62–1.38) among male petrochemical workers considered to be exposed to acrylonitrile in 43 facilities in Italy. [Although the study used incidence data, the Working Group noted limitations in the exposure assessment and probable downward healthy-worker biases.]

(b) Case–control studies

Karami et al. (2011) reported the results of a hospital-based case–control study conducted in seven centres across Czechia, Poland, Romania, and the Russian Federation. The study included 1097 newly diagnosed histologically confirmed cases of renal cell carcinoma diagnosed between August 1999 and January 2003 in people aged 20–88 years living in the study area for ≥ 1 year. The study included 1476 controls selected from patients admitted to hospital for diagnoses unrelated to smoking or urological diseases between August 1998 and March 2003. Controls were frequency-matched on age, sex, and place of residence. A standardized questionnaire was administered by trained interviewers to obtain occupational information on jobs held for ≥ 12 months. Specialized occupational questionnaires were also used for specific jobs or industries that were likely to have entailed exposure to agents of interest, such as acrylonitrile. Exposure to an agent was determined by expert judgement. Risk estimates were adjusted for sex, age, centre, smoking status, self-reported hypertension (yes/no), BMI, and family history of cancer. The authors reported findings for acrylonitrile exposure and renal cell carcinoma (OR, 2.5; 95% CI, 0.9–7.1). An analysis of cumulative exposure using those unexposed to acrylonitrile as the referent was also included (exposure below the median: OR, 1.6; 95% CI, 0.4–6.4; exposure at or above the median: OR, 4.3; 95% CI, 0.9–22.1; *P* for trend, 0.06). [The Working Group noted that only 10 cases were exposed to acrylonitrile and that the risk estimates were imprecise.]

2.3.3 Other solid cancers

[The Working Group noted that for some mortality outcomes the number of observations was small, leading to imprecise analytical results.]

Five cohort studies and one case–control study reported findings for acrylonitrile exposure in relation to tumours of the brain and central nervous system. Symons et al. (2008) (see Section 2.1.2) reported findings for mortality for tumours of the central nervous system (SMR, [0.71]; 95% CI, [0.26–1.54]) among all members of a cohort of male workers exposed to acrylonitrile for ≥ 6 months in two acrylic fibre facilities, using the US population as the referent. They also reported an adjusted HR for tumours of the central nervous system per 100 ppm-year increase in 5-year lagged cumulative exposure (HR, 1.38; 95% CI, 0.43–4.47). Swaen et al. (2004) (see Section 2.1.12) reported mortality findings for brain cancer (SMR, [1.25]; 95% CI, [0.456–2.706]) among workers considered to be exposed to acrylonitrile in eight chemical companies, using the population of the Netherlands as the referent. Mastrangelo et al. (1993) (see Section 2.1.9) reported mortality findings for brain cancer in a cohort of men employed for ≥ 12 months in an acrylic fibre factory, using a regional referent (SMR, [2.6]; 95% CI, [0.1–15)]). This result was based on one observation. Geĭko et al. (1996) (see Section 2.1.11) reported brain cancer mortality findings (SMR, [5.556]; 95% CI, [0.14–30.95]) in a cohort of workers exposed to acrylonitrile in the Russian Federation, using the city of Dzerzhinsk as the referent.

Koutros et al. (2019) (see Section 2.1.11) reported mortality results for cancers of the brain and nervous system among workers considered to be exposed to acrylonitrile (SMR, 0.94; 95% CI, 0.69–1.25) and employed at eight

acrylonitrile-producing facilities in the USA, using the US population as the referent. No trend was observed in internal analyses using the Cox model with cumulative acrylonitrile exposure (through 1983) and a 10-year lag (*P* for trend, 0.50).

Thomas et al. (1987) conducted a population-based case–control study on brain tumours associated with occupational exposures. The cases included 300 men aged ≥ 30 years, identified through death certificates, with a diagnosis of glioblastoma multiforme, astrocytoma, or mixed glioma with astrocytic cells, in geographical areas where high proportions of the workforce were employed in petroleum refining and chemical manufacturing in the USA. The controls included 386 men who died from causes other than brain tumour, epilepsy, cerebrovascular disease, suicide, or homicide. Occupational history was assessed through interviews with the next of kin, and jobs were classified according to their potential for exposure to acrylonitrile and other agents of interest. Risk estimates were adjusted for age at death, history of cigarette smoking, history of alcoholism, ethnic background, and highest level of education. The results for ever having a job that might have involved exposure to acrylonitrile and astrocytic tumours were presented (OR, 0.9; 95% CI, 0.5–1.6). [The Working Group noted the inconsistent findings between studies for acrylonitrile exposure and brain cancer. The results were probably imprecise because brain tumours are rare. Furthermore, outcome definitions varied from study to study.]

Six cohort studies included findings for acrylonitrile exposure and cancer of the liver and bile ducts. Thiess et al. (1980) (see Section 2.1.4) reported mortality findings for liver cancer (SMR, [2.38]; 95% CI, [0.06–13.2]) in a cohort of workers employed for ≥ 6 months in 12 companies that used different methods to process acrylonitrile in Germany, using the Rheinhessen-Pfalz region as the referent. In analyses that excluded workers from plant 5, the authors reported results for liver cancer among workers with 5–9 years of exposure (SMR, [13.9]; 95% CI, [0.35–77.4]). Swaen et al. (2004) (see Section 2.1.12) reported mortality findings for cancer of the liver and bile ducts (SMR, [1.380]; 95% CI, [0.371–3.491]) among workers considered to be exposed to acrylonitrile in eight chemical companies, using the population of the Netherlands as the referent. Geĭko et al. (1996) (see Section 2.1.11) reported findings for liver cancer mortality (SMR, [10.0]; 95% CI, [0.25–55.7]) in a cohort of workers exposed to acrylonitrile in the Russian Federation, using the city of Dzerzhinsk as the referent. In a cohort of workers employed at eight acrylonitrile-producing facilities in the USA, Koutros et al. (2019) (see Section 2.1.11) reported a mortality deficit for cancer of the liver (SMR, 0.70; 95% CI, 0.49–0.97). No trend was observed in internal analyses using the Cox model with cumulative acrylonitrile exposure (through 1983) and a 10-year lag (*P* for trend, 0.30). Marsh and Zimmerman (2015) (see Section 2.1.13) reported mortality findings for cancer of the liver and bile ducts (SMR, 1.41; 95% CI, 0.17–5.10) among workers considered to be exposed to acrylonitrile in an acrylonitrile-manufacturing plant in the USA, using local county rates as referents. Budroni et al. (2010) (see Section 2.1.15) reported results for incidence of cancer of the liver and bile ducts (SIR, 1.24; 95% CI, 0.73–2.09) among male petrochemical workers considered to be exposed to acrylonitrile in 43 facilities in Italy. [The Working Group noted that estimates for most studies were highly imprecise.]

One study reported findings for breast cancer. Koutros et al. (2019) (see Section 2.1.11) observed a deficit in mortality for breast cancer in workers considered to be exposed to acrylonitrile (SMR, 0.59; 95% CI, 0.34–0.95) in a cohort of workers employed at eight acrylonitrile-producing facilities in the USA. No trend was observed in internal analyses using the Cox model with cumulative acrylonitrile exposure (through 1983) and a 10-year lag (*P* for trend, 0.52).

One study reported findings for melanoma of the skin. Koutros et al. (2019) (see Section 2.1.11) reported mortality for melanoma in workers considered to be exposed to acrylonitrile (SMR, 0.96; 95% CI, 0.66–0.1.34) in a cohort of workers employed at eight acrylonitrile-producing facilities in the USA. No trend was observed in internal analyses using the Cox model with cumulative acrylonitrile exposure (through 1983) and a 10-year lag (*P* for trend, 0.94).

2.4 Cancers of lymphatic and haematopoietic tissue

See Table 2.5.

Several retrospective cohort studies have described mortality among workers with potential exposure to acrylonitrile (Ott et al., 1980, 1989; Thiess et al., 1980; Delzell and Monson, 1982; Mastrangelo et al., 1993; Benn and Osborne, 1998; Swaen et al., 2004; Symons et al., 2008; Budroni et al., 2010; Marsh and Zimmerman, 2015; Koutros et al., 2019).

Symons et al. (2008) (see Section 2.1.2) described mortality in a cohort of workers in two company facilities that used acrylonitrile during fibre production in the USA. The cohort included 2548 male workers with ≥6 months of acrylonitrile exposure between 1947 and through 1991 and was followed through 2002. Cumulative exposure was estimated for each worker by company facility representatives. Results were presented for cancers of all lymphatic and haematopoietic tissues combined (ICD-9 codes 200–208). Adjusted HR estimates for a 100 ppm-year increase in cumulative exposure were as follows: unlagged HR, 0.90 (95% CI, 0.51–1.60; 20 deaths); 5-year lagged HR, 1.09 (95% CI, 0.59–2.01; 16 deaths); 10-year lagged HR, 1.29 (95% CI, 0.66–2.54; 14 deaths); and 15-year lagged HR, 1.27 (95% CI, 0.57–2.86; 13 deaths). [The Working Group considered that this was one of the more informative studies for the evaluation because of the use of internal comparisons and cumulative exposure measures over well-defined time periods, although it probably had downward healthy-worker biases.]

Ott et al. (1980) (see Section 2.1.3) evaluated mortality in a cohort of 2904 men employed in the manufacture of styrene-based products between 1937 and 1970 in a plant in the USA. Acrylonitrile-exposed jobs were categorized into several exposure groups, some of which also had exposures to styrene and ethylbenzene and others that were also exposed to extrusion vapours, polymer dusts (from cutting), and colourants. After excluding 164 workers with exposure to arsenicals, asbestos, or high levels of vinyl chloride, the reported SMR for malignant neoplasms of the lymphohaematopoietic system was [1.32] (95% CI, [0.53–2.72]) and for leukaemia was [1.76] (95% CI, [0.65–3.83]). [The Working Group noted that the presence of co-exposures to other agents made it difficult to disentangle the effects of acrylonitrile exposure. Thus, this study was considered to be minimally informative.]

Thiess et al. (1980) (see Section 2.1.4) conducted a mortality study among 1469 employees employed for ≥ 6 months in 12 plants that used different methods to process acrylonitrile in Germany. A total of four neoplasms of the lymphatic and haematopoietic system was observed (1.664 was expected, based on mortality statistics for the population of Germany). SMRs were computed by plant, which was used to assign exposure, and revealed some excesses for Hodgkin lymphoma, based on 2 deaths. [The Working Group noted the low number of outcomes and likelihood of downward healthy-worker biases.]

Benn and Osborne (1998) (see Section 2.1.6) evaluated mortality in a cohort of 2763 men employed in six facilities involved in the polymerization of acrylonitrile and spinning of acrylic fibre in the UK. Exposure was assigned by organizing workers into one of six qualitative exposure groupings based on job title. The

Table 2.5 Epidemiological studies on exposure to acrylonitrile and cancers of lymphatic and haematopoietic tissue

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Symons et al. (2008) Waynesboro (VA) and Camden (SC), USA Enrolment, 1947–1991/follow-up, through 2002 Cohort	2548 male workers who were exposed to AN for ≥ 6 mo in two company facilities (Waynesboro, and Camden) manufacturing acrylic fibre, in 1947–1991. Exposure assessment method: See Table 2.1.	Lymphatic and haematopoietic (ICD-9, 200–208), mortality	SMR (US referent): Total cohort	20	[0.64 (0.39–0.99)]	Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations:* See Table 2.1.
		Lymphatic and haematopoietic (ICD-9, 200–208), mortality	Cumulative AN exposure (HR): Continuous (per 100 ppm-yr), no lag	20	0.9 (0.51–1.6)	Birth period, employment in SC start-up group, age	
			Continuous (per 100 ppm-yr), 5-yr lag	16	1.09 (0.59–2.01)		
			Continuous (per 100 ppm-yr), 10-yr lag	14	1.29 (0.66–2.54)		
			Continuous (per 100 ppm-yr), 15-yr lag	13	1.27 (0.57–2.86)		
		Lymphatic and haematopoietic (ICD-9, 200–208), mortality	Mean AN exposure intensity, men with cumulative exposure > 10 ppm-yr (HR): < 10 ppm	6	1	Birth period, employment in SC start-up group, age	
			≥ 10 ppm	13	1.09 (0.4–2.96)		

Table 2.5 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ott et al. (1980) USA Enrolment, 1937–1970/follow-up, 1940–1975 Cohort	2904 (2740 without exposure to arsenicals, asbestos, or vinyl chloride); employed for ≥ 1 yr in the manufacture of styrene-based products between 1937 and 1970. Exposure assessment method: See Table 2.1.	Lymphatic and haematopoietic (excluding leukaemia), mortality	SMR (US White male referent):			Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
			Cohort, less workers with arsenicals, asbestos, or high vinyl chloride exposure	7	[1.32 (0.53–2.72)]		
		Leukaemia	SMR (US White male referent):				
			Cohort, less workers with arsenicals, asbestos, or high vinyl chloride exposure	6	[1.76 (0.65–3.83)]		
Thiess et al. (1980) Germany Enrolment, varied/follow-up, through 15 May 1978 Cohort	1469 workers employed for ≥ 6 mo at 12 AN-using plants from 1954 through 15 May 1978. Exposure assessment method: See Table 2.1.	Lymphatic and haematopoietic, mortality	SMR (Germany referent):			Age	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
			Total cohort	4	[2.4 (0.65–6.15)]		
		Hodgkin lymphoma, mortality	SMR (Rheinhessen-Pfalz referent):				
			Total cohort	2	[5.99 (0.73–21.6)]		
		Hodgkin lymphoma, mortality	Duration of exposure, excluding plant 5, SMR (Rheinhessen-Pfalz referent):				
			0–4 yr	0	NC		
			5–9 yr	1	[27 (0.68–151)]		
			≥ 10 yr	1	[17.2 (0.44–96)]		

Table 2.5 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Benn and Osborne (1998) England, Wales, Scotland, Northern Ireland Enrolment, phase I (1950–1968); phase II (1969–1978)/follow-up, through 1991 Cohort	2763 male workers employed for ≥ 1 yr and exposed to polymerization of AN or the spinning of acrylic fibre in six factories in the UK identified within a study conducted by the HSE and the Chemical Industries Association. Exposure assessment method: Questionnaire; see Table 2.1.	Lymphatic and haematopoietic, mortality	SMR (national referent): Total cohort	 5	 [0.499 (0.16–1.16)]	Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1. *Other comments*: Unclear reference group for Northern Ireland.
Delzell and Monson (1982) Akron (OH), USA Enrolment, 1940–1971/follow-up, 1940 through 1 July 1978 Cohort	327 White male production workers employed for ≥ 2 yr between 1940 and 1971 at a rubber-manufacturing plant in Akron who had worked in the chemicals division in departments 5578 or 5579 with potential AN exposure. Exposure assessment method: See Table 2.1.	Lymphatic and haematopoietic (ICD-7, 200–205), mortality	SMR (US referent): Total cohort	 4	 2.3 (0.6–5.8)	Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
Ott et al. (1989) USA 1940–1978 Nested case–control	Cases: 52 NHL, 20 multiple myeloma, 39 non-lymphocytic leukaemia, 18 lymphocytic leukaemia; mortality from social security administration, motor vehicle registry, and listings of war deaths. Death certificates obtained (cases). Controls: NR; selected using group-matched incidence density sampling and frequency-matched 5:1 to cases on decade of first employment for each disease category. Exposure assessment method: See Table 2.1.	NHL, mortality Multiple myeloma, mortality Leukaemia (non-lymphocytic), mortality Leukaemia (lymphocytic), mortality	AN exposure (OR): Never Ever AN exposure (OR): Never Ever AN exposure (OR): Never Ever AN exposure (OR): Never Ever	 NR 6 NR 3 NR 1 NR 1	 1 2.5 1 2 1 0.4 1 2.6	Survival time, decade of hire	*Strengths*: See Table 2.1. *Limitations*: See Table 2.1.

Table 2.5 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Swaen et al. (2004) Netherlands Enrolment, varied by plant, starting 1959–1973/follow-up, through 1 January 2001 Cohort	2842 men, citizens of the Netherlands, working in eight chemical companies with AN exposure for > 6 mo. Exposure assessment method: See Table 2.1.	Multiple myeloma, mortality Leukaemia, mortality	SMR (national referent): Total cohort SMR (national referent): Total cohort	3 5	[1.304 (0.262–3.742)] [1.19 (0.384–2.757)]	Age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. *Other limitations*: Small numbers of deaths.
Mastrangelo et al. (1993) Italy Enrolment, 1959–1988/follow-up, 1959–1990 Cohort	671 men employed for ≥ 12 mo at any time since the opening of the factory in 1959. Clerks and some workers with past exposure to vinyl chloride monomer or to benzidine and its compounds were excluded, as were workers employed after 31 December 1988. Exposure assessment method: See Table 2.1.	Leukaemia, mortality	SMR (regional referent): Total cohort	1	[2.5 (0.1–14)]	Age, calendar period	*Strengths*: See Table 2.1. *Limitations*: See Table 2.1.
Koutros et al. (2019) USA Enrolment, 1952–1983/follow-up, through 2011 Cohort	25 460 (16 889 AN-exposed); all workers employed ≥ 1 day at any of the eight participating plants after the start of AN operations (1952–1965). Work histories covered the date of hire (i.e. 1942–1983) through date of record abstraction at the plant (1983). Exposure assessment method: See Table 2.1.	Lymphoma (type not specified, includes Hodgkin and NHL), mortality	Cumulative AN exposure (through 1983), 10-yr lag (56 total exposed cases) (HR): Unexposed Tertile 1 Tertile 2 Tertile 3 Trend-test *P* value, 0.67	 NR NR NR NR	 1 0.94 (0.52–1.7) 0.86 (0.47–1.56) 1.26 (0.68–2.33)	Age, race, sex, birth year, salary–wage classification	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.

Table 2.5 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2019) (cont.)		NHL, mortality	Cumulative AN exposure (through 1983), 10-yr lag (52 total exposed cases) HR):			Age, race, sex, birth year, salary–wage classification	
			Unexposed	NR	1		
			Tertile 1	NR	0.91 (0.49–1.69)		
			Tertile 2	NR	0.97 (0.51–1.82)		
			Tertile 3	NR	1.38 (0.72–2.61)		
			Trend-test *P* value, 0.41				
		Leukaemia (all types), mortality	Cumulative AN exposure (through 1983), 10-yr lag (64 total exposed cases) (HR):				
			Unexposed	NR	1		
			Tertile 1	NR	0.64 (0.37–1.11)		
			Tertile 2	NR	0.98 (0.56–1.7)		
			Tertile 3	NR	0.97 (0.56–1.7)		
			Trend-test *P* value, 0.86				
		Multiple myeloma, mortality	Cumulative AN exposure (through 1983), 10-yr lag (36 total exposed cases) (HR):				
			Unexposed	NR	1		
			Tertile 1	NR	1.59 (0.68–3.71)		
			Tertile 2	NR	1.34 (0.57–3.12)		
			Tertile 3	NR	1.64 (0.69–3.86)		
			Trend-test *P* value, 0.34				
		NHL (chronic lymphocytic leukaemia), mortality	Cumulative AN exposure (through 1983), 10-yr lag (12 total exposed cases) (HR):				
			Unexposed	NR	1		
			≤ Median	NR	0.48 (0.16–1.45)		
			> Median	NR	0.77 (0.26–2.33)		
			Trend-test *P* value, 0.63				
		Leukaemia (acute myeloid leukaemia), mortality	Cumulative AN exposure (through 1983), 10-yr lag (28 total exposed cases) (HR):				
			Unexposed	NR	1		
			≤ Median	NR	0.9 (0.41–1.95)		
			> Median	NR	1.33 (0.6–2.96)		
			Trend-test *P* value, 0.48				

Table 2.5 (continued)

Reference, location enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Marsh and Zimmerman (2015) Lima (OH), USA Enrolment, starting in 1955/follow-up, 1955–2011 Cohort	2096 (789 with AN exposure); employees (any time length) in Lima factory between 1955 and 2011. The Lima site was included in the study by Koutros et al. (2019), but the overlap between the two Lima cohorts was only partial. Exposure assessment method: See Table 2.1.	Hodgkin lymphoma, mortality	Exposure group (SMR, county referent):			Sex, race, age, calendar period	*Exposure assessment critique*: See Table 2.1. *Other strengths*: See Table 2.1. *Other limitations*: Small numbers of deaths. *Other comments*: It was unclear which reference population was used for the female component.
			Unexposed	1	1.65 (0.04–9.2)		
			AN-exposed	0	0 (0–11.6)		
		NHL (ICD-9, 200, 202.0–202.1, 202.8–202.9), mortality	Exposure group (SMR, county referent):				
			Unexposed	1	0.21 (0.01–1.15)		
			AN-exposed	1	0.37 (0.01–2.07)		
		Leukaemia and aleukaemia (ICD-9, 204–208), mortality	Exposure group (SMR, county referent):				
			Unexposed	5	1.19 (0.39–2.79)		
			AN-exposed	4	1.73 (0.47–4.42)		
Budroni et al. (2010) Porto Torres, Sardinia, Italy 1990–2006 Cohort	5350 (2336 with AN exposure) male workers with ≥ 6 mo of employment in one or more chemical plants in the Porto Torres industrial area, working in the plants and alive as of 1 January 1990. Workers that joined between 1 January 1990 and 31 December 2001 were also included. Exposure assessment method: See Table 2.1.	Hodgkin lymphoma, incidence	No. of cases			None	*Exposure assessment critique*: See Table 2.1. *Other strengths and limitations*: See Table 2.1.
			AN-exposed	0	NC		
		NHL, incidence	SIR (local referent):			Age, calendar period	
			AN-exposed	10	1.78 (0.96–3.3)		
		Leukaemia (lymphoid), incidence	SIR (local referent):				
			AN-exposed	3	1.19 (0.38–3.69)		
		Leukaemia (myeloid), incidence	SIR (local referent):				
			AN-exposed	3	1.05 (0.34–3.27)		
		Multiple myeloma, incidence	SIR (local referent):				
			AN-exposed	4	1.47 (0.55–3.93)		

AML, acute myeloid leukaemia; AN, acrylonitrile; CI, confidence interval; HR, hazard ratio; HSE, Health and Safety Executive; HWSB, healthy-worker survivor bias; ICD, International Classification of Diseases; mo, month(s); NC, not calculated; NHL, non-Hodgkin lymphoma; NR, not reported; OH, Ohio; OR, odds ratio; ppm, parts per million; SC, South Carolina; SIR, standardized incidence ratio; SMR, standardized mortality ratio; UK, United Kingdom; US, United States; USA, United States of America; VA, Virginia; yr, year(s).

observed number of cancers of the lymphatic and haematopoietic system (n = 5) was about half the expected number, although the estimate was imprecise, with an SMR of [0.499] (95% CI, [0.16–1.16]). [The Working Group noted that downward healthy-worker biases were likely.]

Delzell and Monson (1982) (see Section 2.1.7) analysed mortality among 327 male employees from a rubber-manufacturing plant in the USA. SMRs based on national death rates were estimated for workers in two departments where there was potential for exposure to acrylonitrile. The SMR based on 4 observed cancers of the lymphatic and haematopoietic system was 2.3, although the estimate was imprecise (95% CI, 0.6–5.8). [The Working Group considered that the information provided by this study was limited by imprecise effect estimates, inadequate assessment of exposure, and downward healthy-worker biases.]

Ott et al. (1989) (see Section 2.1.8) reported on a set of case–control studies of mortality from NHL, multiple myeloma, non-lymphocytic leukaemia and lymphocytic leukaemia nested in a cohort of men from three facilities in the USA. Ever exposure and duration of exposure to acrylonitrile were assessed by two retired senior manufacturing personnel using job records. The ORs for ever exposure to acrylonitrile all had $P > 0.05$ (NHL, 6 deaths; multiple myeloma, 3 deaths; non-lymphocytic leukaemia, 1 death; and lymphocytic leukaemia, 1 death). [The Working Group noted that the effect estimates were imprecise; thus, the study was considered to be minimally informative.]

Swaen et al. (2004) (see Section 2.1.12) conducted a retrospective cohort study of 2842 men employed by one of eight chemical plants with exposure to acrylonitrile in the Netherlands. A quantitative assessment of exposure to acrylonitrile was conducted using job records and expert assessment for each combination of job, workplace, and time period. SMRs were calculated for ever exposure for multiple myeloma (SMR, [1.304]; 95% CI, [0.262–3.742]; 3 deaths) and leukaemia (SMR, [1.190]; 95% CI, [0.384–2.757]; 5 deaths). [The Working Group noted the good-quality quantitative exposure assessment, although information on exposure in 1980–2000 was missing, which may have introduced nondifferential exposure misclassification and bias towards the null. Additional downward healthy-worker biases were likely.]

Mastrangelo et al. (1993) (see Section 2.1.9) reported 1 death from leukaemia in a retrospective cohort study in 671 male workers with ≥ 12 months of exposure to acrylonitrile in an acrylic fibre factory in Italy between 1959 and 1988. [The Working Group noted that this study was minimally informative because of the small numbers of exposed cases, resulting in imprecise SMR estimates.]

Koutros et al. (2019) (see Section 2.1.11) presented an extended mortality follow-up of a cohort of 25 460 workers employed at eight acrylonitrile-producing facilities in the USA. Quantitative estimates of acrylonitrile exposure were developed using work histories, plant records, and monitoring data to develop 8-hour TWA estimates of acrylonitrile exposure for each job/department/plant combination by time period. Potential exposure to 340 other substances was also assessed. HRs for cumulative acrylonitrile exposure were calculated, adjusting for age, race, sex, birth year, and salary–wage classification. A total of 56 deaths from lymphoma and 64 deaths from leukaemia were reported in the 10-year lagged analysis. No positive trends in the HRs were observed between categories of increasing cumulative exposure (ppm-years), lagged 10 years, and risk of death from NHL (52 exposed deaths; P for trend, 0.41), chronic lymphocytic leukaemia (12 exposed deaths; P for trend, 0.63), multiple myeloma (36 exposed deaths; P for trend, 0.34), or acute myeloid leukaemia (28 exposed deaths; P for trend, 0.48). [The Working Group considered this study to be highly informative given

its large size, reporting of internal comparisons, high-quality estimates of cumulative exposure to acrylonitrile in well-defined cohorts, and accounting for possible co-exposures.]

Marsh and Zimmerman (2015) (see Section 2.1.13) conducted a retrospective cohort mortality study in an acrylonitrile-manufacturing plant in Lima, Ohio, USA. The cohort was expanded three times (in March 1999, March 2002, and March 2015) to include a final total of 2096 workers who had been employed at the facility between 1955 and 2011 (789 with acrylonitrile exposure). Quantitative estimates of acrylonitrile were developed using a combination of approaches over the follow-up period. SMRs were calculated using national and county-based mortality rates. Among the acrylonitrile-exposed, SMRs were reported with minimal precision for 0 deaths from Hodgkin lymphoma, 1 death from NHL, and 4 deaths from leukaemia and aleukaemia (ICD-9, 204–208). [The Working Group noted that a large proportion of this cohort, 894/2096 individuals, overlapped with that described in Koutros et al. (2019) and considered that the study was prone to healthy-worker biases.]

Budroni et al. (2010) (see Section 2.1.15) conducted a retrospective cohort study of 5350 male petrochemical workers in the industrial area of Porto Torres, Sardinia, Italy, who were followed for cancer incidence between 1990 and 2006. SIRs were calculated for the cohort overall and for workers potentially exposed to acrylonitrile and several other chemical agents on the basis of plant work processes conducted at those facilities. The SIR for NHL among acrylonitrile-exposed workers indicated an excess in incidence (SIR, 1.78 95% CI, 0.96–3.30; 10 cases). Estimates for lymphoid leukaemia, myeloid leukaemia, and myeloma were imprecise, based on only 3 or 4 observed cases each. [The Working Group noted that histopathological examination was used to validate the cancer data, and that incidence is a better representation of the burden of disease than is mortality. Key limitations of this study included the assessment of exposure to acrylonitrile that was based only on the plant (no individual-level assessment), possible co-exposure to other agents, and the absence of internal comparisons.]

2.5 Evidence synthesis for cancer in humans

For the evaluation of cancer in humans exposed to acrylonitrile, the Working Group considered 19 studies in total. Most were retrospective cohort studies (n = 13) reporting on workers who were occupationally exposed to acrylonitrile. Of these retrospective cohort studies, 12 reported on cancer mortality, and one on cancer incidence. A case–control study nested within a retrospective occupational cohort study reported on occupational exposure to acrylonitrile and cancer mortality, and a case–control study nested within a population-based prospective cohort study also reported on biomarkers of acrylonitrile exposure and cancer incidence. In addition, four hospital- or population-based case–control studies reported on occupational exposure to acrylonitrile and either cancer incidence (two studies) or mortality (two studies). Acrylonitrile exposure has been most commonly evaluated with respect to cancers of the respiratory system, genitourinary system, gastrointestinal system, brain, prostate, breast, thyroid, and lymphohaematopoietic system.

2.5.1 Exposure assessment quality and co-exposures

The Working Group considered the quality of the exposure assessment to be an important factor in evaluating the quality and informativeness of studies. The occupational cohort studies were conducted in a variety of industries that included acrylonitrile production or processing, chemical plants using acrylonitrile

in acrylic-fibre production and other processes, as well as those manufacturing styrene-based products and rubber. The case–control studies relied on occupational histories reported by study participants or their next of kin. Details regarding the strengths and limitations of the exposure assessment in the evaluated studies are provided in Section 1.6.1, including consideration of the exposure route, changes in workplace practices, availability of measurements, timing of exposure and its assessment, reported use of personal protective equipment, and type of exposure metrics constructed. Exposure metrics varied markedly among studies, with analyses using qualitative, semiquantitative, and quantitative exposure variables. Nondifferential exposure misclassification was a major source of bias in studies using qualitative exposure metrics, resulting in bias of effect measures towards the null. Thus, the Working Group gave more weight to studies with quantitative estimates of cumulative exposure based on expert assessment and/or studies that included exposure measurements (Swaen et al., 2004; Scélo et al., 2004; Symons et al., 2008; Marsh and Zimmerman, 2015; Koutros et al., 2019).

The potential for co-exposure to other agents, including those known or suspected to be carcinogenic, was variably accounted for across studies (summarized in Table S1.12; see Annex 1, Supplementary material for Section 1, online only, available from: https://publications.iarc.who.int/646). In several cohort studies, exposure to other agents in the workplace was noted; the most frequently reported co-exposure was to styrene (Group 2A, with *limited* evidence for lymphohaematopoietic cancers; IARC, 2019b), particularly in the plastics and rubber industries. The Working Group noted that exposures could be highly correlated and that it was difficult to distinguish the independent effect of acrylonitrile exposure in studies using JEMs or expert assessment with only job title information. Ultimately, the Working Group weighted most heavily those studies in which co-exposures were quantified and confounding of the acrylonitrile–cancer relationship was explored by adjusting for other chemicals, such as in the NCI pooled cohort study by Koutros et al. (2019).

2.5.2 Healthy-worker biases

The assessment of associations between exposure to acrylonitrile and cancer was most often available from studies of occupational cohorts. Estimates from these studies are often subject to healthy-worker biases, including the HWE (because of the selection of healthy individuals into the workforce) and HWSB. The HWE was seen where occupational populations were compared with external reference populations and an SMR or SIR was calculated in the analysis. Because of the HWE, the Working Group gave greater weight to findings from occupational cohorts for which the assessment of the carcinogenicity of acrylonitrile used analyses with an internal reference group of workers (Symons et al., 2008; Marsh and Zimmerman, 2015; Koutros et al., 2019). The additional impact of the HWSB, whereby workers with poorer health status or sensitivity to acrylonitrile tend to leave employment, was noted by the Working Group as a source of bias in most studies, including those in which external comparisons were made but a minimum employment duration of ≥ 6 months was required. Only in the NCI pooled cohort study, for the analysis of lung cancer mortality, was the impact of this bias assessed quantitatively, and it was found to be notably downward (Keil et al., 2024).

2.5.3 Lung cancer (including trachea and bronchus)

Results from early epidemiological studies suggested slightly elevated death rates from lung cancer, primarily among working populations exposed to acrylonitrile. Consequently, lung

cancer mortality has been explored in several retrospective cohorts of exposed workers. Several studies included a comparison of incidence or mortality in workers exposed to acrylonitrile with that in external reference populations (Kiesselbach et al., 1979; Ott et al., 1980; Thiess et al., 1980; Waxweiler et al., 1981; Delzell and Monson, 1982; Mastrangelo et al., 1993; Geĭko et al., 1996; Benn and Osborne, 1998; Swaen et al., 2004; Budroni et al., 2010). Most of these studies also required a minimum employment duration of ≥ 6 months. These results were likely to be subject to healthy-worker biases, which probably diminished the effect estimates for the relation between acrylonitrile exposure and lung cancer. Despite this downward bias, excesses have been reported by Waxweiler et al. (1981) (SMR, 1.49; 95% CI, 1.07–2.01), Thiess et al. (1980) (SMR, [1.86]; 95% CI, [0.93–3.33]), Delzell and Monson (1982) (SMR, 1.5; 95% CI, 0.7–2.9), and Geĭko et al. (1996) (SMR, [2.273]; 95% CI, [0.62–5.82]). In several of these studies, the numbers of deaths from lung cancer in exposed workers were also small, which resulted in imprecise estimates. Among the studies in which external comparisons were used, the largest numbers of observed deaths were reported by Swaen et al. (2004) (67 deaths in exposed workers) and Benn and Osborne (1998) (53 deaths in exposed workers). Among this group of studies, only that by Swaen et al. (2004) considered additional semiquantitative analytic metrics of intensity. Swaen et al. (2004) reported SMRs for increasing cumulative exposure – low (< 1 ppm-year), SMR, [0.921] (95% CI, [0.369–1.890]); moderate (1–10 ppm-year), SMR, [1.065] (95% CI, [0.746–1.474]); and high (> 10 ppm-year), SMR, [1.148] (95% CI, [0.736–1.709]) – but did not calculate direct comparisons between the exposed and unexposed. Although these studies contributed to the literature on the association between acrylonitrile and lung cancer, the conclusions that could be drawn were hampered by HWEs, limited exposure assessment, and imprecise SMR or SIR estimates.

Four studies were available that evaluated semiquantitative or quantitative metrics of exposure to acrylonitrile in exposed and unexposed workers to allow for internal comparisons (Scélo et al., 2004; Symons et al., 2008; Marsh and Zimmerman, 2015; Koutros et al., 2019). The industry-conducted study by Marsh and Zimmerman (2015) was not independent since it overlapped (42% overlap) with the population reported by Blair et al. (1998) and Koutros et al. (2019). Furthermore, with only 15 deaths from lung cancer in exposed workers, most estimates lacked precision. Another industry-conducted study from the USA (Symons et al., 2008) reported a null HR, based on a continuous model, for an increase of 100 ppm-years in cumulative acrylonitrile exposure and lung cancer mortality (unlagged HR, 0.95; 95% CI, 0.73–1.23; 88 deaths). No data on smoking or co-exposure were available for confounding assessment, and the reported HRs for lung cancer were imprecise. Despite the more well-defined characterization of exposure in the studies by Symons et al. (2008) and Marsh and Zimmerman (2015), these additional weaknesses limited the informativeness of these studies with respect to the association between acrylonitrile exposure and lung cancer. Also, they did not account for the HWSB. The study by Scélo et al. (2004) was the only case–control study to report on the association between acrylonitrile and lung cancer. Using occupational histories from 5979 participants and a semiquantitative assessment of exposure, they reported an association between acrylonitrile exposure and lung cancer after adjustment for smoking and other potential confounding variables (OR, 2.20; 95% CI, 1.11–4.36, 39 ever-exposed cases) and a positive exposure–response relation (*P* for trend for cumulative exposure, 0.06). The Working Group noted several strengths of this study, including the exposure–response assessment and adjustment for smoking and co-exposures.

The Working Group noted that a direct comparison between exposure levels in this study and those in occupational cohorts, particularly those with measurements, was difficult, because of differences in the exposure assessment methods used. There were strong correlations between acrylonitrile, styrene, and vinyl chloride in this study, suggesting that these exposures may be difficult to disentangle; however, there is scant evidence that these agents are lung carcinogens.

The most informative study regarding the relation between acrylonitrile exposure and lung cancer mortality was a retrospective cohort study that pooled 25 460 workers from eight facilities in the USA (Blair et al., 1998; Koutros et al., 2019). Exposure was assessed on the basis of job, department, plant, and time period. Work history records, plant records, and monitoring data were used to estimate TWA exposure, and metrics were developed for cumulative and average exposure, and duration of exposure. Cox regression analyses showed an elevated risk of lung and bronchial cancer (for ≥ 32 ppm-years versus unexposed, HR, 1.47; 95% CI, 1.07–2.02; *P* for trend, 0.02; 88 deaths), which was robust in sensitivity analyses that adjusted for smoking or co-exposures including asbestos. The primary limitation in the study was that the exposure assessment ended in 1983, whereas cancer follow-up ended in 2011. Approximately 30% of workers were still actively working in 1983 and with additional sensitivity analyses censoring those workers at their last known date, the observed association was similar, if not slightly stronger (with censoring, for ≥ 32 ppm-years versus unexposed, HR, 1.64; 95% CI, 1.11–2.42; *P* for trend, 0.04), suggesting that some nondifferential exposure misclassification may be biasing estimates towards the null. Additional analyses adjusting for the HWSB suggested that this bias was operating to underestimate the causal association between acrylonitrile exposure and lung cancer (Keil et al., 2024). Thus, the association between acrylonitrile exposure and lung cancer mortality may be larger than reported in this study.

The Working Group concluded that there was persuasive evidence that exposure to acrylonitrile increases the risk of lung cancer. Results from eight facilities representing many of the major sectors of acrylonitrile production operations (monomer, fibre, and resin) showed consistent evidence of a quantitative exposure–response relation after accounting for major sources of confounding and bias, including smoking, co-exposures, and the HWSB. In one additional case–control study, an exposure–response relation was observed after adjusting for smoking and co-exposures, and excesses in mortality were also reported in four retrospective cohort studies. Some studies in which a positive relation between acrylonitrile exposure and lung cancer was not observed provided imprecise effect estimates and were probably biased downward because of HWE and HWSB and towards the null because of nondifferential exposure misclassification.

2.5.4 Bladder cancer

Although positive associations were observed in some instances, inconsistencies were observed between and within studies for the association between exposure to acrylonitrile and bladder cancer. Koutros et al. (2019) reported no excess mortality from cancer of the urinary bladder among workers considered to be exposed to acrylonitrile (SMR, 0.86; 95% CI, 0.61–1.17). In internal analyses using the Cox model to assess the exposure–response relation, the authors reported a trend between average acrylonitrile exposure lagged 10 years and bladder cancer ($P = 0.02$); workers in the top tertile of average exposure (> 2.56 ppm, 10-year lag) had an elevated risk of bladder cancer (HR, 2.96; 95% CI, 1.38–6.34), although an elevation was not observed in the lower two tertiles compared with workers who were never exposed. In additional

analyses including 16 deaths for which bladder cancer was listed as a contributing cause, the HR was increased for tertile 3 versus unexposed (HR, 2.62; 95% CI, 1.33–5.15); no trends were observed for cumulative exposure and duration of exposure when these additional deaths were included. The Working Group noted that the results of this study were not adjusted for smoking; however, the positive association between acrylonitrile exposure and bladder cancer was credible, considering that smoking was not an important confounder of the relationship between acrylonitrile exposure and lung cancer in the same study. In an industry-sponsored study, Symons et al. (2008) reported an SMR of 1.00 (95% CI, 0.57–1.62) for bladder and kidney cancers combined, using the US population as the referent. In internal analyses using the Cox model, the authors reported an HR for a 100 ppm-year increase in cumulative exposure of 0.98 (95% CI, 0.53–1.79) for bladder and kidney cancers combined. Thiess et al. (1980), Delzell and Monson (1982), Swaen et al. (2004), and Marsh and Zimmerman (2015) reported some elevated mortality for bladder cancer, although these estimates were imprecise because of the very small number of exposed deaths (≤ 5). Since individuals with bladder cancer frequently die of other causes, mortality outcomes do not fully capture disease occurrence. Using incidence data, Budroni et al. (2010) reported a SIR of 1.26 (95% CI, 0.84–1.87) in exposed workers compared with a local population referent. The Working Group noted that this study was probably affected by downward healthy-worker biases.

Overall, the Working Group considered that a positive association between acrylonitrile exposure and bladder cancer was credible. Associations in individual studies were frequently elevated, although they were often imprecise and based on a small number of exposed cases or deaths. Further study limitations included nondifferential exposure misclassification. Since smoking was not an important confounder of the relationship between acrylonitrile and lung cancer in the study by Koutros et al. (2019), smoking was considered unlikely to be a confounder for bladder cancer in this cohort. The Working Group considered that chance, bias, and confounding could not be ruled out with reasonable confidence.

2.5.5 Other cancers of the genitourinary system

Koutros et al. (2019) reported mortality findings for kidney and renal pelvis cancer (SMR, 0.92; 95% CI, 0.67–1.24) among workers considered to be exposed to acrylonitrile, using the US population as the referent. They did not observe a trend in hazard for kidney and renal cancer using cumulative acrylonitrile exposure with a 10-year lag (*P* for trend, 0.47). Results for bladder and kidney cancer combined, reported by Symons et al. (2008), have been described in Section 2.5.7. In a hospital-based case–control study, Karami et al. (2011) reported risk between ever exposure to acrylonitrile and renal cell carcinoma (OR, 2.5; 95% CI, 0.9–7.1). An analysis of trends in cumulative exposure, using no acrylonitrile exposure as the referent, suggested a positive trend (OR for exposure below the median, 1.6; 95% CI, 0.4–6.4; and OR for exposure at or above the median, 4.3; 95% CI, 0.9–22.1; *P* for trend, 0.06), but only 10 cases were exposed to acrylonitrile.

For prostate cancer, Koutros et al. (2019) reported mortality results for acrylonitrile-exposed workers, using the US population as the referent (SMR, 0.98; 95% CI, 0.81–1.16). The authors did not observe a trend in Cox proportional hazard analyses using cumulative acrylonitrile exposure with a 10-year lag (*P* for trend, 0.94). Marsh and Zimmerman (2015) reported imprecise estimates for mortality for cancers of the prostate (SMR, 1.32; 95% CI, 0.43–3.09), and testis (SMR, 4.97; 95% CI, 0.12–27.70) among acrylonitrile-exposed workers, compared with a county population referent.

Overall, the Working Group considered that the available studies in humans were not

sufficiently informative to permit a conclusion to be drawn about the presence of a causal association between exposure to acrylonitrile and cancers of the kidney, renal pelvis, prostate, or testis.

2.5.6 Laryngeal cancer

There were three studies on mortality from or incidence of cancer of the larynx in workers (Swaen et al., 2004; Budroni et al., 2010; Marsh and Zimmerman, 2015). The estimates from these studies were imprecise and were based on limited numbers of exposed participants ($n \leq 5$), and they lacked internal comparisons. The Working Group considered that the available data from studies in humans were insufficient to permit a conclusion to be drawn regarding the association between acrylonitrile exposure and cancer of the larynx.

2.5.7 Mesothelioma

One study has reported on the association between acrylonitrile exposure and mesothelioma (Koutros et al., 2019). Findings based on quantitative estimates of acrylonitrile exposure showed no exposure–response relation for duration of exposure, average exposure, or cumulative exposure when comparing exposed and unexposed workers, but estimates were imprecise. The Working Group considered that the data were insufficient to permit a conclusion to be drawn regarding the association between acrylonitrile exposure and mesothelioma.

2.5.8 Cancers of the digestive tract

Two informative studies reported findings on the association between acrylonitrile exposure and gastrointestinal cancers.

In two retrospective cohort studies, Koutros et al. (2019) observed no trend in stomach cancer mortality with increasing cumulative acrylonitrile exposure, using a 10-year lagged proportional hazard model (when comparing unexposed, below median, and above median exposure, *P* for trend, 0.66), and Budroni et al. (2010) observed a reduced, but imprecise, incidence of cancer of the stomach.

Findings from studies on oesophageal cancer were mixed. Koutros et al. (2019) observed no excess of mortality from oesophageal cancer among workers considered to be exposed to acrylonitrile compared with the general population. Likewise, no trend was observed using proportional hazard models of cumulative acrylonitrile exposure, lagged 10-years (*P* for trend, 0.68; 56 exposed deaths). Conversely, in a case–control study on oesophageal cancer that was nested within a prospective population-based cohort, Etemadi et al. (2024) compared concentrations at the 90th versus the 10th percentile of two specific urinary metabolites of acrylonitrile (CHEMA and CEMA). Among participants not using tobacco at baseline, the authors reported elevated adjusted ORs for CHEMA (OR, 2.1; 95% CI, 0.9–4.9) and CEMA (OR, 4.3; 95% CI, 1.4–13.5).

For cancer of the colon and rectum, in an industry-sponsored study, Symons et al. (2008) reported mortality findings, using the US population as the referent (SMR, 0.85; 95% CI, 0.57–1.24). In a proportional hazards model, the HR for a 100 ppm-year increase in cumulative exposure was 1.16 (95% CI, 0.75–1.81). Koutros et al. (2019) did not observe a trend in incidence for cumulative acrylonitrile exposure, lagged 10 years (*P* for trend, 0.09), whereas Budroni et al. (2010) reported an imprecise finding for incidence of cancer of the colon and rectum (SIR, 1.29; 95% CI, 0.92–1.83).

Koutros et al. (2019) did not observe a trend for cumulative acrylonitrile exposure, lagged 10-years, for pancreatic cancer, using a proportional hazards model (*P* for trend, 0.70). In a population-based case–control study on pancreatic cancer and exposure to chemical and physical agents in Finland, Kauppinen et al. (1995)

reported an association with acrylonitrile exposure (OR, 2.1; 95% CI, 0.9–4.7), after controlling for age, sex, smoking, history of diabetes, and alcohol intake. However, these findings were based on potential rather than probable exposure to acrylonitrile, since 1% of cases and 0.3% of controls were considered to be potentially exposed to acrylonitrile, but none were considered probably exposed.

For other retrospective cohort studies (Thiess et al., 1980; Delzell and Monson, 1982; Ǧeǐko et al., 1996; Benn and Osborne, 1998; Swaen et al., 2004; and Marsh and Zimmerman, 2015), results were presented for gastrointestinal cancers, but the reported associations were inconsistent and imprecise. These studies were of inadequate size, had short follow-up periods, or did not report internal analyses. In addition, there were limitations in the exposure assessment and minimal control for co-exposures.

Overall, the Working Group considered that the available studies in humans were not sufficiently informative to permit a conclusion to be drawn about the presence or absence of a causal association between exposure to acrylonitrile and cancers of the stomach, oesophagus, colon and rectum, and pancreas.

2.5.9 Other solid cancers

Koutros et al. (2019) reported that there was no trend with increasing cumulative exposure to acrylonitrile, lagged 10 years, and risk of brain and other nervous system cancers (*P* for trend, 0.50). Ǧeǐko et al. (1996) and Swaen et al. (2004) reported on brain cancer mortality, but these findings were imprecise and based on few deaths. In a population-based case–control study, Thomas et al. (1987) reported no excess risk of glioblastoma multiforme, astrocytoma, or mixed glioma with astrocytic cells (OR, 0.9; 95% CI, 0.5–1.6) among people ever exposed to acrylonitrile, as reported by their next of kin.

Only Koutros et al. (2019) reported mortality findings for breast cancer among acrylonitrile-exposed workers, using the US population as the referent (SMR, 0.59; 95% CI, 0.34–0.95). No trend with increasing cumulative exposure to acrylonitrile, lagged 10 years, was observed (*P* for trend, 0.52; 16 exposed deaths).

Koutros et al. (2019) also reported mortality findings for melanoma of the skin (SMR, 0.96; 95% CI, 0.66–1.34) among acrylonitrile-exposed workers, using the US population as the referent. Cox proportional hazard analyses using cumulative acrylonitrile exposure with a 10-year lag showed no trend with increasing cumulative exposure to acrylonitrile (*P* for trend, 0.94).

The Working Group considered that the available studies in humans were not sufficiently informative to permit a conclusion to be drawn about the presence or absence of a causal association between exposure to acrylonitrile and cancers of the brain, nervous system, breast, or melanoma of the skin.

2.5.10 Lymphatic and haematopoietic cancers

Symons et al. (2008) reported findings for cancers of lymphatic and haematopoietic tissues combined. The adjusted HR estimates per 100 ppm-year increase in cumulative acrylonitrile exposure did not show an increase (unlagged HR, 0.90; 95% CI, 0.51–1.60). The HRs for increasing lagging durations were increasingly imprecise.

Koutros et al. (2019) did not observe a trend across tertiles of cumulative exposure in proportional hazard analyses with a 10-year lag for NHL (*P* for trend, 0.41) or multiple myeloma (*P* for trend, 0.34), or when comparing unexposed, below median, and above median exposure for chronic lymphocytic leukaemia (*P* for trend, 0.63) and acute myeloid leukaemia (*P* for trend, 0.48). Budroni et al. (2010) reported an excess in NHL incidence among acrylonitrile-exposed workers

(SIR, 1.78; 95% CI, 0.96–3.30). The estimates for lymphoid leukaemia, myeloid leukaemia, and myeloma were imprecise and based on only 3 or 4 observed cases each.

Ott et al. (1980), Thiess et al. (1980), Benn and Osborne (1998), Delzell and Monson (1982), Swaen et al. (1992), Mastrangelo et al. (1993), and Marsh and Zimmerman (2015) reported inconsistent and imprecise SMRs for lymphohaematopoietic cancers.

Overall, the Working Group considered that the available studies in humans were not sufficiently informative to permit a conclusion to be drawn about the presence or absence of a causal association between exposure to acrylonitrile and NHL, chronic lymphocytic leukaemia, multiple myeloma, and acute myeloid leukaemia.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

Oral administration (gavage)

In a well-conducted study of chronic toxicity and carcinogenicity (NTP, 2001; also reported by Ghanayem et al., 2002) that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female B6C3F$_1$ mice (age, 6 weeks) were treated with acrylonitrile (purity, > 99%) at doses of 0 (control), 2.5, 10, or 20 mg/kg body weight (bw) in deionized water by gavage, 5 days per week, for 104–105 weeks. At study termination (104–105 weeks), survival in males was 38/50, 42/50, 39/50, and 14/50 and survival in females was 39/50, 32/50, 39/50, and 23/50 for the groups at 0, 2.5, 10, and 20 mg/kg bw, respectively. Survival of male and female mice at 20 mg/kg bw was significantly lower than that of the controls. Mean body weights of males and females at 20 mg/kg bw were generally less than those of mice in the vehicle control groups throughout most of the study; however, mean body weights of surviving females at 20 mg/kg bw were similar to those of the controls during the last 25 weeks of the study. A complete histopathological examination was performed on grossly visible lesions and major organs and tissues from all mice.

In males, there was a significant positive trend ($P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test]) in the incidence of squamous cell papilloma of the forestomach, of squamous cell carcinoma of the forestomach, and of squamous cell papilloma or carcinoma (combined) of the forestomach. The incidence of squamous cell papilloma of the forestomach – 3/50, 4/50, 19/50, and 25/50 in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively – was significantly higher ($P < 0.001$, poly-3 test; [$P < 0.0001$, Fisher exact test]) at 10 mg/kg bw and 20 mg/kg bw than in the control group. The incidence of squamous cell carcinoma of the forestomach – 0/50, 0/50, 8/50, and 9/50 in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively – was significantly higher at 10 mg/kg bw ($P = 0.004$, poly-3 test; [$P = 0.0029$, Fisher exact test]) and 20 mg/kg bw ($P < 0.001$, poly-3 test; [$P = 0.0013$, Fisher exact test]) than in the control group. The incidence of squamous cell papilloma or carcinoma (combined) of the forestomach – 3/50, 4/50, 26/50, and 32/50 in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively – was significantly higher ($P < 0.001$, poly-3 test; [$P < 0.0001$, Fisher exact test]) at 10 mg/kg bw and 20 mg/kg bw than in the control group. There was a significant positive trend ($P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test]) in the incidence of adenoma of the Harderian gland and of adenoma or carcinoma (combined) of the Harderian gland. The incidence of adenoma of the Harderian gland – 5/50, 16/50, 24/50, and 27/50 in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively – was significantly higher at 2.5 mg/kg bw ($P = 0.006$, poly-3 test; [$P = 0.0008$, Fisher exact test]), and at 10 and

Table 3.1 Studies of carcinogenicity in experimental animals exposed to acrylonitrile

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 Mouse, B6C3F$_1$ (M) 6 wk 104–105 wk NTP (2001)	Oral administration (gavage) Acrylonitrile, > 99% Deionized water (10 mL/kg bw) (NTP-2000 diet) 0, 2.5, 10, 20 mg/kg bw 5 d/wk for 104–105 wk 50, 50, 50, 50 38, 42, 39, 14	*Forestomach* Squamous cell papilloma 3/50, 4/50, 19/50*, 25/50* Squamous cell carcinoma 0/50, 0/50, 8/50*, 9/50** Squamous cell papilloma or carcinoma (combined) 3/50, 4/50, 26/50*, 32/50*	$P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test; [$P < 0.0001$, Fisher exact test] $P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test] *$P = 0.004$, poly-3 test; [$P = 0.0029$, Fisher exact test] **$P < 0.001$, poly-3 test; [$P = 0.0013$, Fisher exact test] $P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test; [$P < 0.0001$, Fisher exact test]	*Principal strengths*: GLP study, multiple-dose study, study covered most of the lifespan, adequate duration of exposure and observation, adequate number of animals, both sexes used, complete histopathological examination.

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	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6 wk 104–105 wk NTP (2001) (cont.)		*Harderian gland* Adenoma 5/50, 16/50*, 24/50**, 27/50**	$P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test] *$P = 0.006$, poly-3 test; [$P = 0.0008$, Fisher exact test] **$P < 0.001$, poly-3 test; [$P < 0.0001$, Fisher exact test]	
		Carcinoma 1/50 (2%), 1/50 (2%), 4/50 (8%), 3/50 (6%)	NS	
		Adenoma or carcinoma (combined) 6/50, 16/50*, 27/50**, 30/50**	$P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test] *$P = 0.014$, poly-3 test; [$P = 0.0041$, Fisher exact test] **$P < 0.001$, poly-3 test; [$P < 0.0001$, Fisher exact test]	

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	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 104–105 wk NTP (2001)	Oral administration (gavage) Acrylonitrile, > 99% Deionized water (10 mL/kg bw) (NTP-2000 diet) 0, 2.5, 10, 20 mg/kg bw 5 d/wk 50, 50, 50, 50 39, 32, 39, 23	*Forestomach* Squamous cell papilloma 3/50, 6/50, 24/50*, 19/50*	$P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test; [$P < 0.0001$, Fisher exact test]	*Principal strengths*: GLP study, multiple-dose study, study covered most of the lifespan, adequate duration of exposure and observation, adequate number of animals used, both sexes used, complete histopathological examination.
		Squamous cell carcinoma 0/50, 1/50, 1/50, 11/50*	$P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test; [$P = 0.0003$, Fisher exact test]	
		Squamous cell papilloma or carcinoma (combined) 3/50, 7/50, 25/50*, 29/50*	$P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test; [$P < 0.0001$, Fisher exact test]	
		Harderian gland Adenoma 10/50, 10/50, 25/50*, 23/50**	$P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test; [$P = 0.0015$, Fisher exact test] **$P < 0.001$, poly-3 test; [$P = 0.0004$, Fisher exact test]	

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	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 104–105 wk NTP (2001) (cont.)		Carcinoma 1/50 (2%), 0/50, 3/50 (6%), 2/50 (4%)	NS	
		Adenoma or carcinoma (combined) 11/50, 10/50, 26/50*, 25/50**	$P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test; [$P = 0.0017$, Fisher exact test] **$P < 0.001$, poly-3 test; [$P = 0.0032$, Fisher exact test]	
		Lung Bronchioloalveolar adenoma 4/50 (8%), 1/50 (2%), 8/50 (16%), 5/50 (10%)	NS	
		Bronchioloalveolar carcinoma 2/50 (4%), 5/50 (10%), 6/50 (12%), 4/50 (8%)	NS	
		Bronchioloalveolar adenoma or carcinoma (combined) 6/50 (12%), 6/50 (12%), 14/50 (28%)*, 9/50 (18%)	$P = 0.029$, poly-3 trend test *$P = 0.039$, poly-3 test; [$P = 0.0392$, Fisher exact test]	

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	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 104–105 wk NTP (2001) (cont.)		*Ovary* Benign granulosa cell tumour 0/50, 0/50, 3/50 (6%), 1/50 (2%) Malignant granulosa cell tumour 0/50, 0/50, 1/50 (2%), 0/50 Benign or malignant granulosa cell tumour (combined) 0/50, 0/50, 4/50 (8%), 1/50 (2%)	NS NS NS	
Full carcinogenicity Rat, CD (Sprague-Dawley) (M) NR 24 mo Gallagher et al. (1988)	Oral administration (drinking-water) Acrylonitrile, NR Tap water 0, 20, 100, 500 ppm Ad libitum 20, 20, 20, 20 4, 7, 3, 0	*Zymbal gland* Tumours [mostly squamous carcinoma] 0/18, 0/20, 1/19, 9/18* *Forestomach* Papilloma 0/20, 0/20, 0/20, 4/20	[$P < 0.0001$, Cochran–Armitage trend test]; *$P < 0.005$, χ^2 test; [$P = 0.0005$, Fisher exact test] [$P = 0.0002$, Cochran–Armitage trend test]	*Principal strengths*: Multiple-dose study, covered most of the lifespan, adequate duration of exposure and observation. *Principal limitations*: Small number of animals per group, limited histopathological evaluation, study in one sex only, lack of information on purity.
Full carcinogenicity Rat, Sprague-Dawley (Spartan substrain) (M) 8–12 wk 2 yr (746 d) Quast (2002)	Oral administration (drinking-water) Acrylonitrile, > 99% Drinking-water 0, 35, 100, 300 ppm Ad libitum 80, 47, 48, 48 7, 5, 5, 0	*Nervous system* Focal or multifocal glial cell proliferation (suggestive of early tumour) 0/80, 4/47*, 3/48, 7/48* Focal or multifocal glial cell tumour (astrocytoma) 1/80, 8/47*, 19/48*, 23/48*	*$P < 0.05$, Fisher exact test *$P < 0.05$, Fisher exact test	*Principal strengths*: Multiple-dose study, study covered most of the lifespan, adequate duration of exposure and observation, adequate number of animals, both sexes used, complete histopathological examination for animals exposed at 0 and 300 ppm. *Principal limitations*: Incomplete histopathological examination for animals exposed at 35 and 100 ppm.

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	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (Spartan substrain) (M) 8–12 wk 2 yr (746 d) Quast (2002) (cont.)		Focal or multifocal glial cell proliferation or astrocytoma (combined) 1/80, 12/47*, 22/48*, 30/48* *Tongue* Papilloma or carcinoma (combined) of the squamous epithelium 1/80, 2/47, 4/48, 5/48* *Forestomach* Squamous cell papilloma [0/80], [2/47], [17/48]*, [25/48]* Squamous cell carcinoma [0/80], [0/47], [10/48]*, [25/48]* Squamous cell papilloma or carcinoma (combined) 0/80, 2/47, 23/48*, 39/48* *Zymbal gland* Carcinoma 3/80, 4/47, 3/48, 16/48*	*$P < 0.05$, Fisher exact test *$P < 0.05$, Fisher exact test *[$P < 0.0001$, Fisher exact test] *[$P < 0.0001$, Fisher exact test] *$P < 0.05$, Fisher exact test *$P < 0.05$, Fisher exact test	

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	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (Spartan substrain) (F) 8–12 wk 2 yr (746 d) Quast (2002)	Oral administration (drinking-water) Acrylonitrile, > 99% Drinking-water 0, 35, 100, 300 ppm Ad libitum 80, 48, 48, 48 20, 4, 1, 0	*Nervous system* Focal or multifocal glial cell proliferation (suggestive of early tumour) 0/80, 3/48, 3/48, 7/48*	*$P < 0.05$, Fisher exact test	*Principal strengths*: Multiple-dose study, covered most of the lifespan, adequate duration of exposure and observation, adequate number of animals, both sexes used, complete histopathological examination for animals exposed at 0 and 300 ppm. *Principal limitations*: Incomplete histopathological examination for animals exposed at 35 and 100 ppm.
		Focal or multifocal glial cell tumour (astrocytoma) 1/80, 17/48*, 22/48*, 24/48*	*$P < 0.05$, Fisher exact test	
		Focal or multifocal glial cell proliferation or astrocytoma (combined) 1/80, 20/48*, 25/48*, 31/48*	*$P < 0.05$, Fisher exact test	
		Tongue Papilloma or carcinoma (combined) of the squamous epithelium 0/80, 1/48, 2/48, 12/48*	*$P < 0.05$, Fisher exact test	
		Forestomach Squamous cell papilloma [1/80], [1/48], [12/48]*, [29/48]*	*[$P < 0.0001$, Fisher exact test]	
		Squamous cell carcinoma [0/80], [0/48], [0/48], [12/48]*	*[$P < 0.0001$, Fisher exact test]	
		Squamous cell papilloma or carcinoma (combined) 1/80, 1/48, 12/48*, 30/48*	*$P < 0.05$, Fisher exact test	

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	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (Spartan substrain) (F) 8–12 wk 2 yr (746 d) Quast (2002) (cont.)		*Zymbal gland* Adenoma 0/80, 1/48, 3/48, 4/48*	*$P < 0.05$, Fisher exact test	
		Carcinoma 1/80, 4/48*, 6/48*, 14/48*	*$P < 0.05$, Fisher exact test	
		Adenoma or carcinoma (combined) 1/80, 5/48*, 9/48*, 18/48*	*$P < 0.05$, Fisher exact test	
		Mammary gland Benign tumours (fibroadenoma or adenoma) [57/80], [41/48], [39/48], [25/48]	[NS]	
		Carcinoma [6/80], [7/48], [9/48], [13/48]*	*[$P = 0.0033$, Fisher exact test]	
		Benign tumours (fibroadenoma or adenoma) or carcinoma (combined) 58/80, 42/48*, 42/48*, 35/48	*$P < 0.05$, Fisher exact test	
		Small intestine Mucous cystadenocarcinoma without metastasis 0/80, 1/48, 4/48*, 4/48*	*$P < 0.05$, Fisher exact test	

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	Significance	Comments
Full carcinogenicity Rat, F344 (M) 9–10 wk ≤ 26 mo Johannsen and Levinskas (2002a)	Oral administration (drinking-water) Acrylonitrile, 100% Distilled water 0, 1, 3, 10, 30, 100 ppm Ad libitum for 26 mo (equivalent doses: 0, 0.1, 0.3, 0.8, 2.5, 8.4 mg/kg bw) 200, 100, 100, 100, 100, 101 NR	*Brain* Astrocytoma 2/200, 2/100, 1/100, 2/100, 10/99*, 21/99* *Spinal cord* Astrocytoma 1/196, 0/99, 0/92, 0/98, 0/99, 4/93* *Zymbal gland* Squamous cell papilloma, adenoma or carcinoma (combined) 2/189, 1/97, 0/93, 2/88, 7/94*, 16/93* *Forestomach* Squamous cell papilloma or carcinoma (combined) 0/199, 1/100, 4/97*, 4/100*, 4/100*, 1/101	*$P < 0.01$, Fisher exact test *$P < 0.05$, Fisher exact test *$P < 0.01$, Fisher exact test *$P < 0.05$, Fisher exact test	*Principal strengths*: Multiple-dose study, covered most of the lifespan, adequate duration of exposure and observation, high number of animals per group, both sexes used. *Principal limitations*: Survival estimate was given as a fraction on a graph, histopathology involved limited sampling of non-target tissues from rats at the lowest dose.

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	Significance	Comments
Full carcinogenicity Rat, F344 (F) 9–10 wk ≤ 23–24 mo Johannsen and Levinskas (2002a)	Oral administration (drinking-water) Acrylonitrile, 100% Distilled water 0, 1, 3, 10, 30, 100 ppm Ad libitum for 23–24 mo (equivalent doses: 0, 0.1, 0.4, 1.3, 3.7, 10.9 mg/kg bw) 200, 100, 100, 100, 100, 99 NR	*Brain* Astrocytoma 1/199, 1/100, 2/100, 4/95, 6/100*, 23/98*	*$P < 0.01$, Fisher exact test	*Principal strengths*: Multiple-dose study, covered most of the lifespan, adequate duration of exposure and observation, high number of animals per group, both sexes used. *Principal limitations*: Survival estimate was given as a fraction on a graph, histopathology involved limited sampling of non-target tissues from rats at the lowest dose.
		Zymbal gland Squamous cell papilloma, adenoma or carcinoma (combined) 0/193, 0/94, 2/92, 4/90*, 5/94*, 10/86*	*$P < 0.01$, Fisher exact test	
		Forestomach Squamous cell papilloma or carcinoma (combined) 1/199, 1/100, 2/100, 2/97, 4/100*, 2/97	*$P < 0.05$, Fisher exact test	
Full carcinogenicity Rat, Spartan (Sprague-Dawley-derived) (M) 8–9 wk ≤ 22 mo Johannsen and Levinskas (2002b)	Oral administration (drinking-water) Acrylonitrile, 100% Distilled water 0, 1, 100 ppm Ad libitum for 22 mo (equivalent doses: 0, 0.09 and 8.0 mg/kg bw) 100, 100, 100 NR	*Zymbal gland* Adenoma 0/100, 0/91, 5/93*	*$P < 0.05$, Fisher exact test	*Principal strengths*: Study covered most of the lifespan, adequate duration of exposure and observation, high number of animals per group, both sexes used, use of multiple doses. *Principal limitations*: Survival estimate was given as a fraction on a graph, histopathology involved limited sampling of non-target tissues from rats at the lowest dose.
		Carcinoma 1/100, 0/91, 14/93*	*$P < 0.01$, Fisher exact test	

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	Significance	Comments
Full carcinogenicity Rat, Spartan (Sprague-Dawley-derived) (F) 8–9 wk ≤ 19 mo Johannsen and Levinskas (2002b)	Oral administration (drinking-water) Acrylonitrile, 100% Distilled water 0, 1, 100 ppm Ad libitum for 19 mo (equivalent doses: 0, 0.15, and 10.7 mg/kg bw) 100, 100, 100 NR	*Brain* Astrocytoma 0/99, 1/100, 32/97*	*$P < 0.01$, Fisher exact test	*Principal strengths*: Study covered most of lifespan, adequate duration of exposure and observation, use of a high number of animals per group, male and female animals used, two-dose study. *Principal limitations*: Survival estimate was given as a fraction on a graph, histopathology involved limited sampling of non-target tissues from rats at the lowest dose.
		Spinal cord Astrocytoma 0/96, 0/99, 7/98*	*$P < 0.01$, Fisher exact test	
		Zymbal gland Carcinoma 0/99, 0/95, 7/98*	*$P < 0.01$, Fisher exact test	
		Forestomach Squamous cell papilloma 1/100, 4/99, 7/99*	*$P < 0.05$, Fisher exact test	
Full carcinogenicity Rat, Charles River [CRL:CDBS CD (SD)BR] (F) 36 d 48 wk Friedman and Beliles (2002)	Oral administration (drinking-water) Acrylonitrile, NR Deionized water 0, 100, 500 ppm Ad libitum for 48 wk (equivalent doses: 0, 20 ± 3 and 40 ± 8 mg/kg bw) 19, 20, 25 NR	*Brain or spinal cord* Astrocytoma 0/19, 1/20, 2/25	NS	*Principal strengths*: Two-dose study. *Principal limitations*: Small number of animals per group, limited histopathological evaluation, three-generation reproduction study not specifically designed to evaluate the carcinogenicity of AN, several statistical test not reported, lack of information on purity.
		Zymbal gland Tumours 0/19, 0/20, 2/24	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	Significance	Comments
Full carcinogenicity Rat, Charles River [CRL:CDBS CD (SD)BR] (F) Embryos Prenatal then postnatal exposure, followed by oral administration for 48 wk from age 21 d (weanling) Friedman and Beliles (2002)	Prenatal then postnatal exposure, followed by oral administration (drinking-water) Acrylonitrile, NR Deionized water 0, 100, 500 ppm Ad libitum 20, 19, 17 NR	*Brain or spinal cord* Astrocytoma 0/20, 1/19, 4/17* *Zymbal gland* Tumours 0/20, 2/19, 3/17*	 *$P < 0.05$, statistical test NR; [$P = 0.036$, Fisher exact test] *$P < 0.05$, statistical test NR; [NS, Fisher exact test]	*Principal strengths*: Two-dose study. *Principal limitations*: Small number of animals per group, limited histopathological evaluation, three-generation reproduction study not specifically designed to evaluate the carcinogenicity of AN, several statistical test not reported, lack of information on purity.
Full carcinogenicity Rat, Charles River [CRL:CDBS CD (SD)BR] (F) Embryos Prenatal then postnatal exposure, followed by oral administration for 48 wk from age 21 d (weanling) Friedman and Beliles (2002)	Prenatal then postnatal exposure, followed by oral administration (drinking-water) Acrylonitrile, NR Deionized water 0, 100, 500 ppm Ad libitum 20, 20, 20 NR	*Brain or spinal cord* Astrocytoma 0/20, 1/20, 1/20 *Zymbal gland* Tumours 0/20, 0/20, 3/20	 NS NS	*Principal strengths*: Two-dose study. *Principal limitations*: Small number of animals per group, limited histopathological evaluation, three-generation reproduction study not specifically designed to evaluate the carcinogenicity of AN, several statistical test not reported, lack of information on purity.
Full carcinogenicity Rat, Sprague-Dawley (M) 10 wk Lifetime (≤ 131 wk) Maltoni et al. (1988)	Oral administration (gavage) Acrylonitrile, > 99.9% Olive oil 0, 5 mg/kg bw 1 × /d, 3 d/wk, for 52 wk 75, 40 NR	*Adrenal gland* Pheochromocytoma 2/75, 4/40 *Zymbal gland* Carcinoma 0/75, 1/40	 NS NS	*Principal strengths*: Male and female animals used, study covered lifespan, complete histopathological examination, adequate duration of observation, both sexes used. *Principal limitations*: Use of a single dose, 1-yr exposure.

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	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 10 wk Lifetime (≤ 131 wk) Maltoni et al. (1988)	Oral administration (gavage) Acrylonitrile, > 99.9% Olive oil 0, 5 mg/kg bw 1 × /d, 3 d/wk, for 52 wk 75, 40 NR	*Adrenal gland* Pheochromocytoma 2/75, 1/40	NS	*Principal strengths*: Male and female animals used, study covered lifespan, complete histopathological examination, adequate duration of observation, both sexes used. *Principal limitations*: Use of a single dose, 1-yr exposure.
Full carcinogenicity Rat, Spartan (Sprague-Dawley-derived) (M) 6–7 wk ≤ 20 mo Johannsen and Levinskas (2002b)	Oral administration (gavage) Acrylonitrile, 100% Distilled water 0, 0.1, 10 mg/kg bw 1 × /d 100, 100, 100 NR	*Brain* Astrocytoma 2/100, 0/99, 16/97* *Zymbal gland* Adenoma 0/96, 1/93, 5/96* Carcinoma 1/96, 0/93, 10/96* Squamous cell papilloma 0/96, 0/93, 3/96 *Forestomach* Squamous cell papilloma 2/99, 6/97, 22/99* Squamous cell carcinoma 0/99, 0/97, 18/99* *Intestine* Adenocarcinoma 0/100, 1/100, 6/100*	 *$P < 0.01$, Fisher exact test *$P < 0.05$, Fisher exact test *$P < 0.01$, Fisher exact test NS *$P < 0.01$, Fisher exact test *$P < 0.01$, Fisher exact test *$P < 0.05$, Fisher exact test	*Principal strengths*: Study covered most of lifespan, adequate duration of exposure and observation, use of a high number of animals per group, male and female animals used, two doses used. *Principal limitations*: Survival estimate was given as a fraction on a graph, histopathology involved limited sampling of non-target tissues from rats at the lowest dose.

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	Significance	Comments
Full carcinogenicity Rat, Spartan (Sprague-Dawley-derived) (F) 6–7 wk ≤ 20 mo Johannsen and Levinskas (2002b)	Oral administration (gavage) Acrylonitrile, 100% Distilled water 0, 0.1, 10 mg/kg bw 1 × /d for 735 d (except weekends or holidays) [equivalent doses: 0, 43, or 173 mg/m³] 100, 100, 100 NR	*Brain* Astrocytoma 1/100, 2/98, 17/100*	*$P < 0.01$, Fisher exact test	*Principal strengths*: Study covered most of lifespan, adequate duration of exposure and observation, use of a high number of animals per group, male and female animals used, two doses used. *Principal limitations*: Survival estimate was given as a fraction on a graph, histopathology involved limited sampling of non-target tissues from rats at the lowest dose.
		Zymbal gland Carcinoma 0/85, 0/94, 9/94*	*$P < 0.01$, Fisher exact test	
		Adenoma 1/85, 0/94, 5/94	NS	
		Squamous cell papilloma 0/85, 0/94, 1/94	NS	
		Forestomach Squamous cell papilloma 2/99, 4/99, 16/99*	*$P < 0.05$, Fisher exact test	
		Mammary gland Carcinoma 8/100, 6/100, 22/100*	*$P < 0.01$, Fisher exact test	
Full carcinogenicity Rat, Sprague-Dawley (Spartan substrain) (M) 8–10 wk 2 yr (735 d) US EPA (1980)	Inhalation (whole-body exposure) Acrylonitrile, NR [> 99%] Air 0, 20, 80 ppm 6 h/d, 5 d/wk, for 735 d (except weekends or holidays) [equivalent doses: 0, 43, or 173 mg/m³] 100, 100, 100 18, 14, 4	*Nervous system* Focal or multifocal glial cell proliferation (suggestive of early tumour) (only) 0/100, 0/99, 7/99*	*$P < 0.05$, Fisher exact test	*Principal strengths*: Study covered most of lifespan, adequate duration of exposure and observation, male and female animals used, high number of animals per group, complete histopathological examination for animals exposed at 0 and 80 ppm, two doses used. *Principal limitations*: Incomplete histopathological examination for animals exposed at 20 ppm, lack of reporting for animals bearing more than one tumour of the nervous system, lack of information on purity.
		Focal or multifocal glial cell tumour (astrocytoma) (only) 0/100, 4/99, 15/99*	*$P < 0.05$, Fisher exact test	
		Focal or multifocal glial cell proliferation (suggestive of early tumour) or astrocytoma 0/100, 4/99, 22/99*	*$P < 0.05$, Fisher exact test	

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	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (Spartan substrain) (M) 8–10 wk 2 yr (735 d) US EPA (1980) (cont.)		*Tongue* Papilloma or carcinoma (combined) of the squamous epithelium 1/96, 0/14, 7/89* *Small intestine* Mucinous cystadenocarcinoma 2/99, 2/20, 14/98* *Small intestine or large intestine* Tumours 4/100, 3/100, 17/100* *Zymbal gland* Adenoma 1/100, 1/100, 0/100 Carcinoma 1/100, 3/100, 11/100* Adenoma or carcinoma (combined) 2/100, 4/100, 11/100*	*$P < 0.05$, Fisher exact test *$P < 0.05$, Fisher exact test *$P < 0.05$, Fisher exact test NS *$P < 0.05$, Fisher exact test *$P < 0.05$, Fisher exact test	

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	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (Spartan substrain) (F) 8–10 wk 2 yr (735 d) US EPA (1980)	Inhalation (whole-body exposure) Acrylonitrile, NR [> 99%] Air 0, 20, 80 ppm 6 h/d, 5 d/wk, for 735 d (except weekends or holidays) [equivalent doses: 0, 43, or 173 mg/m^3] 100, 100, 100 22, 9, 1	*Nervous system* Focal or multifocal glial cell proliferation (suggestive of early tumour) (only) 0/100, 4/100, 4/100 Focal or multifocal glial cell tumour (astrocytoma) (only) 0/100, 4/100, 16/100* Focal or multifocal glial cell proliferation (suggestive of early tumour) or astrocytoma 0/100, 8/100, 20/100* *Zymbal gland* Adenoma 0/100, 1/100, 0/100 Carcinoma 0/100, 0/100, 11/100* Adenoma or carcinoma (combined) 0/100, 1/100, 11/100* *Mammary gland* Fibroadenoma 79/100, 95/100*, 75/100 Benign and/or malignant tumours 88/100, 95/100, 85/100	 NS *$P < 0.05$, Fisher exact test *$P < 0.05$, Fisher exact test NS *$P < 0.05$, Fisher exact test *$P < 0.05$, Fisher exact test *$P < 0.05$, Fisher exact test NS	*Principal strengths*: Study covered most of lifespan, adequate duration of exposure and observation, male and female animals used, high number of animals per group, complete histopathological examination for animals exposed at 0 and 80 ppm, two doses used. *Principal limitations*: Incomplete histopathological examination for animals exposed at 20 ppm, lack of reporting for animals bearing more than one tumour of the nervous system, lack of information on purity.

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	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (Spartan substrain) (F) 8–10 wk 2 yr (735 d) US EPA (1980) (cont.)		Benign and malignant tumours (in the same animal) 4/100, 7/100, 15/100*	*$P < 0.05$, Fisher exact test	
		Benign tumours (fibroma, adenoma or fibroadenoma) 79/100, 88/100*, 63/100	*[$P = 0.0057$, Fisher exact test]	
		Malignant tumours (carcinomas) (only) 5/100, 0/100, 7/100	NS (for increase)	
		Adenocarcinoma 9/100, 7/100, 20/100*	*$P < 0.05$, Fisher exact test	
Full carcinogenicity Rat, Sprague-Dawley (M) 12 wk Lifetime (≤ 136 wk) Maltoni et al. (1988)	Inhalation (whole-body exposure) Acrylonitrile, > 99.9% Filtered air 0, 5, 10, 20, 40 ppm 4 h/d, 5 d/wk, for 52 wk [equivalent doses: 0, 11, 22, 43, or 87 mg/m^3] 30, 30, 30, 30, 30 NR	*Adrenal gland* Benign pheochromocytoma 6/30, 6/30, 8/30, 2/30, 6/30	NS	*Principal strengths*: Multiple-dose study, both sexes used, study covered the lifespan, complete histopathological examination, adequate duration of observation. *Principal limitations*: Small number of animals per group, rats may have tolerated exposure concentrations higher than 40 ppm, exposure for 52 wk only.
		Encephalic Glioma (oligodendroglioma) 0/30, 0/30, 0/30, 1/30, 2/30	NS	
		Zymbal gland Carcinoma 0/30, 0/30, 2/30, 0/30, 0/30	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	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 12 wk Lifetime (≤ 136 wk) Maltoni et al. (1988)	Inhalation (whole-body exposure) Acrylonitrile, > 99.9% Filtered air 0, 5, 10, 20, 40 ppm 4 h/d, 5 d/wk, for 52 wk [equivalent doses: 0, 11, 22, 43, or 87 mg/m³] 30, 30, 30, 30, 30 NR	*Adrenal gland* Benign pheochromocytoma 1/30, 5/30, 7/30*, 2/30, 0/30 *Encephalic* Glioma (oligodendroglioma) 0/30, 0/30, 0/30, 1/30, 1/30 *Zymbal gland* Carcinoma 0/30, 0/30, 1/30, 1/30, 0/30	*$P < 0.05$, statistical test NR; [$P = 0.0262$, Fisher exact test] NS NS	*Principal strengths*: Multiple-dose study, both sexes used, study covered the lifespan, complete histopathological examination, adequate duration of observation. *Principal limitations*: Small number of animals per group, rats may have tolerated exposure concentrations higher than 40 ppm, exposure for 52 wk only.
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk-breeders Lifetime (≤ 150 wk) Maltoni et al. (1988)	Inhalation (whole-body exposure) Acrylonitrile, > 99.9% Filtered air 0, 60 ppm 4 h/d, 5 d/wk, for 7 wk; then 7 h/d, 5 d/wk, for 97 wk [equivalent doses: 0, 130 mg/m³] 60, 54 NR	*Mammary gland* Benign and malignant tumours 24/60, 37/54	NS	*Principal strengths*: Adequate duration of exposure and observation, study covered lifespan, adequate number of animals used, complete histopathological examination. *Principal limitations*: Use of a single dose.

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	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) Embryos (12 d) Lifetime (≤ 150 wk) Maltoni et al. (1988)	Inhalation (whole-body exposure) Acrylonitrile, > 99.9% Filtered air 0, 60 ppm Prenatal then perinatal exposure; followed by 4 h/d, 5 d/wk, for 7 wk; then 7 h/d, 5 d/wk, for 97 wk 158, 67 NR	*Encephalic* Glioma (oligodendroglioma) 2/158, 11/67* *Zymbal gland* Carcinoma 2/158, 10/67* *Liver* Hepatomas 1/158, 5/67*	*$P < 0.01$, statistical test NR; [$P < 0.0001$, Fisher exact test] *$P < 0.01$, statistical test NR; [$P = 0.0001$, Fisher exact test] *$P < 0.05$, statistical test NR; [$P = 0.0096$, Fisher exact test]	*Principal strengths*: Adequate duration of exposure and observation, study covered lifespan, adequate number of animals used, male and female animals used, complete histopathological examination. *Principal limitations*: Use of a single dose.
Full carcinogenicity Rat, Sprague-Dawley (F) Embryos (12 d) Lifetime (≤ 150 wk) Maltoni et al. (1988)	Inhalation (whole-body exposure) Acrylonitrile, > 99.9% Filtered air 0, 60 ppm Prenatal then perinatal exposure; followed by 4 h/d, 5 d/wk, for 7 wk; then 7 h/d, 5 d/wk, for 97 wk 149, 54 NR	*Encephalic* Glioma (oligodendroglioma) 2/149, 10/54* *Extrahepatic* Angiosarcoma 0/149, 3/54* *Mammary gland* Malignant tumours Tumour incidence: 8/149, 9/54* Adenocarcinoma 6/149, 8/54*	*$P < 0.01$, statistical test NR; [$P < 0.0001$, Fisher exact test] *$P < 0.05$, statistical test NR; [$P = 0.0181$, Fisher exact test] *$P < 0.05$, statistical test NR; [$P = 0.0146$, Fisher exact test] *[$P = 0.0122$, Fisher exact test]	*Principal strengths*: Adequate duration of exposure and observation, study covered lifespan, adequate number of animals used, male and female animals used, complete histopathological examination. *Principal limitations*: Use of a single dose.

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	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) Embryos (12 d) Lifetime (≤ 150 wk) Maltoni et al. (1988)	Inhalation (whole-body exposure) Acrylonitrile, > 99.9% Filtered air 0, 60 ppm Prenatal then perinatal exposure; followed by 4 h/d, 5 d/wk, for 7 wk; then 7 h/d, 5 d/wk, for 8 wk 158, 60 NR	*Zymbal gland* Carcinoma 2/158, 4/60	NS	*Principal strengths*: Study covered lifespan, adequate number of animals used, male and female animals used, adequate duration of observation, complete histopathological examination. *Principal limitations*: Short duration of exposure, use of a single dose.
Full carcinogenicity Rat, Sprague-Dawley (F) Embryos (12 d) Lifetime (≤ 150 wk) Maltoni et al. (1988)	Inhalation (whole-body exposure) Acrylonitrile, > 99.9% Filtered air 0, 60 ppm Prenatal then perinatal exposure; followed by 4 h/d, 5 d/wk, for 7 wk; then 7 h/d, 5 d/wk, for 8 wk 149, 60 NR	*Haematopoietic and lymphoid tissues* Leukaemia 1/149, 6/60*	*[$P = 0.0025$, Fisher exact test]	*Principal strengths*: Study covered lifespan, adequate number of animals used, male and female animals used, adequate duration of observation, complete histopathological examination. *Principal limitations*: Short duration of exposure, use of a single dose.

bw, body weight; d, day(s); F, female; F344, Fischer 344; GLP, Good Laboratory Practice; h, hour(s); M, male; mo, month(s); NR, not reported; NS, not significant; ppm, parts per million; wk, week(s); yr, year(s).

20 mg/kg bw ($P < 0.001$, poly-3 test; [$P < 0.0001$, Fisher exact test]) than in the control group. The incidence of adenoma or carcinoma (combined) of the Harderian gland – 6/50, 16/50, 27/50, and 30/50 in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively – was significantly higher at 2.5 mg/kg bw ($P = 0.014$, poly-3 test; [$P = 0.0041$, Fisher exact test]), and at 10 and 20 mg/kg bw ($P < 0.001$, poly-3 test; [$P < 0.0001$, Fisher exact test]) than in the control group. The incidence of carcinoma of the Harderian gland was 1/50 (2%), 1/50 (2%), 4/50 (8%), and 3/50 (6%) in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively, exceeding the upper bound of the range for historical controls at 10 mg/kg bw and 20 mg/kg bw, which was 4/659 (0.6%); range, 0–4% (all routes of administration); from 2-year studies.

In females, there was a significant positive trend ($P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test]) in the incidences of squamous cell papilloma of the forestomach, of squamous cell carcinoma of the forestomach, and of squamous cell papilloma or carcinoma (combined) of the forestomach. The incidence of squamous cell papilloma of the forestomach – 3/50, 6/50, 24/50, and 19/50 in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively – was significantly higher ($P < 0.001$, poly-3 test; [$P < 0.0001$, Fisher exact test]) at 10 and 20 mg/kg bw than in the control group. The incidence of squamous cell carcinoma of the forestomach – 0/50, 1/50, 1/50, and 11/50 in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively – was significantly higher at 20 mg/kg bw ($P < 0.001$, poly-3 test; [$P = 0.0003$, Fisher exact test]) than in the control group. The incidence of squamous cell papilloma or carcinoma (combined) of the forestomach – 3/50, 7/50, 25/50, and 29/50 in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively – was significantly higher ($P < 0.001$, poly-3 test; [$P < 0.0001$, Fisher exact test]) at 10 and 20 mg/kg bw than in the control group. There was a significant positive trend ($P < 0.001$, poly-3 trend test; [$P < 0.001$, Cochran–Armitage trend test]) in the incidence of adenoma of the Harderian gland and of adenoma or carcinoma (combined) of the Harderian gland. The incidence of adenoma of the Harderian gland – 10/50, 10/50, 25/50, and 23/50 in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively – was significantly higher at 10 mg/kg bw ($P < 0.001$, poly-3 test [$P = 0.0015$, Fisher exact test]) and 20 mg/kg bw ($P < 0.001$, poly-3 test; [$P = 0.0004$, Fisher exact test]) than in the control group. The incidence of adenoma or carcinoma (combined) of the Harderian gland – 11/50, 10/50, 26/50, and 25/50 in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively – was significantly higher at 10 mg/kg bw ($P < 0.001$, poly-3 test; [$P = 0.0017$, Fisher exact test]) and 20 mg/kg bw ($P < 0.001$, poly-3 test; [$P = 0.0032$, Fisher exact test]) than in the control group. The incidence of carcinoma of the Harderian gland was 1/50 (2%), 0/50, 3/50 (6%), and 2/50 (4%) in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively, exceeding the upper bound of the range for historical controls at 10 mg/kg bw – 9/659 (1.3%); range, 0–4% (all routes of administration); from 2-year studies, while it equalled the upper bound at 20 mg/kg bw.

In females, there was a significant positive trend ($P = 0.029$, poly-3 trend test) in the incidence of bronchioloalveolar adenoma or carcinoma (combined). The incidence of bronchioloalveolar adenoma or carcinoma (combined) – 6/50 (12%), 6/50 (12%), 14/50 (28%), and 9/50 (18%) in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively – was significantly higher at 10 mg/kg bw ($P = 0.039$, poly-3 test; [$P = 0.0392$, Fisher exact test]) than in the control group, exceeding the upper bound of the range for historical controls at 10 mg/kg bw and 20 mg/kg bw – 53/654 (7.6%); range, 0–12% (all routes of administration); from 2-year studies. The incidence of bronchioloalveolar adenoma was 4/50 (8%), 1/50 (2%), 8/50 (16%), and 5/50 (10%) in the groups at 0 (control), 2.5, 10, and

20 mg/kg bw, respectively, exceeding the upper bound of the range for historical controls at 10 mg/kg bw – 37/654 (5.4%); range, 0–12% (all routes of administration); from 2-year studies. The incidence of bronchioloalveolar carcinoma was 2/50 (4%), 5/50 (10%), 6/50 (12%), and 4/50 (8%) in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively, exceeding the upper bound of the range for historical controls at 2.5, 10, and 20 mg/kg bw – 17/654 (2.3%); range, 0–6% (all routes of administration); from 2-year studies.

The incidence of benign or malignant granulosa cell tumour (combined) of the ovary was 0/50, 0/50, 4/50 (8%), and 1/50 (2%) in groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively, exceeding the upper bound of the range for historical controls at 10 mg/kg bw – 5/626 (0.7%); range, 0–3% (all routes of administration); from 2-year studies. The incidence of benign granulosa cell tumour of the ovary was 0/50, 0/50, 3/50 (8%), and 1/50 (2%) in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively, exceeding the upper bound of the range for historical controls at 10 mg/kg bw – 5/626 (0.7%); range, 0–3% (all routes of administration); from 2-year studies. The incidence of malignant granulosa cell tumour of the ovary was 0/50, 0/50, 1/50, and 0/50 in the groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively. [The incidence of malignant granulosa cell tumour in historical controls (all routes of administration) from 2-year studies was not reported.]

Regarding non-neoplastic lesions in males, there was a significant increase in the incidence of mild focal or multifocal epithelial hyperplasia (combined) of the forestomach at 20 mg/kg bw and the incidence of mild diffuse or focal hyperkeratosis (combined) of the forestomach in the group at 20 mg/kg bw compared with controls. There was also a significant increase in the incidence of Harderian gland hyperplasia in the group at 10 mg/kg bw compared with controls. Regarding non-neoplastic lesions in females, there was a significant increase in the incidence of mild focal or multifocal epithelial hyperplasia (combined) of the forestomach in the group at 20 mg/kg bw compared with controls, and a significant increase in the incidence of atrophy and cyst of the ovary in the groups at 2.5 (cyst only), 10, and 20 mg/kg bw compared with controls.

[The Working Group noted that this was a well-conducted GLP study, multiple doses were used, the study covered most of lifespan, the duration of exposure and observation was adequate, the number of animals per group used was adequate, both males and females were used, and a complete histopathological examination was performed.]

3.2 Rat

3.2.1 Oral administration (drinking-water)

In a lifetime study of oral administration (Bigner et al., 1986), groups of 50 male and 50 female Fischer 344 rats (age, 6 weeks) were given drinking-water containing acrylonitrile [purity not reported] at a concentration of 100 or 500 ppm (mg/L) for life. Two additional groups of 51 males and 49 females served as controls and were given drinking-water only. Comparative survival studies were made for the groups above. [Body weights and survival were not reported but were described as being lower than those of controls for both sexes and both doses.] Tumour incidence was investigated in an additional group of 147 males and 153 females given drinking-water containing acrylonitrile at a concentration of 500 ppm, and subsets of animals in this group were euthanized periodically during the study. The authors reported that 215 rats exposed to acrylonitrile at 500 ppm and that either died or were euthanized between months 6 and 18 were examined microscopically for brain lesions. Of these, 49 rats [sex distribution not reported] had primary brain

tumours described as "similar to astrocytomas or anaplastic astrocytomas". Tumours of the Zymbal gland, stomach and skin papillomas, and brain tumours were more frequently seen in acrylonitrile-treated rats than in controls [incidence was not reported]. [The Working Group noted the very limited reporting, in particular, the lack of data on tumour incidence in controls, the unclear reporting of brain tumour incidence in acrylonitrile-treated animals, and the lack of data on tumour incidence in organs other than the brain. Therefore, the Working Group considered that this study was uninformative for the evaluation of the carcinogenicity of acrylonitrile and it was not tabulated.]

In a study by Gallagher et al. (1988), groups of 20 male CD (Sprague-Dawley) rats [age not reported] were given drinking-water (tap water, ad libitum) containing acrylonitrile [purity not reported] at a concentration of 0 (control), 20, 100, or 500 ppm (mg/L) for 24 months. At the end of the study (24 months), survival [read from graph] was 4/20, 7/20, 3/20, and 0/20 for the groups at 0, 20, 100, and 500 ppm, respectively. The authors reported that rats exposed to acrylonitrile at 500 ppm died sooner and that body-weight gain was consistently retarded by acrylonitrile exposure [numerical data were not provided]. The mean body weight of rats exposed to acrylonitrile at 100 ppm or 500 ppm showed a slower increase than that of rats in the control group in the first year of the study, and a greater decrease than that of rats in the control group during the second year of the study. Histopathological examination was performed on grossly visible lesions and on the liver, stomach, kidney, lung, brain, heart, adrenal gland, pituitary gland, and testes for all animals.

There was a significant positive trend [$P < 0.0001$, Cochran–Armitage trend test] in the incidence of tumours of the Zymbal gland [mostly squamous cell carcinomas]; the incidence – 0/18, 0/20, 1/19, and 9/18 in groups at 0 (control), 20, 100, and 500 ppm, respectively – was significantly higher at 500 ppm ($P < 0.005$, χ^2 test; [$P = 0.0005$, Fisher exact test]) than in the control group. There was also a significant positive trend [$P = 0.0002$, Cochran–Armitage trend test] in the incidence of squamous cell papilloma of the forestomach – 0/20, 0/20, 0/20, 4/20 in groups at 0 (control), 2.5, 10, and 20 mg/kg bw, respectively.

[The Working Group noted the use of multiple doses, the adequate duration of exposure and observation, and that the study covered most of lifespan. However, it was limited by the small number of animals per group, the use of one sex only, the lack of information on purity, and that the histopathological evaluation was limited to some organs.]

In a study by Quast (2002), groups of 48 male and 48 female Sprague-Dawley rats (Spartan substrain) (age, 8–12 weeks) were given drinking-water (ad libitum) containing acrylonitrile (purity, > 99%) at a concentration of 35, 100, or 300 ppm (mg/L) for up to 746 days. Two additional groups of 80 male and 80 female rats each served as controls. At the start of the study, the concentrations used were 0, 35, 85, and 210 ppm, on the basis of findings of significant "adverse effects" [the term used in the original paper, not further specified] at concentrations above 85 ppm in a 90-day study, but were increased to 0, 35, 100, and 300 ppm after day 21, on the basis of an "insufficient adverse effect" [not further specified] at 210 ppm before day 21 in the 2-year study. The equivalent doses of acrylonitrile were 0, 3.4, 8.5, and 21.2 mg/kg bw per day for the groups of males at 0, 35, 100, and 300 ppm, respectively; and 0, 4.4, 10.8, and 25.0 mg/kg bw per day for the groups of females at 0, 35, 100, and 300 ppm, respectively. One male rat at 35 ppm was found to have been incorrectly sexed as female and was removed from the study on day 56. At the end of the study (746 days), survival in males was 7/80, 5/47, 5/48, and 0/48 in the groups at 0, 35, 100, and 300 ppm, respectively; survival in females was 20/80, 4/48, 1/48 and 0/48 in the

groups at 0, 35, 100, and 300 ppm, respectively. Survival was significantly decreased in males at 300 ppm and in females at 35, 100, and 300 ppm compared with controls. Mean body weights were significantly decreased in males at 300 ppm and females at 300 ppm, compared with controls, within the first 5 days of the study until termination. A complete histopathological examination was performed on major organs and tissues (more than 40) and grossly visible lesions from all animals in the groups at 0 (controls) and 300 ppm. Histopathological examination was carried out on 22 specifically selected organs or tissues from nearly all [not further specified] rats exposed to acrylonitrile at 35 ppm or 100 ppm. All tumour or tumour-like lesions from all control or exposed rats were selected for histopathological evaluation.

In males, there was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of focal or multifocal glial cell proliferation (suggestive of early tumour) of the nervous system (brain, including cerebral cortex, cerebellum and brain stem; and spinal cord, combined) in groups at 35 ppm (4/47) and 300 ppm (7/48) compared with controls (0/80), of focal or multifocal glial cell tumour (described as astrocytoma) of the nervous system (brain and spinal cord, combined) at 35 (8/47), 100 (19/48) and 300 ppm (23/48) compared with controls (1/80), and of focal or multifocal glial cell proliferation (suggestive of early tumour) or focal or multifocal glial cell tumour (astrocytoma) (combined) of the nervous system [mainly of the brain] at 35 (12/47), 100 (22/48), and 300 ppm (30/48) compared with controls (1/80). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of papilloma or carcinoma (combined) of the squamous epithelium of the tongue in the groups at 300 ppm (5/48) compared with controls (1/80). There was a significant increase in the incidence of squamous cell papilloma of the forestomach at 100 [17/48] and 300 ppm [25/48] [$P < 0.0001$, Fisher exact test] compared with controls (0/80), of squamous cell carcinoma of the forestomach at 100 [10/48] and 300 ppm [25/48] [$P < 0.0001$, Fisher exact test] compared with controls (0/80), and of squamous cell papilloma or carcinoma (combined) of the forestomach at 100 (23/48) and 300 ppm (39/48) ($P < 0.05$, Fisher exact test) compared with controls (0/80). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of carcinoma of the Zymbal gland in the group at 300 ppm (16/48) compared with controls (3/80).

In females, there was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of focal or multifocal glial cell proliferation (suggestive of early tumour) of the nervous system (brain and spinal cord, combined) in the group at 300 ppm (7/48) compared with controls (0/80), of focal or multifocal glial cell tumour (described as astrocytoma) of the nervous system (brain and spinal cord, combined) at 35 (17/48), 100 (22/48), and 300 ppm (24/48) compared with controls (1/80), and of focal or multifocal glial cell proliferation (suggestive of early tumour) or focal or multifocal glial cell tumour (astrocytoma) (combined) of the nervous system [mainly of the brain] at 35 (20/48), 100 (25/48), and 300 ppm (31/48) compared with controls (1/80). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of papilloma or carcinoma (combined) of the squamous epithelium of the tongue at 300 ppm (12/48) compared with controls (0/80). There was a significant increase in the incidence of squamous cell papilloma of the forestomach at 100 (12/48) and 300 ppm (29/48) [$P < 0.0001$, Fisher exact test] compared with controls (1/80), of squamous cell carcinoma of the forestomach at 300 ppm [12/48] [$P < 0.0001$, Fisher exact test] compared with controls (0/80), and of squamous cell papilloma or carcinoma (combined) of the forestomach at 100 (12/48) and 300 ppm (30/48) ($P < 0.05$, Fisher exact test) compared with controls (1/80). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of adenoma of the Zymbal gland at 300 ppm (4/48)

compared with controls (0/80), of carcinoma of the Zymbal gland at 35 (4/48), 100 (6/48), and 300 ppm (14/48) compared with controls (1/80), and of adenoma or carcinoma (combined) of the Zymbal gland at 35 (5/48), 100 (9/48), and 300 ppm (18/48) compared with controls (1/80). There was a significant increase in the incidence of carcinoma of the mammary gland at 300 ppm [13/48] [$P = 0.0033$, Fisher exact test] compared with controls (6/80), and of benign tumours (fibroadenoma, adenoma) and/or carcinoma (combined) of the mammary gland at 35 (42/48) and 100 ppm (42/48) ($P < 0.05$, Fisher exact test) compared with controls (58/80). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of mucous cystadenocarcinoma without metastasis of the small intestine [this cystadenocarcinoma is always presented as with or without metastasis in pathological context] in the groups at 100 (4/48) and 300 ppm (4/48) compared with controls (0/80).

Regarding non-neoplastic lesions in males, there was a significant increase in the incidence of hyperplasia or hyperkeratosis (combined) of the forestomach in the groups at 100 and 300 ppm compared with controls. Regarding non-neoplastic lesions in females, there was a significant increase in the incidence of gliosis and perivascular cuffing of the brain in the groups at 35 and 100 ppm and of hyperplasia or hyperkeratosis (combined) of the forestomach at 35, 100 and 300 ppm compared with controls.

[The Working Group noted the use of multiple doses, the adequate duration of exposure and observation, the adequate number of animals per group, the use of both males and females, the complete histopathological examination for rats in the control group or exposed at 300 ppm, and that the study covered most of the lifespan. However, it was limited by the histopathological examination for rats at 35 and 100 ppm that was not as comprehensive as that for the controls and rats at 300 ppm.]

In a study by Johannsen and Levinskas (2002a), groups of 100 male and 100 female Fischer 344 rats (age, 9–10 weeks) were given drinking-water (distilled water, ad libitum) containing acrylonitrile (purity, 100%) at a concentration of 1, 3, 10, 30, or 100 ppm (mg/L) for up to 26 months (males) or up to 23–24 months (females). Two additional groups of 200 male and 200 female rats each were used as controls. The equivalent doses of acrylonitrile were 0, 0.1, 0.3, 0.8, 2.5 and 8.4 mg/kg bw per day for males at 0, 1, 3, 10, 30, and 100 ppm, respectively; and 0, 0.1, 0.4, 1.3, 3.7, and 10.9 mg/kg bw per day for females at 0, 1, 3, 10, 30, and 100 ppm, respectively. One male rat at 100 ppm was found to have been incorrectly sexed as female and was reassigned (actual numbers in the group at 100 ppm, 101 males and 99 females). One female at 3 ppm died on day 15 and was replaced.

In each group, 10 males and 10 females were euthanized at 6, 12, 18 months, and at study termination for histopathological evaluation. At study termination, all surviving animals were euthanized (males, at 26 months; and females, at 23–24 months) to ensure that at least 10 males and 10 females per group were available for histopathology.

At the end of the study (males, at 26 months; females, at 23–24 months), there was a significant decrease in survival [numerical values were not reported; survival estimate was given as a fraction on a graph] in males at 100 ppm and in females at 30 ppm and 100 ppm compared with controls. A significant decrease in mean body weights was observed in males and females at 100 ppm and in males at 30 ppm.

All animals, whether found dead or killed in a moribund condition, underwent complete histopathological examination of major organs and tissues (approximately 40) and grossly visible lesions. Although 10 males and 10 females per group were euthanized at each of the interim time points and at termination of the study, major organs and tissues were examined only

for rats in the groups at the highest dose and for half of the rats in the unexposed control group (5 males and 5 females from each control group); potential target organs such as the brain, ear canal, stomach, and spinal cord, and all grossly visible lesions, were examined microscopically in all animals.

In males, there was a significant increase in the incidence of brain astrocytoma ($P < 0.01$, Fisher exact test) at 30 ppm (10/99) and 100 ppm (21/99) compared with controls (2/200), and of spinal cord astrocytoma ($P < 0.05$, Fisher exact test) at 100 ppm (4/93) compared with controls (1/196). There was a significant increase ($P < 0.01$, Fisher exact test) in the incidence of squamous cell papilloma, adenoma, or carcinoma (combined) of the Zymbal gland at 30 ppm (7/94) and 100 ppm (16/93) compared with controls (2/189). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach at 3 (4/97), 10 (4/100), and 30 ppm (4/100) compared with controls (0/199).

In females, there was a significant increase ($P < 0.01$, Fisher exact test) in the incidence of brain astrocytoma at 30 ppm (6/100) and 100 ppm (23/98) compared with controls (1/199). There was a significant increase ($P < 0.01$, Fisher exact test) in the incidence of squamous cell papilloma, adenoma, or carcinoma (combined) of the Zymbal gland at 10 (4/90), 30 (5/94), and 100 ppm (10/86) compared with controls (0/193). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach at 30 ppm (4/100) compared with controls (1/199).

Regarding non-neoplastic lesions, an increase in the incidence of hyperplasia or hyperkeratosis of the squamous epithelium of the forestomach was observed in male and female rats at 3, 10, and 30 ppm.

[The Working Group noted that multiple doses were used, the study covered most of the lifespan, the duration of exposure and observation was adequate, and a high number of animals per group and both males and females were used. The Working Group also noted the limited sampling for non-target tissues in the groups other than the control and high-dose groups, and that survival was reported only as a fraction on a graph.]

In a study by Johannsen and Levinskas (2002b), groups of 100 male and 100 female Sprague-Dawley rats (Spartan substrain) (age, 8–9 weeks) were given drinking-water (distilled water, ad libitum) containing acrylonitrile (purity, 100%) at a concentration of 0 (control), 1, or 100 ppm (mg/L) for up to 22 months (males) or up to 19 months (females). The equivalent doses of acrylonitrile were 0, 0.09 and 8.0 mg/kg bw per day for males at 0, 1, and 100 ppm, respectively; and 0, 0.15, and 10.7 mg/kg bw per day for females at 0, 1, and 100 ppm, respectively. In each group, 10 males and 10 females were euthanized at 6, 12, 18 months, and at study termination for histopathological evaluation. At study termination, all surviving animals were euthanized to ensure that at least 10 males and 10 females per group were available for histopathology. At the end of the study (males, at 22 months; females, at 19 months), there was a significant decrease in survival [numerical values were not reported; survival estimate was given as a fraction on a graph] in females at 100 ppm compared with controls. A significant decrease in mean body weights was observed in males at 100 ppm. All animals were necropsied, whether they were found dead or moribund, or were euthanized by design. Although 10 males and 10 females per group were killed at each of the were euthanized at each of the interim time points and at termination of the study, major organs and tissues (approximately 40) were examined only for rats in the groups at the highest dose and from the unexposed controls; potential target organs such as the brain, ear canal, stomach, and spinal cord, and all grossly visible lesions were examined microscopically for all animals.

In males, there was a significant increase ($P < 0.01$, Fisher exact test) in the incidence of adenoma of the Zymbal gland (5/93) and of carcinoma of the Zymbal gland (14/93) in the group at 100 ppm compared with controls (0/100).

In females, there was a significant increase ($P < 0.01$, Fisher exact test) in the incidence of carcinoma of the Zymbal gland in the group at 100 ppm (7/98) compared with controls (0/99). There was a significant increase ($P < 0.01$, Fisher exact test) in the incidence of brain astrocytoma (32/97) and of spinal cord astrocytoma (7/98) in the group at 100 ppm compared with controls (0/99, 0/96, respectively). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of squamous cell papilloma of the forestomach in the group at 100 ppm (7/99) compared with controls (1/100).

Regarding non-neoplastic lesions, an increase in the severity of squamous cell hyperplasia of the forestomach was observed in male and female rats at 100 ppm.

[The Working Group noted that the study covered most of the lifespan, the duration of exposure and of observation was adequate, and a high number of animals per group, both males and females, and multiple doses were used. The Working Group also noted the limited sampling of non-target tissues from rats in the group at the lowest dose, and that survival was reported only as a fraction on a graph.]

In a three-generation study of reproductive toxicity (Friedman and Beliles, 2002), groups of male and female Charles River [CRL:CDBS CD (SD) BR] rats (F_0 generation), (age, 36 days), were given drinking-water containing acrylonitrile [purity unspecified] at a concentration of 0 (control), 100, or 500 ppm (mg/L). The equivalent doses of acrylonitrile were 0, 11 ± 5, and 37 ± 10 mg/kg bw for males at 0, 100, and 500 ppm, respectively; and 0, 20 ± 3, and 40 ± 8 mg/kg bw per day for females at 0, 100, and 500 ppm, respectively. At 500 ppm, acrylonitrile reduced the body-weight gain of males and females of the F_0 generation during the first 10 weeks of the study.

After 100 days, the rats were paired for mating for 6 days. Females of the F_0 generation were remated 2 weeks after removal of the F_{1a} pups to produce the F_{1b} offspring. At weaning (21 days), F_{1b} males and females were selected as potential breeders for the F_2 generation. The dams and the females that did not produce a litter were maintained for 20 weeks after weaning of the F_{1b} pups. The F_0 females were exposed for approximately 48 weeks. After that period, the three groups of 19–25 females at 0, 100, or 500 ppm were euthanized and subsequently necropsied, and histopathological examination was carried out on the sciatic nerve, gastrocnemius muscle, brain, and any abnormal tissues. The authors also indicated that an [unspecified] number of retained F_{1a} and F_{1b} rats were euthanized and subsequently necropsied at week 95, and that the stomach and intestines were inspected for papillomas [no results or further details or discussion on these animals were provided in the article].

As mentioned above, males and females from the F_{1b} generation were selected as parents for the F_2 generation. The animals were maintained as previously described. At age 100 days, the rats were mated, and two litters were produced as previously described. Female F_1 parental rats were maintained for 20 weeks after the weaning of the F_{2b} litter. The F_1 females were exposed for approximately 48 weeks. After that period, the three groups of 17–20 females exposed at 0, 100, or 500 ppm prenatally, perinatally, and via drinking-water were euthanized and subsequently necropsied, and histopathological examination was carried out on the sciatic nerve, gastrocnemius muscle, brain, and any abnormal tissues.

Males and females from the F_{2b} generation were selected as parents for the F_3 generation and maintained as previously described. At age 100 days, the rats were mated, and two litters were produced as previously described. Female F_2 parental rats were maintained for 20 weeks after

the weaning of the F_{3b} litter. The F_2 females were exposed for approximately 48 weeks. After that period, the three groups of 20 females exposed at 0, 100, or 500 ppm prenatally, perinatally, and via drinking-water were euthanized and subsequently necropsied, and histopathological examination was carried out on the sciatic nerve, gastrocnemius muscle, brain, and any abnormal tissues. In addition, 10 male and 10 female weanling F_{3b} rats in the control group and at 500 ppm that were probably exposed prenatally and perinatally, and via drinking-water, were submitted for histopathological examination.

Overall, F_0 female rats were exposed by oral administration for 48 weeks; F_1 and F_2 female rats were exposed prenatally, postnatally, and then by oral administration from age 21 days (weanling) for 48 weeks; and F_3 rats were exposed prenatally and postnatally.

In F_1 females exposed at 500 ppm, there was a significant increase ($P < 0.05$; [statistical test not reported by the authors, P values were calculated by the Working Group]; [$P = 0.036$, Fisher exact test, calculated by the Working Group]) in the incidence of astrocytoma of the brain or spinal cord (4/17) compared with controls (0/20). The authors also reported that in F_1 females at 500 ppm, there was a significant increase ($P < 0.05$, [statistical test not reported; not significant, Fisher exact test performed by the Working Group]) in the incidence of tumours of the Zymbal gland (3/17) compared with controls (0/20). There was no significant increase in the incidence of tumours in rats of the F_0 and F_2 generation at 100 ppm or 500 ppm compared with controls. No neoplasms were observed in rats of the F_{3b} generation.

[The Working Group also noted that this three-generation study of reproductive toxicity was not specifically designed to evaluate the carcinogenicity of acrylonitrile.] [The Working Group noted the use of two doses. However, this study was limited by the use of a small number of animals per group, no information on purity, statistical analysis was not provided, and the histopathological evaluation was restricted to a few selected organs.]

3.2.2 Oral administration (gavage)

In studies by Maltoni et al. (1977, 1982, 1987, 1988) [experiment BT 203], two groups of 40 male and 40 female Sprague-Dawley rats (age, 10 weeks) were treated with acrylonitrile (purity, > 99.9%) at a dose of 5 mg/kg bw in olive oil by gavage, once per day, three times per week, for 52 weeks. Two other groups of 75 males and 75 females each received olive oil alone and served as controls. Rats were allowed to live out their lifespan. Exposure to acrylonitrile did not affect body-weight gain or survival. Histopathological examination was performed on grossly visible lesions and major organs and tissues on all animals.

At the end of the study, no significant increase in the incidence of tumours at any site was observed in exposed males or exposed females. [The Working Group noted that this was a lifetime study, the duration of observation was adequate, both males and females were used, and a complete histopathological examination was performed. However the study was limited by the use of a single dose was used, and the rats were exposed for 1 year only.]

In a study by Johannsen and Levinskas (2002b), groups of 100 male and 100 female Sprague-Dawley rats (Spartan substrain) (age, 6–7 weeks) were treated with acrylonitrile (purity, 100%) at doses of 0 (control), 0.1, or 10 mg/kg bw in distilled water by gavage for up to 20 months. In each group, 10 males and 10 females were euthanized at 6, 12, 18 months, and at study termination, for histopathological examination. At study termination, all surviving animals were euthanized to ensure that at least 10 males and 10 females per group were available for histopathology; consequently, all groups were terminated at 20 months. At the end of the study, there

was a significant decrease in survival [numerical values were not reported; survival estimate was given as a fraction on a graph] in males and females at 10 mg/kg bw compared with controls. A significant decrease in mean body weight was observed in males at 10 mg/kg bw. All animals, whether found dead, moribund, or euthanized by design, were necropsied. Although 10 males and 10 females per group were euthanized at each of the interim time points and at termination of the study, major organs and tissues (approximately 40) were examined only for rats in the groups at the highest dose and from the unexposed controls; potential target organs such as the brain, ear canal, stomach, and spinal cord, and all grossly visible lesions were examined microscopically for all animals.

In males, there was a significant increase ($P < 0.01$, Fisher exact test) in the incidence of brain astrocytoma in the group at 10 mg/kg bw (16/97) compared with controls (2/100). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of adenoma of the Zymbal gland (5/96) and of carcinoma of the Zymbal gland (10/96) in the group at 10 mg/kg bw compared with controls (0/96 and 1/96, respectively). There was a significant increase ($P < 0.01$, Fisher exact test) in the incidence of squamous cell papilloma (22/99) and squamous cell carcinoma (18/99) of the forestomach in the group at 10 mg/kg bw compared with controls (2/99 and 0/99, respectively). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of adenocarcinoma of the intestine in the group at 10 mg/kg bw (6/100) compared with controls (0/100).

In females, there was a significant increase ($P < 0.01$, Fisher exact test) in the incidence of brain astrocytoma in the group at 10 mg/kg bw (17/100) compared with controls (1/100). There was a significant increase ($P < 0.01$, Fisher exact test) in the incidence of carcinoma of the Zymbal gland in the group at 10 mg/kg bw (9/94) compared with controls (0/85). There was a significant ($P < 0.05$, Fisher exact test) increase in the incidence of squamous cell papilloma of the forestomach in the group at 10 mg/kg bw (16/99) compared with controls (2/99). There was a significant increase ($P < 0.01$, Fisher exact test) in the incidence of carcinoma of the mammary gland in the group at 10 mg/kg bw (22/100) compared with controls (8/100).

Regarding non-neoplastic lesions, an increase in the severity of forestomach squamous cell hyperplasia was observed in male and female rats at 10 mg/kg bw compared with controls.

[The Working Group noted the adequate duration of exposure and observation, the use of a high number of animals per group, both males and females, and two doses, and that the study covered most of the lifespan. The Working Group also noted the limited sampling of non-target tissues from rats at the lowest dose, and that survival was reported only as a fraction on a graph.]

3.2.3 Inhalation

In a study by Quast and colleagues (US EPA, 1980), groups of 100 male and 100 female Sprague-Dawley rats (Spartan substrain) (age, 8–10 weeks) were exposed to acrylonitrile [purity, > 99%] at concentrations of 0 (control), 20, or 80 ppm (mg/L) [equivalent concentrations, 0, 43, or 173 mg/m^3] by inhalation (whole-body exposure) for up to 735 days (except weekends or holidays). The total number of days of exposure was 507 days for the chamber at 20 ppm and 508 days for the chamber at 80 ppm. At the end of the study (735 days), survival in males was 18/100, 14/100, and 4/100 for the groups at 0, 20, and 80 ppm, respectively; survival in females was 22/100, 9/100, and 1/100 for the groups at 0, 20, and 80 ppm, respectively. Survival was significantly decreased in males at 80 ppm and in females at 20 and 80 ppm compared with controls. Mean body weights were significantly decreased in males and females at 80 ppm, compared with controls, within the first 6 days

of the study. During the latter part of the study (612 days onwards), body weight was no longer significantly decreased in females at 80 ppm compared with controls [probably because many of these rats had developed tumour masses that masked the effect of exposure on body weight]. A complete histopathological examination was performed on all organs and tissues from all animals at 0 (controls) and 80 ppm. Twenty-three organs or tissues were selected for histopathological examination for >80% [not further specified] of the animals at 20 ppm. Histopathological examination was also carried out on all grossly visible lesions from all control or exposed rats.

In males, there was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of (only) focal or multifocal glial cell proliferation (suggestive of early tumour) of the nervous system (brain only: cerebral cortex, cerebellum and brain stem) in the group at 80 ppm (7/99), of (only) focal or multifocal glial cell tumour (astrocytoma) of the nervous system (brain and spinal cord, combined; [mainly brain]) at 80 ppm (15/99), and of focal or multifocal glial cell proliferation (suggestive of early tumour) or astrocytoma of the nervous system (brain and spinal cord, combined; [mainly brain]) in the group at 80 ppm (22/99) compared with controls (0/100). [The Working Group noted the lack of reporting of animals that had more than one tumour of the nervous system.] There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of papilloma or carcinoma (combined) of the squamous epithelium of the tongue in the group at 80 ppm (7/89) compared with controls (1/96). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of mucinous cystadenocarcinoma of the small intestine (with or without metastasis) in the group at 80 ppm (14/98) compared with controls (2/99). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of tumours of the small and large intestine in the group at 80 ppm (17/100) compared with controls (4/100). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of carcinoma of the Zymbal gland in the group at 80 ppm (11/100) compared with controls (1/100).

In females, there was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of (only) focal or multifocal glial cell tumour (astrocytoma) of the nervous system (brain and spinal cord, combined; [mainly brain]) in the group at 80 ppm (16/100), and of focal or multifocal glial cell proliferation (suggestive of early tumour) or astrocytoma of the nervous system (brain and spinal cord, combined; [mainly brain]) in the group at 80 ppm (20/100) compared with controls (0/100). [The Working Group noted the lack of reporting of animals that had more than one tumour of the nervous system.] There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of carcinoma of the Zymbal gland in the group at 80 ppm (11/100) compared with controls (0/100). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of fibroadenoma of the mammary gland in the group at 20 ppm (95/100) compared with controls (79/100). There was a significant increase [$P = 0.0057$, Fisher exact] test in the incidence of benign tumours (fibroma, adenoma, or fibroadenoma) of the mammary gland in the group at 20 ppm (88/100) compared with controls (79/100). There was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of adenocarcinoma of the mammary gland in the group at 80 ppm (20/100) compared with controls (9/100).

Regarding non-neoplastic lesions in males and females, there was a significant increase in the incidence of gliosis and perivascular cuffing of the brain in males at 80 ppm compared with controls.

[The Working Group noted the adequate duration of exposure and observation, the use of a high number of animals per group, both males and females, and two doses, the complete histopathological examination for rats exposed at 0 and 80 ppm, and that the study covered most

of the lifespan. However, the study was limited by the lack of information on purity, the incomplete histopathological examination for animals exposed at 20 ppm, and the lack of reporting of animals bearing more than one tumour of the nervous system.]

In studies by Maltoni et al. (1977, 1982, 1987, 1988) [experiment BT 201], groups of 30 male and 30 female Sprague-Dawley rats, (age, 12 weeks), were exposed to acrylonitrile (purity, >99.9%) at a concentration of 0 (control), 5, 10, 20, or 40 ppm (mg/L) [equivalent doses, 0, 11, 22, 43, or 87 mg/m^3] by inhalation (whole-body exposure) for 4 hours per day on 5 days per week for 52 weeks. Rats were allowed to live out their lifespan. Exposure to acrylonitrile did not affect body-weight gain or survival. A complete histopathological examination was performed on grossly visible lesions and major organs and tissues from all animals.

At the end of the study, there was a significant increase ($P < 0.05$, statistical test not reported; [$P = 0.0262$, Fisher exact test]) in the incidence of benign pheochromocytoma of the adrenal gland in female rats at 10 ppm (7/30) compared with controls (1/30).

No significant increase in the incidence of tumours at any site was observed in exposed males. [The Working Group that noted this was a lifetime study, multiple doses were used, the duration of observation was adequate, there was a complete histopathological examination, and both males and females were used. However, it was limited by the use of a small number of animals per group, a duration of exposure of52 weeks, and that rats may have tolerated exposure concentrations higher than 40 ppm.]

In a two-generation study by Maltoni et al. (1987, 1988) [experiment BT 4003,4006], a group of 54 female Sprague-Dawley rats (age, 13 weeks) was exposed to acrylonitrile (purity, 99.9%) at a concentration of 60 ppm [equivalent dose, 130 mg/m^3] by inhalation (whole-body exposure) for 4 hours per day on 5 days per week for 7 weeks. Of these rats, 22 were pregnant at the start of the study and delivered litters. Male offspring were divided into groups of 67 (group A, 104-week exposure experiment) and 60 (group B, 15-week exposure experiment) animals, and female offspring into groups of 54 (group A) and 60 (group B) animals. All groups were exposed for 4 hours per day on 5 days per week for the first 7 weeks after birth. Subsequently, the duration of exposure was increased to 7 hours per day, and breeders and group A offspring were exposed on 5 days per week for 97 weeks. Group B offspring were similarly exposed for 8 weeks. [The offspring could also have been exposed transplacentally, were exposed during weaning by inhalation, and possibly also via ingestion of milk.] A group of 60 female rats (age, 13 weeks) (24 were pregnant) was exposed to filtered air and served as controls, and a group of 158 male offspring and a group of 149 female offspring were also exposed to filtered air and served as controls. Rats were allowed to live out their lifespan. Exposure to acrylonitrile did not affect body-weight gain or survival. A complete histopathological examination was performed on grossly visible lesions and major organs and tissues from all animals.

At the end of the study, in male offspring exposed for 104 weeks, there was a significant increase in the incidence of encephalic glioma (oligodendroglioma) (11/67) ($P < 0.01$, statistical test NR; [$P < 0.0001$, Fisher exact test]), of Zymbal gland carcinoma (10/67) ($P < 0.01$, statistical test not reported; [$P = 0.0001$, Fisher exact test]) compared with controls (2/158). There was also a significant increase in the incidence of hepatoma (5/67) ($P < 0.05$, statistical test not reported; [$P = 0.0096$, Fisher exact test]) compared with controls (1/158). Hepatomas comprised proliferative lesions of the liver parenchyma, namely diffused hyperplasia, nodular hyperplasia and nodular dysplasia (neoplastic nodules). They were in some cases multicentric, presented medullary and trabecular patterns (e.g. hepatocellular

carcinoma), and showed various degrees of malignancy.

In female offspring exposed for 104 weeks, there was a significant increase in the incidence of encephalic glioma (oligodendroglioma) (10/54) ($P < 0.01$, statistical test not reported; [$P < 0.0001$, Fisher exact test]), of extrahepatic angiosarcoma (3/54) ($P < 0.05$, statistical test not reported; [$P = 0.0181$, Fisher exact test]), and of malignant tumours of the mammary gland (9/54) ($P < 0.05$, statistical test not reported; [$P = 0.0146$, Fisher exact test]), more specifically of adenocarcinoma of the mammary gland (8/54) [$P = 0.0122$, Fisher exact test], compared with controls (2/149, 0/149, and 8/149, respectively).

In female offspring exposed for 15 weeks, there was a significant increase (6/60) [$P = 0.0025$, Fisher exact test] in the incidence of leukaemia [a variety of tumours of the haematopoietic and lymphoid tissues at different sites], compared with controls (1/149).

There was no significant increase in the incidence of tumours at any site in male offspring exposed for 15 weeks or in exposed female breeders, compared with controls.

Regarding non-neoplastic lesions, there was a significant increase in the incidence of encephalic glial cell hyperplasia or dysplasia (combined) in male offspring and female offspring exposed for 104 weeks compared with controls.

[The Working Group noted that this was a lifetime study, that the durations of exposure (when the animals were exposed for 104 weeks) and of observation was adequate, there was a complete histopathological examination, and both males and females were used. However, it was limited by the use of a single dose, and the short duration of exposure (when the animals were exposed for 15 weeks).]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of acrylonitrile has been assessed in male and female B6C3F$_1$ mice treated by oral administration (gavage) in a well-conducted study that complied with GLP (NTP, 2001; also reported by Ghanayem et al., 2002). The carcinogenicity of acrylonitrile was also assessed in studies that did not comply with GLP. There was one study (Gallagher et al., 1988) in male CD (Sprague-Dawley) rats, two studies (Johannsen and Levinskas, 2002b; Quast, 2002) in male and female Sprague-Dawley rats (Spartan substrain), two studies (Bigner et al., 1986; Johannsen and Levinskas, 2002a) in male and female Fischer 344 rats, and a three-generation study of reproductive toxicity (not specifically designed to evaluate the carcinogenicity of acrylonitrile) (Friedman and Beliles, 2002) in male and female Charles River [CRL:CDBS CD (SD)BR] rats treated by oral administration (drinking-water). There was one study (Johannsen and Levinskas, 2002b) in male and female Sprague-Dawley rats (Spartan substrain) and one study (Maltoni et al., 1977, 1982, 1987, 1988) in male and female Sprague-Dawley rats exposed by oral administration (gavage). There was one study (US EPA, 1980) in male and female Sprague-Dawley rats (Spartan substrain) and one study (Maltoni et al., 1977, 1982, 1987, 1988) in male and female Sprague-Dawley rats exposed by inhalation (whole-body exposure). In addition, there was one transgenerational study (Maltoni et al., 1987, 1988) in which pregnant female Sprague-Dawley rats and their male and female offspring were exposed by inhalation (whole-body exposure).

In the well-conducted GLP study of oral administration (gavage) in male and female B6C3F$_1$ mice (NTP, 2001; also reported by Ghanayem et al., 2002), in males, there was a significant positive trend in the incidence of squamous cell papilloma of the forestomach, of squamous cell carcinoma of the forestomach,

and of squamous cell papilloma or carcinoma (combined) of the forestomach, with the incidence being significantly higher at the intermediate and highest doses than in the controls. In males, there was a significant positive trend in the incidence of adenoma of the Harderian gland and of adenoma or carcinoma (combined) of the Harderian gland, with a significant increase in incidence at all doses compared with controls. In females, there was a significant positive trend in the incidence of squamous cell papilloma of the forestomach, of squamous cell carcinoma of the forestomach, and of squamous cell papilloma or carcinoma (combined) of the forestomach. There was a significant increase in the incidence of squamous cell papilloma of the forestomach and of squamous cell papilloma or carcinoma (combined) of the forestomach at the intermediate and highest doses compared with controls. There was a significant increase in the incidence of squamous cell carcinoma of the forestomach at the highest dose compared with controls. There was a significant positive trend in the incidence of adenoma of the Harderian gland and of adenoma or carcinoma (combined) of the Harderian gland, with incidence being significantly higher in the groups at the intermediate and highest doses than in the controls. There was also a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined), and there was a significant increase in incidence at the intermediate dose compared with controls.

In the study in male CD (Sprague-Dawley) rats treated by oral administration (drinking-water) (Gallagher et al., 1988), there was a significant positive trend in the incidence of tumours [mostly squamous cell carcinomas] of the Zymbal gland, a significant increase in incidence at the highest dose compared with controls, and a significant positive trend in the incidence of papilloma of the forestomach.

In the study in male and female Sprague-Dawley rats (Spartan substrain) treated by oral administration (drinking-water) (Quast, 2002), in males, there was a significant increase in the incidence of focal or multifocal glial cell proliferation suggestive of early tumour of the nervous system (brain and spinal cord, combined) at the lower and higher doses compared with controls, and of focal or multifocal glial cell tumour (astrocytoma) of the nervous system, and of focal or multifocal glial cell proliferation (suggestive of early tumour) or astrocytoma (combined) of the nervous system, at all doses compared with controls. There was a significant increase in the incidence of papilloma or carcinoma (combined) of the squamous epithelium of the tongue and of Zymbal gland carcinoma at the highest dose compared with controls. There was a significant increase in the incidence of squamous cell papilloma of the forestomach, of squamous cell carcinoma of the forestomach, and of squamous cell papilloma or carcinoma (combined) of the forestomach at the intermediate and highest doses compared with controls. In females, there was a significant increase in the incidence of focal or multifocal glial cell proliferation (suggestive of early tumour) of the nervous system (brain and spinal cord, combined) at the highest dose compared with controls, and of focal or multifocal glial cell tumour (astrocytoma) of the nervous system, and of focal or multifocal glial cell proliferation (suggestive of early tumour) or astrocytoma (combined) of the nervous system at all doses compared with controls. There was a significant increase in the incidence of papilloma or carcinoma (combined) of the squamous epithelium of the tongue at the highest dose compared with controls. There was a significant increase in the incidence of squamous cell papilloma of the forestomach and of squamous cell papilloma or carcinoma (combined) of the forestomach at the intermediate and highest doses compared with controls, and of squamous cell carcinoma of the forestomach at the highest

dose compared with controls. There was a significant increase in the incidence of adenoma of the Zymbal gland at the highest dose compared with controls, of carcinoma of the Zymbal gland at the intermediate and highest doses, and of adenoma or carcinoma (combined) of the Zymbal gland at all doses compared with controls. There was a significant increase in the incidence of carcinoma of the mammary gland at the highest dose compared with controls, and of benign tumours or carcinoma (combined) of the mammary gland at the lowest and intermediate doses compared with controls. There was a significant increase in the incidence of mucous cystadenocarcinoma without metastasis of the small intestine at the intermediate and highest doses compared with controls.

In the study in male and female Fischer 344 rats treated by oral administration (drinking-water) (Johannsen and Levinskas, 2002a), in males, there was a significant increase in the incidence of brain astrocytoma at the high-intermediate and highest doses, and of spinal cord astrocytoma at the highest dose compared with controls. In males, there was a significant increase in the incidence of squamous cell papilloma, adenoma, or carcinoma (combined) of the Zymbal gland at the high-intermediate and highest doses compared with controls. There was a significant increase in the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach at the low-intermediate, intermediate, and high-intermediate doses compared with controls. In females, there was a significant increase in the incidence of brain astrocytoma at the high-intermediate and highest doses compared with controls. There was a significant increase in the incidence of squamous cell papilloma, adenoma, or carcinoma (combined) of the Zymbal gland at the intermediate and highest doses compared with controls. There was a significant increase in the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach at the high-intermediate dose compared with controls.

In the study in male and female Sprague-Dawley rats (Spartan substrain) treated by oral administration (drinking-water) (Johannsen and Levinskas, 2002b), in males, there was a significant increase in the incidence of adenoma and of carcinoma of the Zymbal gland at the highest dose compared with controls. In females, there was a significant increase in the incidence of astrocytoma of the brain or spinal cord, of carcinoma of the Zymbal gland, and of squamous cell papilloma of the forestomach, at the highest dose compared with controls.

In the three-generation study of reproductive toxicity in male and female Charles River [CRL:CDBS CD (SD)BR] rats treated by oral administration (drinking-water) (Friedman and Beliles, 2002) [not specifically designed to evaluate the carcinogenicity of acrylonitrile], there was a significant increase in the incidence of astrocytoma of the brain or spinal cord in F_1 females at the highest dose compared with controls. There was no significant increase in the incidence of tumours in female rats of the F_0, F_2, and F_3 generations exposed to acrylonitrile.

In the study in male and female Sprague-Dawley rats (Spartan substrain) treated by oral administration (gavage) (Johannsen and Levinskas, 2002b), there was a significant increase in the incidence of brain astrocytoma, of adenoma and carcinoma of the Zymbal gland, of squamous cell papilloma and squamous cell carcinoma of the forestomach, and of adenocarcinoma of the intestine in males at the highest dose compared with controls. In females at the highest dose, there was a significant increase in the incidence of brain astrocytoma, of carcinoma of the Zymbal gland, of carcinoma of the mammary gland, and of squamous cell papilloma of the forestomach, compared with controls.

In the study in male and female Sprague-Dawley rats (Spartan substrain) exposed by inhalation (whole-body exposure) (US EPA, 1980),

there was a significant increase in the incidence of (only) focal or multifocal glial cell proliferation (suggestive of early tumour) of the brain, of (only) focal or multifocal glial cell tumour (astrocytoma) of the nervous system (brain and spinal cord, combined), and of focal or multifocal glial cell proliferation (suggestive of early tumour) or astrocytoma of the nervous system (combined) in males at the highest concentration compared with controls. There was a significant increase in the incidence of papilloma or carcinoma (combined) of the squamous epithelium of the tongue, of mucinous cystadenocarcinoma of the small intestine, of tumours of the small or large intestine, and of carcinoma of the Zymbal gland in males at the highest concentration compared with controls. In females, there was a significant increase in the incidence of (only) focal or multifocal glial cell tumour (astrocytoma) of the nervous system (brain and spinal cord, combined), and of focal or multifocal glial cell proliferation (suggestive of early tumour) or astrocytoma of the nervous system (combined) at the highest concentration compared with controls. There was a significant increase in the incidence of carcinoma of the Zymbal gland in females at the highest concentration compared with controls. There was a significant increase in the incidence of fibroadenoma of the mammary gland at the lowest concentration, of benign tumours of the mammary gland at the lowest concentration compared with controls, and of carcinomas (all types) and adenocarcinoma of the mammary gland at the highest concentration compared with controls.

In the study in male and female Sprague-Dawley rats treated by inhalation (whole-body exposure) (Maltoni et al., 1977, 1982, 1987, 1988), there was a significant increase in the incidence of benign pheochromocytoma of the adrenal gland at 10 ppm in females compared with controls. No significant increase in the incidence of tumours was observed in exposed males.

In the two-generation study (Maltoni et al., 1987, 1988), pregnant female Sprague-Dawley rats were exposed to acrylonitrile by inhalation (whole-body exposure). Male and female offspring [possibly exposed transplacentally and by ingestion via lactation] were exposed to acrylonitrile by whole-body exposure for either 15 weeks or 104 weeks. In female offspring exposed for 15 weeks, there was a significant increase in the incidence of leukaemia (a variety of tumours of the haematopoietic and lymphoid tissues at different sites). There was no significant increase in the incidence of tumours in male offspring exposed for 15 weeks and in exposed female breeders. In male offspring exposed for 104 weeks, there was a significant increase in the incidence of encephalic glioma (oligodendroglioma), of carcinoma of the Zymbal gland, and of hepatoma. In female offspring exposed for 104 weeks, there was a significant increase in the incidence of encephalic glioma (oligodendroglioma), of extrahepatic angiosarcoma, and of malignant tumours of the mammary gland (mostly adenocarcinoma).

In the study in male and female Sprague-Dawley rats treated by oral administration (gavage) (Maltoni et al., 1977, 1982, 1987, 1988), no significant increase in the incidence of tumours was observed in exposed males and females.

The Working Group considered that the study in male and female Fischer 344 rats treated by oral administration (drinking-water) (Bigner et al., 1986) was uninformative for the evaluation of the carcinogenicity of acrylonitrile.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

(a) Absorption and distribution

Acrylonitrile can be readily absorbed from inhaled air as well as through the skin. In two studies on the inhalation of acrylonitrile, the retention of vapour in the respiratory tract was reported as (mean ± SD) 46 ± 1.6% (Rogaczewska and Piotrowski, 1968) and 52 ± 5% (n = 5) (Jakubowski et al., 1987). When undiluted liquid acrylonitrile was applied to the forearm at a dose of 1.07 mg/cm^2, it was absorbed through the skin at a rate of 0.6 mg/cm^2 per hour (Rogaczewska and Piotrowski, 1968). When 45 µL of neat liquid acrylonitrile was applied on dermatomed human abdominal skin ex vivo (a regulatory-approved ex vivo model for skin permeability tests), total cumulative absorption for 300 minutes post-application amounted to 67.0 ± 20.0 µg/cm^2 (mean ± SE; n = 6). The nominal skin diffusion area was 0.13 cm^2, and the skin thickness was 500 µm (Forsberg et al., 2020). [The Working Group noted that these results indicated that skin uptake could be a route of acrylonitrile exposure that contributes significantly to body burden.]

(b) Metabolism and excretion

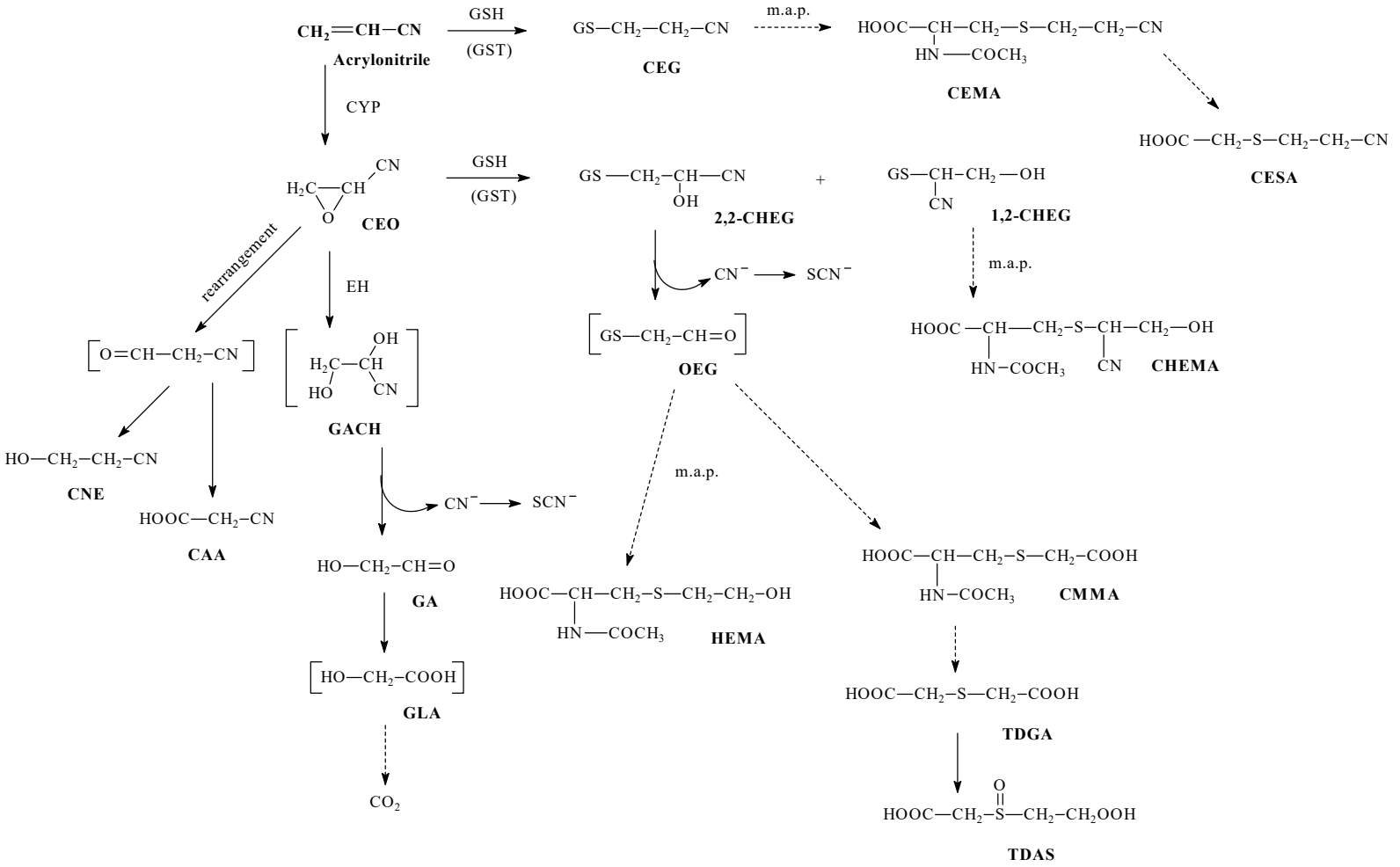
After absorption to the bloodstream, acrylonitrile is metabolized primarily by two pathways: (i) oxidation to glycidonitrile (also known as cyanoethyl oxide, CEO) (Thier et al., 2002); and (ii) conjugation with glutathione (GSH) (Jakubowski et al., 1987; Thier et al., 2001).

Conjugation with GSH gives rise to CEMA (also known as CYMA), which was found in the urine of volunteers experimentally exposed to acrylonitrile vapour (Jakubowski et al., 1987) and of coke oven workers (Frigerio et al., 2020).

After an 8-hour experimental exposure to acrylonitrile at a concentration of 5 and 10 mg/m^3, a significant portion of the retained acrylonitrile (mean ± SD, 21.8 ± 7.6%; n = 6) was excreted as CEMA in the urine (Jakubowski et al., 1987). Smokers had significantly higher levels of CEMA than did non-smokers (Hou et al., 2012; Xiaotao et al., 2014; see also Section 1.4.3 and the references therein). CEO can also undergo conjugation with GSH, leading primarily to two isomeric conjugates, namely, *S*-(2-cyano-2-hydroxyethyl) glutathione (2,2-CHEG) and *S*-(1-cyano-2-hydroxyethyl)glutathione (1,2-CHEG) (Kedderis et al., 1995). Further metabolic conversion of these conjugates can lead to the formation of three mercapturic acids: CHEMA, HEMA, and carboxymethylmercapturic acid (*N*-acetyl-*S*-carboxymethylcysteine, CMMA) (Fig. 4.1).

In a recent study, the three mercapturic acids (CEMA, CHEMA, and HEMA) were determined by sensitive ultraperformance LC-MS/MS in the urine of 80 occupationally exposed workers and 23 controls, including both smokers and non-smokers (Wu et al., 2023). Acrylonitrile exposure levels were 0.21 mg/m^3 (geometric mean) in the group with high exposure (n = 26) and <4 µg/m^3 in the group with low exposure (n = 53). Post-shift values of CEMA, CHEMA, and HEMA in acrylonitrile-exposed workers were significantly greater than pre-shift and control values. Moreover, pre-shift values in exposed workers were also significantly higher than those in the controls, indicating that the excretion and or formation of these mercapturic acids was not complete within 24 hours. Values for urinary CEMA, CHEMA, and the sum of CHEMA plus HEMA correlated with exposure to acrylonitrile, as shown by Spearman correlation coefficients of 0.688 (P < 0.05), 0.525 (P = 0.059), and 0.571 (P < 0.05) for CEMA, CHEMA, and CHEMA plus HEMA, respectively. The authors suggested that CEMA and CHEMA could be used as very sensitive and specific biomarkers of exposure to acrylonitrile. [The Working Group

Fig. 4.1 Metabolic pathways of acrylonitrile



Metabolic pathways of acrylonitrile. Adapted from Sumner et al. (1997), Kedderis et al. (1995), and Lambotte-Vandepaer et al. (1981).
AN, acrylonitrile; CAA, 2-cyanoacetic acid; CEG, *S*-(2-cyanoethyl)glutathione; CEMA, *S*-(2-cyanoethyl) mercapturic acid (also known as CYMA); CEO, cyanoethyl oxide; CESA, 2-[2-(cyanoethyl)sulfanyl]acetic acid; 1,2-CHEG, *S*-(1-cyano-2-hydroxyethyl)glutathione; 2,2-CHEG, *S*-(2-cyano-2-hydroxyethyl)glutathione; CHEMA, *S*-(1-cyano-2-hydroxyethyl)-mercapturic acid; CMMA, carboxymethylmercapturic acid; CNE, cyanoethanol; CYP, cytochrome P450; EH, epoxide hydrolase; GA, glycolaldehyde; GACH, glycolaldehyde cyanohydrin; GLA, glycolic acid; GS, glutathionate; GSH, reduced glutathione; GST, glutathione *S*-transferase; HEMA, 2-hydroxyethylmercapturic acid; m.a.p, mercapturic acid pathway; OEG, *S*-(2-oxoethyl)glutathione; TDAS, thiodiglycolic acid *S*-oxide; TDGA, thiodiglycolic acid.
Adapted by the Working Group.

noted that the correlations between acrylonitrile exposure and CEMA, CHEMA, and CHEMA plus HEMA were rather low, probably because levels of acrylonitrile exposure were close to background levels.]

HEMA has also been found in the urine of tobacco smokers (Hou et al., 2012; Schettgen et al., 2012; Xiaotao et al., 2014). The development of very sensitive analytical methods also enabled the detection of HEMA (Xiaotao et al., 2014) and CHEMA in the urine of both smokers and non-smokers (Schettgen et al., 2012). [The Working Group noted that HEMA is not a specific marker for acrylonitrile exposure since high background levels of HEMA can be derived from the metabolism of endogenous ethylene oxide.]

Thiocyanate (rhodanide, SCN^-), a detoxication product of cyanide, is released spontaneously from CEO after hydrolysis, as well as after the conjugation of CEO with GSH, and was also found at exposure-related concentrations in the urine of humans exposed to acrylonitrile (Sakurai et al., 1978).

In addition to the above-mentioned metabolites, unchanged acrylonitrile was also found in the urine of occupationally exposed workers (Sakurai et al., 1978; Houthuijs et al., 1982; Perbellini et al., 1998). Concentrations of acrylonitrile in the urine peaked near the end of the work shift and remained elevated during days off in workers compared with controls (Houthuijs et al., 1982; Perbellini et al., 1998). Unchanged acrylonitrile was found also in exhaled air of workers from a factory producing and processing rubber (Ulanova et al., 2016).

(c) Toxicokinetics of blood protein adducts

Acrylonitrile forms a stable haemoglobin adduct (see Section 4.2.1) with valine residues at the N-termini of globin.

In humans exposed to acrylonitrile, hydrolytic cleavage of isolated globin releases CEV (Tavares et al., 1996; Bergmark, 1997; Thier et al., 1999; Scherer et al., 2014). Owing to its slow elimination rate, CEV can be used as a biomarker of long-term exposure (see also Sections 1.3.3, 1.4.2, and 1.4.3). Generally, a linear decrease in haemoglobin adduct levels should be observed after cessation of exposure, reflecting physiological turnover of erythrocytes during their lifespan of approximately 126 days (Törnqvist et al., 2002). However, in three cleaning workers accidentally exposed to acrylonitrile in a train depot, CEV levels decreased for about 150 days before reaching the background value, i.e. for 2 weeks longer than the standard lifespan of erythrocytes (Bader and Wrbitzky, 2006). [The Working Group noted that the authors offered a possible explanation for these kinetics with a reference to a previous observation of unchanged acrylonitrile in the urine 1 week after a single accidental exposure (Will et al., 2003); thus, it was hypothesized that free acrylonitrile might circulate in the blood for some time or could be released from unstable adducts (Bader and Wrbitzky, 2006).]

A different elimination rate was observed for the adduct formed with human serum albumin (HSA), namely, *N*-(2-cyanoethyl)aspartic acid (CEA). After accidental exposure of the skin of one worker, the concentration of the CEA adduct in blood fell rapidly from 1300 µg/L to nearly background values within 3–4 days (Thier et al., 2000). [The Working Group noted that the elimination rate of CEA was much shorter than expected, considering that the half-life for HSA is 18–21 days (Mendez et al., 2005), suggesting functional damage to the liver.]

4.1.2 Experimental systems

(a) Absorption and distribution

Acrylonitrile is absorbed and widely distributed by inhalation or by oral administration. After oral administration, acrylonitrile is readily absorbed into the bloodstream and distributed to all major tissues in rats (Sandberg and Slanina, 1980; Ahmed et al., 1982; Sapota, 1982; Kedderis

et al., 1993a; Burka et al., 1994), mice (Kedderis et al., 1993b), and cynomolgus monkeys (Sandberg and Slanina, 1980).

In Fischer 344 (F344) rats treated with [2-^{14}C] acrylonitrile at a dose of 46 mg/kg bw by oral administration (gavage), radiolabel was well absorbed from the gastrointestinal tract and distributed all over the body; the highest levels were found in the forestomach, blood, and urinary bladder (Burka et al., 1994). Treatment with SKF-525A, an inhibitor of cytochrome P450 (CYP), caused elevated tissue retention of radiolabel in the blood, liver, kidney, lung, forestomach, glandular stomach, small intestine, and urinary bladder. In addition, a decrease of about 30% in urinary excretion of the radiolabel was observed within the 24 hours after dosing. Of the administered dose, about 11% was found to be excreted in exhaled air as $^{14}CO_2$ and < 2% as unchanged acrylonitrile. Pretreatment with SKF-525A slightly decreased the exhalation of $^{14}CO_2$ and increased that of unchanged acrylonitrile (Burka et al., 1994).

In rats treated by intravenous administration with [1-^{14}C]acrylonitrile, the highest levels of radioactivity were found in the blood, liver, duodenum, kidneys, and adrenals. The radioactivity concentration increased in the blood, whereas that in the other tissues decreased or remained constant over the 90-minute period post-injection (Silver et al., 1987).

In a study using whole-body radiography, Sandberg and Slanina (1980) showed that radioactivity derived from [1-^{14}C]acrylonitrile accumulated in the bile, liver, kidney, lung, and adrenal cortex in rats treated by oral and intravenous administration, and in cynomolgus monkeys treated by oral administration. However, accumulation in the liver was higher than and well exceeded concentrations in the blood in monkeys. In rats, the distribution patterns after intravenous and oral administration were very similar, except that slower absorption and longer retention were observed after oral administration. Radioactivity in the blood and tissues persisted for 7 days after the end of the treatment. When pregnant rats were treated intravenously or orally with [1-^{14}C]acrylonitrile, the fetal tissues showed low uptake, except that the concentration of radiolabel in the eye lens markedly exceeded that in the maternal blood.

A similar distribution pattern was reported by Sato et al. (1982) in a study on rats dosed intraperitoneally with [2,3-^{14}C]acrylonitrile. Levels of protein-bound radioactivity in erythrocytes decreased gradually over 16 days post-injection and at a much slower rate than that for plasma.

Distribution of the active metabolite of acrylonitrile, [1,2-^{14}C]CEO, administered orally to rats and mice, was very similar in various tissues, with no particular accumulation in any organ, and radioactivity decreased by 71% to 90% within 24 hours after dosing (Kedderis et al., 1993b).

(b) Metabolism

Formation of the key metabolite CEO is catalysed predominantly by hepatic cytochrome P450 2E1 (CYP2E1) and, to a much lesser extent, by lung microsomes. In human hepatic microsomes, oxidation follows Michaelis–Menten kinetics: V_{max} = 129–315 pmol/minute per mg of protein; K_m = 12–18 μM (n = 6) (Kedderis et al., 1993c). In vitro, acrylonitrile was oxidized to CEO by human lung lipoxygenase in the presence of linoleic acid. Cyanide ions (CN^-) were generated during this reaction as an ultimate product of CEO hydrolysis (Roy and Kulkarni, 1999). The metabolism of acrylonitrile to CEO is a prerequisite for cyanide formation. In mice, this pathway is exclusively catalysed by CYP2E1, as shown by experiments in CYP2E1-null mice (Wang et al., 2002).

CEO undergoes spontaneous hydrolysis to glycolaldehyde cyanohydrin (2,3-dihydroxypropanenitrile, GACH), with a half-life at 37 °C and pH 7.3 of approximately 100 minutes. Hydrolysis can also be catalysed by epoxide hydrolase. Human hepatic microsomes, but not hepatic

microsomes from rats and mice, significantly increased the rate of CEO hydrolysis (Kedderis and Batra, 1993).

Both acrylonitrile and CEO are electrophilic species that undergo conjugation with GSH. Both conjugation reactions are catalysed by cytosolic glutathione *S*-transferases (GSTs). Conflicting results were reported for this conjugation reaction in vitro. According to Guengerich et al. (1981), human liver cytosol had a catalytic effect on the conjugation of GSH with CEO but not with acrylonitrile, whereas acrylonitrile was a better substrate for GST than was CEO, according to Kedderis et al. (1995). GSH conjugates are further metabolized to *N*-acetylcysteine derivatives (mercapturic acids), which are then excreted in the urine (Hanna and Anders, 2019).

Gargas et al. (1995) studied the toxicokinetics of acrylonitrile in rats. After intravenous injection, the concentration of CEO steeply increased, and both acrylonitrile and CEO were rapidly eliminated from the blood in a dose-dependent manner. The elimination of acrylonitrile could be ascribed to saturable epoxidation catalysed by CYP, and first-order conjugation with GSH. The elimination of CEO, which was more rapid than the elimination of acrylonitrile, occurred through conjugation with GSH following first-order kinetics.

Acrylonitrile can generate highly toxic cyanide ions upon biotransformation. Both cyanide bound to methaemoglobin and its detoxication product, thiocyanate, were detected in rats, mice, and rhesus monkeys exposed to acrylonitrile by inhalation (Brieger et al., 1952). These findings were confirmed by other authors (Abreu and Ahmed, 1980; Langvardt et al., 1980; Sapota, 1982; Goyal et al., 1989; Fennell et al., 1991). Thiocyanate (SCN^-) can be oxidized by eosinophil peroxidase in vitro to a mixture of hypothiocyanite ($OSCN^-$) and cyanate (OCN^-). This mixture can in turn react very selectively with sulfhydryl (SH) groups. It was shown to deplete GSH in rat erythrocytes and to damage human GST (Arlandson et al., 2001). However, experiments in mice suggested an induction of GST or GSH synthesis in vivo after repeated oral dosing (Sumner et al., 1997; NTP, 2001).

In addition, many other metabolites were identified, forming a rather complex, widely branched metabolic pattern (Fig. 4.1). Acrylonitrile is primarily metabolically activated to the strongly electrophilic epoxide CEO, which was identified in vivo in the blood of rats and mice treated with acrylonitrile by oral administration (Roberts et al., 1991), as well as in vitro after incubation of acrylonitrile with rat liver microsomes (Guengerich et al., 1981; Roberts et al., 1989, 1991; Kedderis et al., 1993c), mouse liver microsomes (Roberts et al., 1991), rat and mouse lung microsomes (Roberts et al., 1991), and in club (Clara) cell-enriched fraction of rat lung (Roberts et al., 1989). Studies in CYP2E1-null mice showed that this particular form of CYP is the main, if not the only, enzyme responsible for the oxidation of acrylonitrile to CEO in mice (Sumner et al., 1999; Wang et al., 2002; El Hadri et al., 2005; Ghanayem and Hoffler, 2007).

[The Working Group noted that CYP2E1 is the CYP form that typically catalyses oxidation of small-molecule substrates such as ethanol, dialkylnitrosamines, and acrylonitrile.]

After oral administration of acrylonitrile at 1–30 mg/kg bw to rats and mice, blood concentrations of CEO were higher in rats than in mice; however, concentrations decreased rapidly in both species. This finding in vivo contrasted with the kinetics of CEO in vitro, since the oxidation rates of acrylonitrile in liver microsomes from mice have been reported to be much higher than those from rats (V_{max} = 2801 and 667 pmol/minute per mg protein, and K_m = 67 and 52 µM, for mice and rats, respectively) (Roberts et al., 1991).

Both acrylonitrile and CEO undergo conjugation with GSH. The resulting conjugates are biologically transformed via the mercapturic acid pathway. Thus, acrylonitrile reacts with GSH to give *S*-(2-cyanoethyl)glutathione (CEG)

(Geiger et al., 1983; Kedderis et al., 1995), which is then converted to CEMA, the major metabolite excreted in the urine of rats, mice, and rabbits (Kopecký et al., 1979; Langvardt et al., 1980; Gut et al., 1981; Lambotte-Vandepaer et al., 1985; Tardif et al., 1987; Goyal et al., 1989).

Conjugation with GSH can proceed efficiently with (but also without) catalysis by GSTs. CEO is a highly reactive compound bearing two electrophilic sites at the α- and β-carbons; thus, the reaction of CEO with GSH can lead to two conjugates: (i) 1,2-CHEG, which results from conjugation of GSH at the α-carbon; and (ii) 2,2-CHEG, which is formed by attack at the β-carbon. [The Working Group noted that these two conjugates have not been isolated, and only one peak has been found by high-performance liquid chromatography (HPLC) analysis of the reaction between GSH and CEO.]

The conjugates were identified by fast atom bombardment-mass spectrometry (FAB-MS) as CHEG, without resolution of the isomers (Kedderis et al., 1995). Their structures can be inferred on the basis of those of mercapturic acids found in the urine of rodents dosed with acrylonitrile, namely, HEMA (van Bladeren et al., 1981; Lambotte-Vandepaer et al., 1985; Linhart et al., 1988; Fennell et al., 1991; Kedderis et al., 1993a), CMMA (Fennell et al., 1991; Kedderis et al., 1993a), and CHEMA (Linhart et al., 1988; Fennell et al., 1991). 1,2-CHEG is a stable compound producing CHEMA as a single mercapturic acid in a straightforward way via the mercapturic acid pathway, whereas 2,2-CHEG is a cyanohydrin that decomposes at physiological conditions to cyanide and *S*-(2-oxomethyl) glutathione (OEG). The formation of HEMA and CMMA (from OEG) can then be deduced via reduction of the aldehydic carbonyl of OEG to hydroxyl and its oxidation to the carboxylic group, respectively. These transformations can occur either directly at the stage of OEG formation or in the course of its conversion to mercapturic acids (Linhart et al., 1988; Fennell et al.,1991). In addition to these mercapturic acids, three additional thioether metabolites were found in the urine of rats and/or mice dosed with acrylonitrile, namely, 2-[2-(cyanoethyl)sulfanyl] acetic acid (CESA) (Fennell et al., 1991; Kedderis et al., 1993a; Sumner et al., 1999), thiodiglycolic acid (TDGA), and thiodiglycolic acid *S*-oxide (TDAS) (Fennell et al., 1991; Sumner et al., 1999). Depletion of GSH increased the formation of cyanide in rats, as indicated by an increase of 2–3-fold in thiocyanide excretion after GSH depletion (Pilon et al., 1988a, b). The ratio of CHEMA to CEMA, which indirectly indicates the proportion of acrylonitrile and CEO available for GSH conjugation in vivo, decreased as acrylonitrile exposure increased (Wu et al., 2023).

The route of administration also has an influence on the metabolic pattern. Production of thiocyanate in rats is higher after inhalation and oral administration than after intravenous or intraperitoneal injection (Silver et al., 1982; Tardif et al., 1987). Similarly, higher excretion of HEMA and lower excretion of CEMA was observed in rats after inhalation than after intravenous or intraperitoneal administration (Tardif et al., 1987). Excretion of thiocyanate in rats, mice, and Chinese hamsters was also lower after intravenous, subcutaneous, and intraperitoneal dosing than after oral administration (Gut et al., 1975). [The Working Group noted that these results suggest that a higher portion of the administered dose of acrylonitrile may be metabolically activated to CEO after inhalation and oral intake than after intravenous and intraperitoneal injection.]

In an in vitro study with 9000 × *g* supernatant (S9) fraction of rat liver homogenate, four metabolites of acrylonitrile were detected by GC or HPLC (Duverger-Van Bogaert et al., 1981). By comparing retention times with those of authentic samples, they were identified as acetic acid, 2-cyanoacetic acid, cyanoethanol, and glycolaldehyde. Glycolaldehyde was detected as 2,4-dinitrophenylhydrazide by HPLC. Two

metabolites, 2-cyanoacetic acid and cyanoethanol, were also reported by the same group of authors to be in the urine of rats dosed intraperitoneally with acrylonitrile (Lambotte-Vandepaer et al., 1981). [The Working Group noted that the identification of these metabolites in vivo should be considered tentative because of the insufficient specificity of the GC method used.]

(c) Excretion

Acrylonitrile is excreted predominantly in the urine, the main metabolites being CEMA and thiocyanate. A small portion is also excreted in exhaled air as carbon dioxide (CO_2). A small amount of acrylonitrile is excreted unchanged in the urine and exhaled air.

Sapota (1982) compared the excretion of radiolabelled acrylonitrile at a dose of 40 mg/kg bw in rats treated by oral administration or intraperitoneally. Urinary excretion was faster after intraperitoneal administration (mean ± SE, 72 ± 6% of the administered dose within 8 hours) than after oral administration (44.5 ± 0.4%). About 90% of the administered dose was excreted in the urine and exhaled air within 24 hours after both oral and intraperitoneal administration.

Rats dosed with acrylonitrile labelled with ^{14}C at either a nitrile or a vinyl group excreted up to 11 % of the administered dose in expired air as CO_2, indicating that a minor part of the administered dose of acrylonitrile enters the intermediary metabolism (Sapota, 1982; Burka et al., 1994). This can be caused by spontaneous release of cyanide ions from the GACH formed by the hydrolysis of CEO, and subsequent oxidation of the glycolaldehyde formed to glycolic acid (Duverger-Van Bogaert et al., 1981; Guengerich et al., 1981; Lambotte-Vandepaer et al., 1981). Glycolic acid is further oxidized to glyoxylic acid then transformed to glycine and CO_2 or oxalic acid (Corley et al., 2005).

Kedderis et al. (1993a) found that 73–100% of the orally administered dose of [2,3-^{14}C]-acrylonitrile was excreted in the urine of rats, and 83–94% was excreted in the urine of mice within 72 hours after administration; only about 5% of the administered dose was found in the faeces. This excretion pattern did not significantly change within a dosing range of 0.09–28.8 mg/kg bw in rats or 0.09–10 mg/kg bw in mice.

Fennell et al. (1991) used ^{13}C-NMR spectroscopy to analyse urine from rats and mice dosed orally with [1,2,3-^{13}C]acrylonitrile. There were marked differences between rats and mice in the proportion of metabolites formed via CEO. In rats dosed with single oral doses of [1,2,3-^{13}C] acrylonitrile at 30 or 10 mg/kg bw, these metabolites (HEMA, CHEMA, TDGA, and TDAS) accounted for approximately 60% of the total metabolites excreted in the urine, whereas in mice dosed orally with [1,2,3-^{13}C]acrylonitrile at 10 mg/kg bw they accounted for nearly 80% of the total metabolites excreted in the urine. Thus, it was observed that mice excreted a higher percentage of the administered dose in the form of metabolites resulting from GSH conjugation with CEO than did rats (Fennell et al., 1991; Sumner et al., 1997). In mice, the percentage of metabolites derived from the conjugation of GSH with both acrylonitrile and CEO significantly increased after repeated dosing (five doses), suggesting induction of GST and/or GSH synthesis (Sumner et al., 1997). These data were consistent with previous observations showing a higher rate of acrylonitrile oxidation in mice than in rats (Roberts et al.,1991).

The ratio of metabolites derived from the reduction and oxidation of OEG was higher in rats than in mice, suggesting differences in the enzymes involved in the biotransformation of this aldehyde (Fennell et al., 1991; Sumner et al., 1997).

4.1.3 *Differences in metabolism and toxicokinetics between humans and animals*

[The Working Group considered that the potential effects of acrylonitrile may be linked to the proportion of the agent that passes along the two primary metabolic pathways, oxidation and conjugation. Oxidation to CEO is a direct metabolic activation that can be associated with the release of cyanide and a potential increase in genotoxicity (see also Section 4.2.2), whereas direct conjugation with GSH represents a detoxification pathway.]

In rat hepatic microsomes, the oxidation of acrylonitrile to CEO followed Michaelis–Menten kinetics; with K_m = 11 ± 1 μM and V_{max} = 366 ± 6 pmol/minute per mg protein (mean ± SD; n = 6). Similar values (V_{max} = 129–315 pmol/minute per mg of protein; K_m = 12–18 μM; n = 6) were observed in human hepatic microsomes (Kedderis et al., 1993c). Roberts et al. (1991) also found similar oxidation rates for acrylonitrile in human and rat hepatic microsomes but a much higher V_{max} in mouse hepatic microsomes. [The Working Group noted that there was high interindividual variability in the kinetics of acrylonitrile oxidation by human hepatic microsomes. Also, despite some kinetic differences, generally the metabolic patterns in humans, rats, and mice appeared to be qualitatively the same.]

The production of CEO in human lung microsomes was lower by about 900 times than that in liver microsomes, and also lower than that in lung microsomes from rats and mice (Roberts et al., 1991). In contrast, the hydrolysis of CEO proceeded more rapidly and efficiently in human hepatic microsomes, and showed significant catalytic activity (K_m ranged from 0.6 to 3.2 mM, and V_{max} from 8.3 to 18.8 nmol/minute per mg protein), whereas no enhancement of spontaneous hydrolysis was observed in rat and mouse microsomes (Kedderis and Batra, 1993).

A linear increase in urinary excretion of metabolites derived from the conjugation of CEO with GSH was reported within the oral dose range of 0.09–28.8 mg/kg bw in rats and 0.09–10 mg/kg bw in mice. At the same time, CEMA excretion increased nonlinearly with dose in both species. Thus, the ratio of metabolites derived from CEO to metabolites derived from acrylonitrile decreased as the dose increased (Kedderis et al., 1993a). A similar trend was also observed in rats (Fennell et al., 1991). [The Working Group noted that the ratio of mercapturic acids CHEMA/CEMA and of (HEMA plus CHEMA)/CEMA indirectly reflected the proportions of CEO and acrylonitrile available for GSH conjugation. Observed increases in the CHEMA/CEMA and (HEMA plus CHEMA)/CEMA ratios with decreasing exposure may suggest a sublinear relationship between exposure and potential key characteristic-related effects, i.e. genotoxicity.]

Kinetic data in vitro for humans and rats on the oxidation of acrylonitrile to CEO (Kedderis et al., 1993c, 1996), CEO hydrolysis (Kedderis and Batra, 1993), and rates of GSH conjugation with acrylonitrile and CEO (Kedderis et al., 1995, 1996) were scaled and used to develop a physiologically based pharmacokinetic (PBPK) model to predict blood and brain concentrations of acrylonitrile and CEO in three scenarios: (i) after inhalation of acrylonitrile vapour at 2 ppm for 8 hours; (ii) after 1 week of continuous inhalation of acrylonitrile at 0.4 ppm; or (iii) after 1 week of periodic exposure to drinking-water containing acrylonitrile at 0.12 mg/L. In the first two scenarios, the predicted concentrations of acrylonitrile and CEO in the blood and brain were similar in rats and humans, whereas in the third scenario after periodic exposure to drinking-water containing acrylonitrile, the peak of acrylonitrile concentration in blood was about 8.5 times as high in rats as in humans. In contrast, average CEO concentrations in the blood and brain were approximately twice as high in humans than in rats. The predicted variability in

blood CEO concentrations in humans exposed via inhalation or drinking-water was modest: 95% of exposed individuals were expected to have blood CEO concentrations that were less than 1.8-times as high as an average individual (Sweeney et al., 2003). [The Working Group noted that, despite the higher prediction of the doses, the authors claimed that the PBPK modelling analysis suggested that humans were not more sensitive than rats to the toxic effects of acrylonitrile.]

Synopsis

[After inhalation, ingestion, or dermal exposure, acrylonitrile is readily absorbed and, through the bloodstream, distributed to all major organs. Most of the absorbed agent is excreted in the form of metabolites in the urine; however, excretion is not complete within 24 hours. Metabolism in humans is very similar to that observed in rats; the main competing pathways are metabolic activation to CEO, predominantly by CYP2E1; and conjugation with GSH, eventually yielding CEMA, which is excreted in the urine. CEO, the key electrophilic metabolite, releases cyanide in the course of biotransformation and is detoxified by two metabolic pathways: (i) epoxide hydrolase-catalysed hydrolysis; and (ii) GSH conjugation. Conjugation leads to the formation of two mercapturic acids, CHEMA and HEMA. The amount of CEO, as reflected by the ratio of CHEMA/CEMA and of (CHEMA + HEMA)/CEMA, shows a sublinear relationship with dose. According to PBPK modelling analysis, the sensitivity of humans to the toxic effects of acrylonitrile appears to be similar to that of rats.]

4.2 Evidence relevant to key characteristics of carcinogens

This section reviews the mechanistic data for the 10 key characteristics of carcinogens (Smith et al., 2016) encompassed by the agent acrylonitrile. Evidence was available on whether acrylonitrile exhibits the key characteristics "is electrophilic or can be metabolically activated to an electrophile", "is genotoxic", "alters DNA repair or causes genomic instability", "induces oxidative stress", "induces chronic inflammation", "is immunosuppressive", and "modulates receptor-mediated effects", "causes immortalization", and "alters cell proliferation, cell death, or nutrient supply". No evidence for "induces epigenetic alterations" was available. The studies in exposed humans that were used to support the mechanistic evidence evaluation were assessed for quality of the study design and exposure assessment, and assay accuracy and precision, and were found to reflect suitable methods for human environmental epidemiological studies. The exposure assessments for the mechanistic studies in humans are described in Section 1.6.2. Further details are presented in Table S1.13 (Annex 1, Supplementary material for Section 1, online only, available from: https://publications.iarc.who.int/646).

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) Humans

(i) Exposed humans

See Table 4.1.

As reported in Section 4.1.2, there is evidence that acrylonitrile can be metabolically activated to an electrophile. In exposed workers, mainly through the activity of CYPs, and confirmed in microsomes in vitro, acrylonitrile is primarily oxidized to the epoxide CEO and conjugated to mercapturic acids.

Table 4.1 End-points relevant to electrophilicity or metabolic activation to an electrophile in humans exposed to acrylonitrile

End-point	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
Hb adducts, (CEV adducts)	Peripheral blood	Portugal Cross-sectional study in CP and MM workers, SM, and newborns		+ CEV (mean ± SD in pmol/g Hb): 9 MM, 1984 ± 2066; 7 CP, 2276 ± 1338; 7 controls, 31.1 ± 18.5; 13 SM, 217 ± 85.1; and 13 newborns, 99.5 ± 53.8	Smoking	Small cohorts, external exposure not measured. Both exposed and control workers were non-smokers. Valuable data on neonates; uncertainty regarding CEV units: pmol/g Hb (as expressed) or pmol/g globin as usual (CEV determined in isolated globin, not Hb).	Tavares et al. (1996)
Hb adducts (CEV in globin)	Peripheral blood	Germany Cross-sectional study within textile industry workers, health supervision	62 workers: 38 smokers and 24 non-smokers (median age, 35 yr). Control group: 10 non-exposed persons (2 smokers, 8 non-smokers) (median age, 30 yr)	(+) Correlation found between cigarettes consumption/d (0–40) and CEV in globin (< 4–256 pmol/g globin), $R^2 = 0.68$	Smoking	CEV used as exposure biomarker for smoking. Mixed exposure – smoking; small number of participants.	Schettgen et al. (2002)
Hb adducts (CEV in globin)	Peripheral blood	Germany Cross-sectional study on acrylamide adducts as markers of transplacental exposure	10 non-smoking and 1 smoking mother in advanced pregnancy (blood) and 11 neonates (umbilical cord blood)	(+) CEV in smoking mothers, 185 pmol/g globin; CEV in neonates, 69 pmol/g globin	Smoking	CEV used only as an indicator of smoking. Very small number of participants tested; valuable report on CEV in neonates. Only smoking mothers and their neonates had measurable CEV levels.	Schettgen et al. (2004)

Table 4.1 (continued)

End-point	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
Hb adducts, (CEV adducts)	Peripheral blood	Germany Cross-sectional, analytical study Participants from Bavaria were not occupationally exposed Included results of adducts for passive smokers and non-smokers	92 non-smokers (52 men, 52 women) and 12 passive smokers (5 men, 7 women); median age, 35 yr	(+) $P = 0.07$	Smoking	External exposure not measured, marginal difference in CEV level between passive smokers and non-smokers. The age range of the group was large.	Schettgen et al. (2010)
Hb adducts (CEV in globin)	Peripheral blood	Belgium Cross-sectional study in emergency responders at a train accident	Total no. of study participants, 841; non-smokers, 635 In 163 non-smokers, CEV was > RV (10 pmol/g globin) In 55 of the 206 smokers, CEV was > RV (200 pmol/g globin)	+ Exposure among the non-smokers could be predicted by the distance to the accident, duration of exposure, and occupational function	Smoking	Large cohorts; external exposure not measured.	Van Nieuwenhuyse et al. (2014)
Hb adducts (CEV in globin)	Peripheral blood	Belgium Cross-sectional study in emergency responders at a train accident	136 non-smokers; extrapolated CEV, mean, 6.9; IQR, 3.4–16.2 pmol/g globin	+ Positive dose–response relation observed between short-term effects and CEV level in globin for non-smokers but not for smokers		External exposure was not determined; nevertheless, the association between AN exposure (massive AN leak) and CEV in globin was convincing. Smoking was neither quantified not verified by any measurement, intensity not reported.	Simons et al. (2016)

Table 4.1 (continued)

End-point	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
Hb adducts (CEV in globin)	Peripheral blood	Belgium Cross-sectional study in residents living near a train accident	142 non-smokers potentially exposed, from various areas 24 controls	+ CEV (mean ± SD, pmol/g globin): 142 non-smokers potentially exposed, 206.7 ± 1163.4, extrapolated to the moment of accident; 24 controls, 4.3 ± 3.3	Smoking	External exposure was not determined; differences in CEV levels between the exposed and control groups were statistically significant only for non-smokers; 53 values for non-smokers were > RV ($P < 0.003$).	De Smedt et al. (2014)
Hb adducts (CEV in globin)	Peripheral blood	Belgium Cross-sectional study in potentially exposed patients at a train accident, analysis of acrylonitrile poisoning	Suspected exposure to AN among 438 patients admitted to the ER: 164 potentially exposed (50 smokers and 114 non-smokers) with data on CEV, and lactate (108), and SCN (71) or all (69)	(+) CEV: smokers, 1.1–29.6 pmol/g globin; non-smokers, 1.1–2.69 pmol/g globin	Smoking	External exposure was not determined; no controls; weak correlation with other internal exposure markers; suspected exposure was not well described.	Colenbie et al. (2017)
Hb adducts (CEV in globin)	Peripheral blood	Germany Case-report study, direct accidental exposure to AN in a train tank wagon	4 exposed participants (cleaning workers); CEV in globin used to estimate internal dose at 25, 85, 115 and 175 d after a high accidental exposure	+ Extrapolated CEV blood levels to the initial value (day of accident) and corrected for smoking were 679–2204 pmol/g globin. The levels decreased in 150 d	Smoking	Small sample size; external exposure was not quantified; however, undoubtedly high direct exposure to the agent.	Bader and Wrbitzky (2006)

Table 4.1 (continued)

End-point	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
Hb adducts (CEV in globin)	Peripheral blood	Germany Cross-sectional, observational study, chemical plant accident (fire), biomonitoring study	816 exposed workers; mean AN concentration in the vicinity of the accident, 7 ppm; CEV concentration (pmol/g globin): mean, 98; median, 20	(+/–) 12 of 816 persons showed CEV concentration > RV of 15 µg/L blood		Inadequate method sensitivity (GC-MS); poor statistics; inconclusive results; no control group. External exposure was not quantified.	Leng and Gries (2014)
HSA adduct (CEA in albumin)	Peripheral blood	Germany Case-report study, accidental exposure through the skin	Exposure level was not reported One exposed participant	+ CEA rapidly eliminated from HSA within 3–4 d		A single participant was described; no control. CEV was also determined but levels did not change significantly after exposure; external exposure was not quantified.	Thier et al. (2000)
Hb adducts (CEV)	Peripheral blood	Germany Cross-sectional study, occupational exposure	Low and unspecified AN exposure 59 exposed workers (38 smokers, 21 non-smokers)	(+)	Smoking	Blood sampled twice. No control group. No statistically significant differences between smokers and non-smokers were observed in levels of CEV. External exposure was not quantified.	Thier et al. (1999)
Hb adducts (CEV in globin)	Peripheral blood	China Cross-sectional study, occupational exposure at a chemical plant	Exposure to acrylamide and AN not quantified. 41 workers exposed, 10 non-exposed workers in the same city	(+) CEV (nmol/g Hb): 0.02–66 in in 41 workers; in 10 controls ND to 0.14		Smoking not controlled in the exposed group; uncertainty in CEV units: nmol/g Hb (as expressed) or nmol/g globin as usual (CEV in isolated globin, not Hb).	Bergmark et al. (1993)

Table 4.1 (continued)

End-point	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
Hb adducts (S-AN adducts in globin)	Peripheral blood	Russian Federation Synthetic rubber plant Biomonitoring study	Exposure levels: 6–9 ppm for 1–10 yr 12 exposed workers	(+) *S*-AN (mainly *S*-(2-cyanoethyl)-cysteine) adducts in globin, adducted AN, ~15 µmol/g globin		Control group was not specified; smoking was not controlled; new analytical method (GC) based on thermal elimination of AN from the adducts was not adequately validated.	Ivanov et al. (1993)
Hb adducts (CEV)	Peripheral blood	USA Cross-sectional study, non-smokers and smokers	14 non-smokers, 32 smokers (1 pack/d), 14 smokers (2 packs/d)	+ Significant difference in CEV levels between smokers and non-smokers ($P < 0.0001$), and between the two groups of smokers ($P < 0.016$)		Relatively small cohort. Exposure to cigarette smoke was quantified using urinary cotinine or self-reported smoking status. Smoking was the primary exposure under evaluation, not a covariate. *GSTM1*, and *GSTT1* polymorphisms were also studied.	Fennell et al. (2000)
Hb adducts (CEV)	Peripheral blood	Sweden Cross-sectional study, university laboratory workers exposed to acrylamide (PAGE); smokers and non-smokers	18 controls: 10 smokers, and 8 non-smokers 17 smokers (1–20 cigarettes/d), including 10 controls and 8 workers 40 workers: 22 at PAGE (for 3 mo to 9 yr), 7 smokers, 15 non-smokers	(+) in control group 2–178 pmol/g globin; $R = 0.94$ (+) also in smoking workers but co-increase with acrylamide and ethylene oxide adducts	Smoking	Small cohort; significant correlation between number of cigarettes/d and CEV in globin (or Hb); uncertainty regarding CEV units: nmol/g Hb (as expressed) or nmol/g globin as usual (CEV determined in isolated globin, not Hb).	Bergmark (1997)

Table 4.1 (continued)

End-point	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
Hb adducts (CEV)	Peripheral blood	Germany Cross-sectional, intervention study of smoking patients AN and 4-ABP biomarker measurements during 183 d, 7 samplings	58 smokers 58 ex-smokers and 57 non-smokers	+ CEV (pmol/g globin): smokers, 6–162; ex-smokers, 1.0–2.5; and controls, 0.2–0.3		Weak correlation between CEMA and CEV.	Scherer et al. (2014)
Albumin adducts	Plasma	USA Cross-sectional study in young healthy participants; age, 18.8–34.5 yr LC-HRMS adductomic measurements	158 participants, smoking/non-smoking 34 pooled plasma samples	+ Significant difference in adduct levels between smokers and non-smokers ($P < 0.0001$); 2-cyanoethyl adducts at Cys-34 locus of HSA	Smoking, race, sex, BMI, consumption of animal fat and vegetable fat	Pooled samples; smoking intensity not reported.	Grigoryan et al. (2016)

4-ABP, 4-aminobiphenyl; AN, acrylonitrile; BMI, body mass index; CEA, *N*-(2-cyanoethyl)aspartic acid; CEMA, 2-cyanoethylmercapturic acid; CEV, *N*-(2-cyanoethyl)valine; CP, continuous polymerization; d, day(s); ER, emergency room; GC, gas chromatography; GC-MS, gas chromatography-mass spectrometry; GST, glutathione *S*-transferase; Hb, haemoglobin; HSA, human serum albumin; IQR, interquartile range; LC-HRMS, liquid chromatography-high-resolution mass spectrometry; MM, maintenance mechanics; mo, month(s); ND, not detected; PAGE, polyacrylamide gel electrophoresis; ppm, parts per million; RV, reference value; S-AN, acrylonitrile-derived mercapturic acid adducts; SCN, thiocyanate; SD, standard deviation; SM, smoking mothers; USA, United States of America; yr, year(s).

[a] +, positive; +/–, equivocal; (+/–), equivocal in a study of limited quality; (+), positive in a study of limited quality.

Despite the formation of the electrophile CEO, no evidence of the formation of DNA adducts or of binding to DNA was reported in exposed humans. However, there was evidence in exposed humans of the formation of protein adducts (see also Section 4.1.2), such as haemoglobin adducts with valine residues at the N-termini of globin, or with aspartic residues of albumin (Tavares et al., 1996; Bergmark, 1997; Thier et al., 1999; Scherer et al., 2014).

Most of the studies were on CEV, a structurally specific haemoglobin–acrylonitrile adduct resulting from the reaction of acrylonitrile at the N-terminal valine of both α- and β-chains of globin, which is therefore often used as a biological marker of long-term exposure to acrylonitrile (Tavares et al., 1996; Schettgen et al., 2002, 2004, 2010). CEV was detected in people exposed by inhalation to acrylonitrile after a train accident in Belgium that caused a massive leak (De Smedt et al., 2014; Van Nieuwenhuyse et al., 2014; Simons et al., 2016; Colenbie et al., 2017). The concentration of CEV in isolated globin was associated with the short-term toxic effects of acrylonitrile (Simons et al., 2016). CEV was also found in four cleaning workers who rescued a colleague who was found dead when decontaminating a tank wagon containing acrylonitrile in a train depot in Germany (Bader and Wrbitzky, 2006).

In an observational study, Leng and Gries (2014) monitored 816 individuals who were potentially exposed to acrylonitrile after an accidental fire in a chemical industry facility in Germany. In 12 individuals, CEV concentrations in globin exceeded the reference value of 15 µg/L blood (Leng and Gries, 2014). Another acrylonitrile-specific adduct, CEA, was detected in the serum albumin of a worker who was accidentally exposed to acrylonitrile via contaminated skin (Thier et al., 2000). [The Working Group noted that a common limitation of these studies was the lack of adequate measurement of external exposure.]

Significantly elevated concentrations of CEV, compared with controls ($P < 0.01$), were also found in workers (direct users) and maintenance workers at an acrylonitrile polymerization factory in Portugal (Tavares et al., 1996). In the same study, CEV was found also in globin from newborn babies of smoking mothers but not of non-smoking mothers. In other studies on haemoglobin adducts in workers occupationally exposed to acrylonitrile, results were marginal or inconclusive owing to low exposure levels that confounded the effects of smoking (Bergmark et al., 1993; Ivanov et al., 1993; Thier et al., 1999; see Table 4.1). Ivanov et al. (1993) used GC to analyse blood from 12 rubber plant workers exposed to acrylonitrile at concentrations of 6–9 ppm in air [the method of sampling and analysis was not described]. Acrylonitrile bound to the cysteine –SH group in haemoglobin was released by high temperatures in the GC injector after oxidation of samples by hydrogen peroxide. In the exposed group, bound acrylonitrile amounted to 3.9–33 µmol/g globin, and none was detected in controls. [The Working Group noted that, as mentioned for studies in accidentally exposed people, external exposure was not always adequately assessed in studies of occupational exposure.]

[The Working Group noted that, since acrylonitrile is a known component of tobacco smoke (Counts et al., 2005) and other combustion products (O'Dell et al., 2020), acrylonitrile-specific haemoglobin adducts are useful biomarkers of exposure to cigarette smoke. Indeed, numerous studies have been published on CEV in haemoglobin in blood collected from smokers (Bergmark, 1997; Thier et al., 1999; Fennell et al., 2000; Schettgen et al., 2002; Scherer et al., 2014). In one study, an albumin adduct was also identified, more specifically, a 2-cyanoethyl adduct at the Cys-34 locus in HSA. This adduct was also detected in non-smokers, albeit at significantly lower levels ($P < 0.0001$) than in smokers (Grigoryan et al., 2016). CEV was also detected

in non-smokers with no apparent previous exposure to acrylonitrile (Schettgen et al., 2010; De Smedt et al., 2014).]

[The Working Group noted that, despite some limitations such as insufficient exposure quantification and non-zero background adduct levels in populations with no apparent previous exposure to acrylonitrile (see Table 4.1), studies in exposed humans overall clearly demonstrated evidence that acrylonitrile can form adducts at cysteine –SH groups as well as at the N-termini of blood proteins such as haemoglobin and HSA in vivo.]

(ii) Human cells in vitro

One report with negative results was focused on the formation of specific DNA adducts with CEO in human cells. Human TK6 cells were exposed during log-phase growth for 2 hours to CEO at 100 µM. No *N*7-(2-oxoethyl)guanine (*N*7-OXE-Gua) was detected by HPLC (LOD, 10 pmol/µmol of unmodified guanine in the DNA isolated from these cells (Walker et al., 2020a) (see Fig. 4.2 and Fig. 4.3).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.2.

In rats dosed with radiolabelled acrylonitrile, radiolabel was detected bound to DNA isolated from the liver (Farooqui and Ahmed, 1983; Peter et al., 1983), brain (Farooqui and Ahmed, 1983; Williams et al., 2017), stomach (Farooqui and Ahmed, 1983; Pilon et al., 1988b; Abdel-Rahman et al., 1994), lung (Ahmed et al., 1992a), and testes (Ahmed et al., 1992a).

One DNA adduct, *N*7-OXE-Gua, has been identified in vivo. Marginal levels of *N*7-OXE-Gua (close to the LOD) were found by HPLC in DNA from target and non-target organs in a study of chronic toxicity in rats exposed to acrylonitrile at 500 ppm in drinking-water over a period of 15 months, as well as in rats exposed at 300 ppm for at least 2 weeks.

At the same time, no etheno-DNA adducts, namely, N^2,3-ethenoguanine (εGua), 1,N^6-ethenodeoxyadenosine (1,N^6-εdA), and 3,N^4-deoxycytidine (εdC) were detected; LODs were εGua, 1.4 pmol/µmol guanine; εdAd, 2.4×10^{-8} pmol/µmol deoxyadenosine; and εdC 3.0×10^{-8} pmol/µmol deoxycytidine (Walker et al., 2020a).

Similarly, *N*7-OXE-Gua but not εdAde was detected in DNA isolated from the liver of rats dosed intraperitoneally with acrylonitrile at 50 mg/kg bw or with CEO at 6 mg/kg bw (Hogy and Guengerich, 1986).

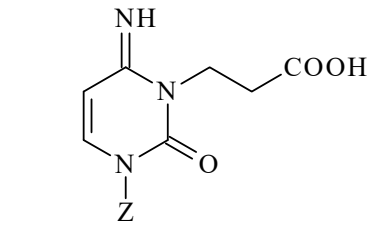
According to a study on acrylonitrile-derived DNA adducts analysed by ^{32}P-postlabelling, no adducts were detected in the brain of rats dosed orally with [2,3-^{14}C]acrylonitrile at 11 mg/kg bw (Williams et al., 2017). [The Working Group noted that the ^{32}P-postlabelling method is not suitable for the detection of easily depurinating adducts such as *N*7-OXE-Gua.]

Negative results for DNA binding were reported after inhalation exposure at a low level corresponding to an acrylonitrile uptake of 4 mg/kg bw (Pilon et al., 1988a), although after the same dose given orally, binding of acrylonitrile was detected in DNA from the rat stomach but not from brain or liver (Pilon et al., 1988b). [The Working Group noted that in this study acrylonitrile uptake was calculated using measurement of the remaining acrylonitrile in inhaled air, which might have led to an underestimation of the absorbed dose.]

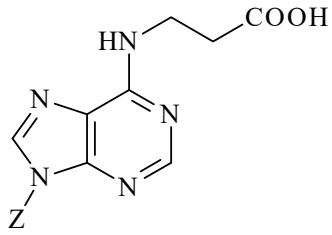
Acrylonitrile was reported to bind to RNA isolated from liver, brain, and stomach of rats exposed to radiolabelled acrylonitrile (Farooqui and Ahmed, 1983; Pilon et al., 1988b).

Binding of radiolabelled acrylonitrile to haemoglobin and multiple tissue proteins was reported in rats. Adducts at terminal valine (Osterman-Golkar et al., 1994; Stevens et al., 1994; Wong et al., 1998) and cysteine (Cys) -SH groups were identified in globin isolated from rat erythrocytes (Ivanov et al., 1993), and specific

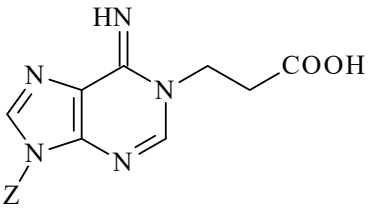
Fig. 4.2 DNA adducts derived from acrylonitrile



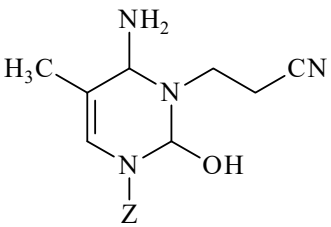
***N3*-CE-Cyt, Z = H[a]**
***N3*-CE-dC, Z = dR[b]**



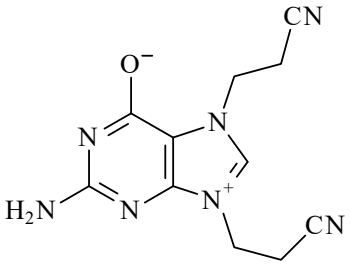
***N*^{6}-CE-Ade, Z = H[a]**
***N*^{6}-CE-dA, Z = dR[b]**



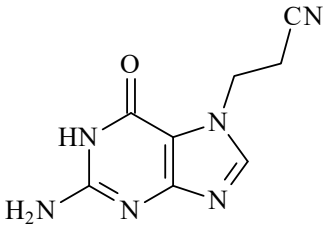
***N1*-CE-Ade, Z = H[a]**
***N1*-CE-dA, Z = dR[b]**



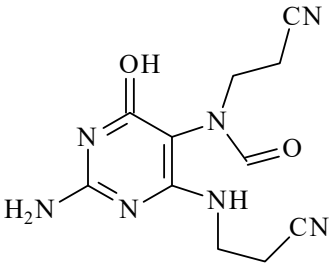
***N3*-AN-Thy, Z = H[a]**
***N3*-AN-dT, Z = dR[b]**



***N7,N9*-bisAN-Gua[a, b]**



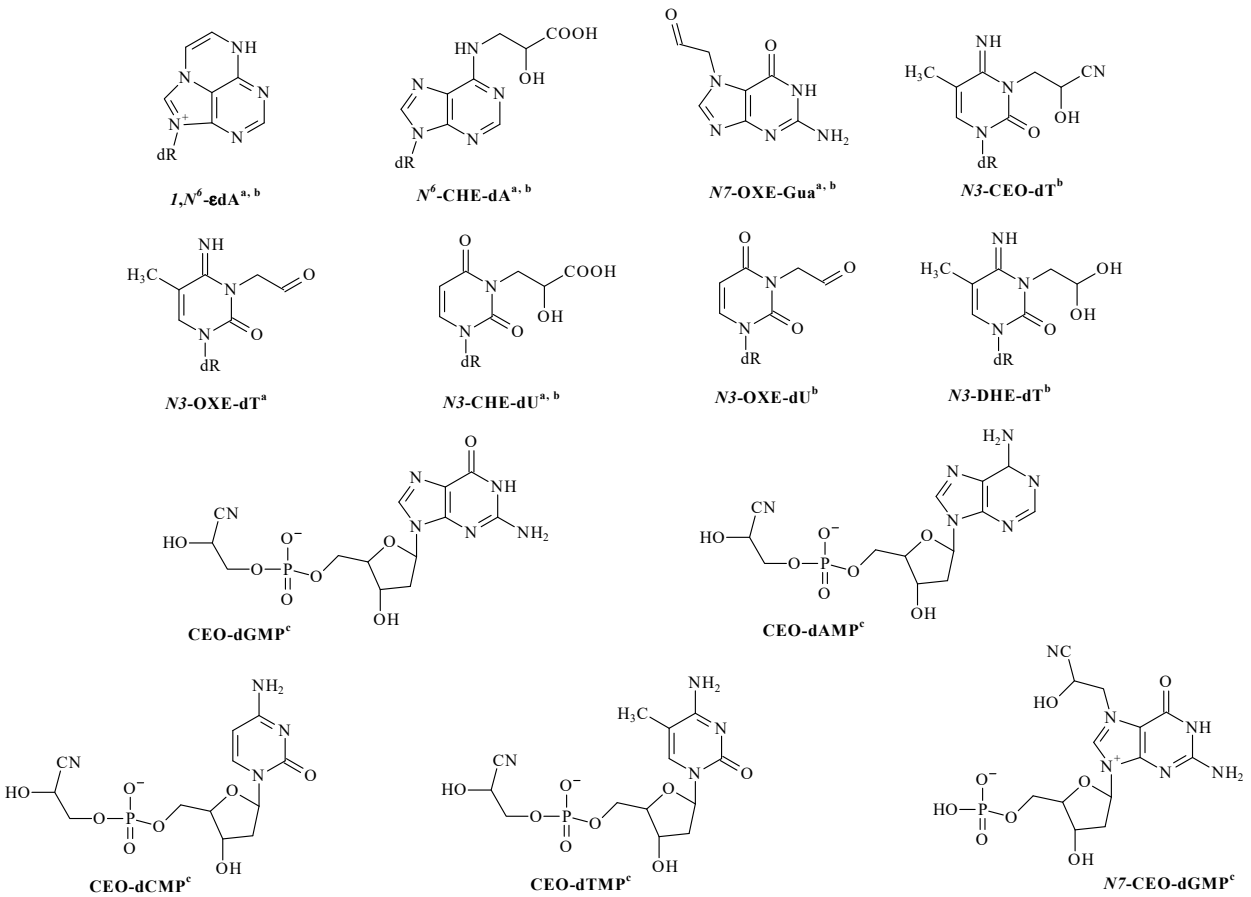
***N7*-AN-Gua[a, b]**



IRO-*N7,N*9-bisAN-Gua[a, b]

Chemical structure of adducts obtained by the reaction of acrylonitrile with (a) DNA followed by acid hydrolysis, or (b) deoxynucleosides. *N*3-CE-Cyt, *N*3-(2-carboxyethyl)cytosine; *N*3-CE-dC, *N*3-(2-carboxyethyl)-2′-deoxycytidine; *N*^{6}-CE-Ade, *N*^{6}-(2-carboxyethyl)adenine; *N*^{6}-CE-dA, *N*^{6}-(2-carboxyethyl)-2′-deoxyadenosine; *N1*-CE-Ade, *N1*-(2-carboxyethyl)adenine; *N1*-CE-dA, *N1*-(2-carboxyethyl)-2′-deoxyadenosine; *N*7-AN-Gua, *N*7-(2-cyanoethyl)guanine; *N*3-AN-Thy, *N*3-(2-cyanoethyl)thymine; *N*3-AN-Thy, *N*3-(2-cyanoethyl)-2′-deoxythymidine; *N*3-AN-dT, *N*3-cyanoethyl-deoxythymidine; *N*7,*N*9-bisAN-Gua, *N*7,*N*9-bis(2-cyanoethyl)guanine; IRO-*N*7,*N*9-bisAN-Gua, imidazole ring-opened *N*7,*N*9-bis(2-cyanoethyl)guanine; dR, 2′-deoxyribosyl.
Created by the Working Group.

Fig. 4.3 DNA adducts derived from cyanoethyl oxide (CEO)



Chemical structures of the adducts obtained by reactions of CEO with (a) DNA followed by enzymatic hydrolysis, or (b) with deoxynucleosides or (c) with deoxynucleoside-5′-phosphates (listed in the order presented in the figure).
1,N^6-εdA, 1,N^6-etheno-2′-deoxyadenosine; N^6-CHE-dA, N^6-(2-carboxy-2-hydroxyethyl)-2′-deoxyadenosine; CEO, cyanoethyl oxide; CEO-dGMP, CEO-dAMP, CEO-dCMP and CEO-dTMP, 2-cyano-2-hydroxyethyl phosphodiester of 2′-deoxyguanosine-5′-phosphate, 2′-deoxyadenosine-5′-phosphate, 2′-deoxycytosine-5′-phosphate, and 2′-deoxythimidine-5′-phosphate, respectively; *N*7-CEO-dGMP, *N*7-(2-cyano-2-hydroxyethyl)-2′-deoxyguanosine-5′-phosphate; *N*3-CEO-dT, *N*3-(2-cyano-2-hydroxyethyl)-2′-deoxythymidine; *N*7-OXE-Gua, *N*7-(2-oxoethyl)guanine; *N*3-OXE-dT, *N*3-(2-oxoethyl)-2′-deoxythymidine; *N*3-CHE-dU, *N*3-(2-carboxy-2-hydroxyethyl)-2′-deoxyuridine; *N*3-OXE-dU, *N*3-(2-oxoethyl)-2′-deoxyuridine; *N*3-DHE-dT, *N*3-(2,2-dihydroxyethyl)-2′-deoxythymidine.
Created by the Working Group.

Table 4.2 End-points relevant to electrophilicity in non-human mammalian systems in vivo exposed to acrylonitrile

End-point	Species, strain (sex) PM	Biosample type	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
*N*7-OXE-Gua	Rat, F344 (M and F)	Isolated DNA from brain, forestomach, liver, and spleen	+	500 ppm	AN at 500 ppm in drinking-water, ~450 d	Long-term study; single concentration; very low levels *N*7-OXE-Gua near the LOD.	Walker et al. (2020a)
Etheno-adducts	Rat, F344 (M)	Isolated DNA from brain, forestomach, liver, spleen	–	500 ppm	AN at 500 ppm in drinking-water, ~450 d	Long-term study; single concentration; sensitive HPLC method.	
*N*7-OXE-Gua, etheno-adducts	Rat, F344 (M)	Isolated DNA from brain, forestomach, spleen	–	300 ppm	AN at 0, 3, 7, 14, 28, 104, 300 ppm in drinking-water, 105 d	Long-term study; multiple concentration, sensitive HPLC method.	
*N*7-OXE-Gua, etheno-adducts	Rat, F344 (M)	Isolated DNA from liver	+ Weakly +	300 ppm	AN at 0, 3, 7, 14, 28, 104, 300 ppm in drinking-water, 105 d Only in the first 14 d of exposure	Long-term study; single AN concentration; very low levels of *N*7-OXE-Gua near the LOD; no etheno-adducts.	
DNA binding	Rat, F344 (M)	Isolated DNA from stomach	+	4 mg/kg bw	[2,3-^{14}C]AN at 4 mg/kg orally by gavage, tissue work-up 6 h after dosing	DNA isolated; radioactivity measured; single dose level.	Pilon et al. (1988b)
RNA binding	Rat, F344 (M)	Isolated RNA plus DNA from brain, stomach, and liver	+	4 mg/kg bw	[2,3-^{14}C]AN at 4 mg/kg orally by gavage	Isolated RNA plus DNA and then removed RNA; no radioactivity detected in DNA except for from stomach.	
DNA binding	Rat, F344 (M)	Isolated DNA from brain, stomach, and liver	–	4 mg/kg bw	Inhalation, finished after 4 mg/kg of [2,3-^{14}C]AN was taken up	DNA isolated, bound radioactivity measured; uptake might be overestimated; single dose level.	Pilon et al. (1988a)
RNA binding	Rat, F344 (M)	Isolated RNA plus DNA from brain, stomach, and liver	+	4 mg/kg bw	Inhalation, finished after 4 mg/kg of [2,3-^{14}C]AN was taken up	Isolated RNA plus DNA and then removed RNA; no radioactivity detected in the DNA; the uptake might be overestimated.	

Table 4.2 (continued)

End-point	Species, strain (sex) PM	Biosample type	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
*N*7-OXE-Gua, *N*1,N^6-εdA	Rat, F344 (M)	Isolated DNA from liver	+	50 mg/kg bw	Single i.p. injection, AN at 50 mg/kg bw, animals killed 2 h after dosing	Single dose at a single dose level; low level of *N*7-OXE-Gua, no *N*1,N^6-εdA detected.	Hogy and Guengerich (1986)
*N*7-OXE-Gua, *N*1,N^6-εdA	Rat, F344 (M)	Isolated DNA from brain	–	50 mg/kg bw	Single i.p. injection, AN at 50 mg/kg bw, animals killed 2 h after dosing	Single dose at a single dose level; short time to enable binding.	
DNA binding, RNA binding	Rat, F344 (M)	Isolated DNA and RNA from liver and brain	–	0.6 mg/kg bw	[2,3-^{14}C]CEO at 0.6 mg/kg bw i.p., animals killed 1 h after dosing	Single low dose at a single dose level; short time to enable binding.	
DNA binding	Rat, F344 (F) and Sprague-Dawley (F)	Brain, isolated DNA	+	11 mg/kg bw	AN at 100 ppm in drinking-water, followed by [2,3-^{14}C]AN at 11 mg/kg bw orally	Rigorously purified isolated DNA; ^{14}C measured by accelerator MS.	Williams et al. (2017)
DNA adducts	Rat, F344 (F) and Sprague-Dawley (F)	Brain, isolated DNA	–	11 mg/kg bw	AN at 100 ppm in drinking-water, followed by [2,3-^{14}C]AN at 11 mg/kg bw orally	Adducts analysed by ^{32}P-postlabelling; single dose level.	
DNA binding	Rat, Sprague-Dawley (M)	Isolated DNA from lung	+	46.5 mg/kg bw	[2,3-^{14}C]AN orally by gavage, tissues taken 0.5, 3, 12, and 72 h after dosing	Single dose level, multiple time points; maximum bound radioactivity detected 12 h after dosing.	Ahmed et al. (1992a)
DNA binding	Rat, Sprague-Dawley (M)	Isolated DNA from testis	+	46.5 mg/kg bw	[2,3-^{14}C]AN orally by gavage, tissues taken at 0.25, 0.5, 1, 3, 6, 12, and 72 h after dosing	Single dose level, multiple time points; maximum bound radioactivity detected 0.5 h after dosing, followed by a gradual decrease.	Ahmed et al. (1992a)
DNA binding, RNA binding	Rat, Wistar (M)	Isolated DNA from liver	+	0.2 mmol	[2,3-^{14}C]AN i.p., tissue taken 12 and 72 h after dosing	Single dose level; low level of bound radioactivity to the DNA; in the RNA, bound radioactivity was found at uracil, adenine, guanine, and cytosine.	Peter et al. (1983)

Table 4.2 (continued)

End-point	Species, strain (sex) PM	Biosample type	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA binding	Rat, Sprague-Dawley (M)	Isolated DNA from brain, stomach, and liver	+	46.5 mg/kg bw	[2,3-^{14}C]AN orally by gavage, tissues taken 1, 6, 24, and 48 h after dosing	DNA was isolated and radioactivity was measured for a single dose level; possible contamination by protein residues.	Farooqui and Ahmed (1983)
RNA binding	Rat, Sprague-Dawley (M)	Isolated RNA from liver, stomach, brain, kidney and spleen	+	46.5 mg/kg bw	[2,3-^{14}C]AN orally by gavage, tissues taken 1, 6, 24 and 48 h after dosing	RNA was isolated, and radioactivity was measured for single dose level; possible contamination by protein residues.	
DNA binding	Rat, Sprague-Dawley (M)	Isolated DNA from stomach	(+)	46.5 mg/kg bw	[2,3-^{14}C]AN orally by gavage, tissues taken at 0.25, 0.5, 1, 3, 6, 12, and 72 h after dosing	Single dose level, multiple time points; maximum bound radioactivity detected 0.25 h after dosing, followed by a sharp decrease; probable contamination by gastric contents.	Abdel-Rahman et al. (1994)
Hb adduct (CEV)	Rat, F334 (M)	Peripheral blood	+	3 ppm in water for 65 d, equivalent to AN total dose of 6.3 mg/kg bw	Oral, in drinking-water, 105 d (erythrocyte lifespan, 65 d)	Clear dose–response relation with AN at up to 300 ppm (513 mg/kg bw).	Osterman-Golkar et al. (1994)
Hb adduct	Rat, F334 (M)	Peripheral blood	+	100 ppm in drinking-water	Oral, in drinking-water, 105 d	Whole globin analysed by ESI-MS; 2-cyanoethyl-adduct predominantly on β-chains of globin.	Stevens et al. (1994)
Hb adduct	Rat, Sprague-Dawley (M)	Peripheral blood	+	1.1 mg/kg bw	[2,3-^{14}C]AN subcutaneously at 1.1, 3.3, 10, 20, 50, 80, and 115 mg/kg bw	New ELISA method compared with GC-MS.	Wong et al. (1998)
Protein binding	Rat, Sprague-Dawley (M)	Multiple tissues	+	1.2 mg/kg bw	[2,3-^{14}C]AN subcutaneously at 1.2–115 mg/kg bw	Multiple dose levels, high dose–response correlation.	Benz et al. (1997)

Table 4.2 (continued)

End-point	Species, strain (sex) PM	Biosample type	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Protein binding	Rat, Wistar (M)	Multiple tissues	+	0.2 mmol	[2,3-^{14}C]AN i.p. at 0.2 mmol/animal	Single dose level.	Peter and Bolt (1981)
Protein binding	Rat, Sprague-Dawley (M)	Proteins from liver, stomach brain, kidney, and spleen	+	46.5 mg/kg bw	[2,3-^{14}C]AN orally by gavage, tissue taken at 1, 6, 24, and 48 h after dosing	Precipitated protein from the extract was used, not further characterized.	Farooqui and Ahmed (1983)
Protein binding	Rat, F344 (M)	Proteins from liver and brain	+	0.6 mg/kg bw	[2,3-^{14}C]CEO at 0.6 mg/kg bw i.p., animals killed 1 h after dosing	Single low dose at a single level; showed high capability of CEO to rapidly bind proteins.	Hogy and Guengerich (1986)
Protein adduct	Rat, Sprague-Dawley (M)	Liver cytosol, carbonic anhydrase III	+	115 mg/kg bw	[2,3-^{14}C]AN subcutaneously, tissue taken 2 h after dosing	Single dose level; adducted AN located mainly at Cys-181 and Cys-186 residues.	Nerland et al. (2003)
Protein adduct	Rat, Sprague-Dawley (M)	Liver cytosol GSTs	+	115 mg/kg bw	[2,3-^{14}C]AN subcutaneously, tissue taken 2 h after dosing	Single dose level; AN located preferably in GSTM1 at Cys-86.	Nerland et al. (2001)
Hb adduct *S*-(2-cyanoethyl)cysteine at globin	Rat, Wistar (M)	Peripheral blood	(+)	5 mg/kg bw	Single i.p. dose of 5, 25, or 50 mg/kg bw	No control group; new analytical method (GC) not adequately validated.	Ivanov et al. (1993)
Hb adduct, *S*-(2-cyanoethyl)cysteine in globin	Rat, Wistar (M)	Peripheral blood	(+)	1 mg/kg bw	Repeated i.p. dosing over six consecutive days, at 1, 5, and 10 mg/kg bw	No control group; new analytical method (GC) not adequately validated.	

AN, acrylonitrile; bw, body weight; CEO, cyanoethyl oxide; CEV, *N*-(2-cyanoethyl)valine; Cys, cysteine; d, day(s); *N*1,*N*^{6}-εdA, *N*1,*N*^{6}-ethenodeoxyadenosine; ELISA, enzyme-linked immunosorbent assay; ESI-MS, electrospray ionization-mass spectrometry; F, female; F344, Fischer 344; GC, gas chromatography; GC-MS, gas chromatography-mass spectrometry; GST, glutathione *S*-transferase; GSTM1, glutathione S-transferase mu class 1; h, hour(s); Hb, haemoglobin; HID, highest ineffective dose; HPLC, high-performance liquid chromatography; i.p., intraperitoneal; LED, lowest effective dose; LOD, limit of detection; M, male; MS, mass spectrometry; *N*7-OXE-Gua, *N*7-(2-oxoethyl)guanine; ppm, parts per million.

[a] +, positive; –, negative; (+), positive in a study of limited quality.

loci of 2-cyanoethyl adduction were identified at the active site of carbonic anhydrase (Cys-181 and Cys-186) (Nerland et al., 2003) and in glutathione *S*-transferase mu class (GSTM1) (Cys-86) (Nerland et al., 2001).

Binding to proteins was reported in multiple tissues from rats treated with radiolabelled acrylonitrile (Peter and Bolt, 1981; Farooqui and Ahmed, 1983; Benz et al., 1997) or with low doses of radiolabelled CEO (Hogy and Guengerich, 1986).

(ii) Acellular systems

See Table 4.3.

Seven adducts with nucleotide bases have been reported to result from the reaction of acrylonitrile with DNA from calf thymus, followed by total acidic hydrolysis, which released both adducted and unchanged nucleotide bases, opened the imidazole ring of adducted guanine, and converted the 2-cyanoethyl group at adenine and cytosine to 2-carboxylethyl (Solomon et al., 1984). Very high concentrations of both DNA and acrylonitrile together with a long reaction time (40 days) were used in this experiment. The adduct structures are shown in Fig. 4.2. [The Working Group noted that these experiments showed a limited reactivity of acrylonitrile towards DNA.]

The reactions of CEO with DNA proceeded much more rapidly than those of acrylonitrile. *N*7-OXE-Gua was released from calf thymus DNA by mild acidic hydrolysis (depurination), as well as from DNA isolated from liver and brain of unexposed rats incubated with CEO. The concentration of the adduct, *N*7-OXE-Gua, increased linearly with that of CEO (Walker et al., 2020a). Reaction of CEO with calf thymus DNA led to the formation of five deoxynucleoside adducts that were released by enzymatic hydrolysis; however, none of them retained an untouched 2-cyano-2-hydroxyethyl moiety that eliminated hydrogen cyanide (HCN) or underwent hydrolysis of the nitrile to carboxyl (Solomon et al., 1993; Table 4.3). [The Working Group noted that experiments in vitro with radiolabelled acrylonitrile showed that metabolic activation plays a key role in the binding of acrylonitrile to DNA and in adduct formation.]

Positive results were observed with calf thymus DNA and metabolic activation via rat liver microsomes, a reconstituted CYP system (Guengerich et al., 1981), and human lung lipoxygenase (Roy and Kulkarni, 1999). Inconclusive results were obtained when calf thymus DNA was mixed with radiolabelled acrylonitrile for a short reaction time (30 minutes) with or without metabolic activation (Peter et al., 1983), or for 4 hours without metabolic activation (Guengerich et al., 1981). In these experiments, marginal levels of DNA-bound radiolabel were obtained. [The Working Group noted that the DNA reactivity of acrylonitrile without metabolic activation appeared to be limited and was unlikely to occur in vivo. In contrast, binding of radiolabelled acrylonitrile to RNA, both with and without metabolic activation, was clearly demonstrated (Guengerich et al., 1981; Peter et al., 1983). Most of the radiolabel was bound to the uracil base (Peter et al., 1983) (Table 4.3).]

The reaction of acrylonitrile with individual deoxynucleosides at 37 °C, pH 7, yielded a set of seven adducts that were analogous to those obtained by reaction with DNA; however, long reaction times of 10 or 40 days were required. The same set of adducts, albeit with much lower yields, was obtained at pH 5 (Fig. 4.2) (Solomon et al., 1984).

In contrast, the reaction of CEO with deoxynucleosides required only 3 hours to afford appreciable yields of the adducts (Fig. 4.3) (Solomon et al., 1993; Yates et al., 1993).

Different adducts were obtained when CEO was reacted with deoxynucleotides at nearly physiological conditions (37 °C, pH 7.0–7.5). In this case, the 2-cyano-2-hydroxyethyl moiety remained untouched, each nucleotide yielding one 2-cyano-2-hydroxyethyl phosphodiester

Table 4.3 End-points relevant to electrophilicity in acellular experimental systems exposed to acrylonitrile

End-point	Conditions	Results[a]		Concentration	Method of detection or characterization	Comments	Reference
		Without metabolic activation	With metabolic activation				
Binding to DNA isolated from rat brain and liver	37 °C, pH 7.4, 2 h	+	NR	0, 1.07, 5.33 or 53.5 µM CEO; DNA, 2 mg/mL	HPLC, fluorescence	*N*7-OXE-Gua concentration in the DNA increased linearly with CEO concentration, $R > 0.99999$.	Walker et al. (2020a)
Binding to DNA isolated from TK6 lymphoblastoid cells	37 °C, pH 7.4, 2 h	+	NR	60 or 210 pmol [2,3-^{14}C] CEO; DNA, 1 mg/mL	Measurement of radioactivity	Dose-dependent binding.	
Binding to calf thymus DNA	37 °C, pH 7.4, 2 h	+	NR	16 µM CEO	HPLC, fluorescence	*N*7-OXE-Gua detected.	
Binding to calf thymus DNA	37 °C, pH 7.4, 3 h	+	NR	150 mM [2,3-^{14}C]CEO; DNA, 1 mg/mL	HPLC-UV and scintillation	DNA hydrolysed to nucleosides, *N*3-CEO-dT identified by HPLC retention-time comparison.	Yates et al. (1993)
Binding to calf thymus DNA	37 °C, pH 7.7, 10 min to 4 h, rat liver microsomes (2 mg of protein/mL), 1.5 mg of DNA/mL	+/–	+	1 mM [2,3-^{14}C]AN; DNA, 1.5 mg/mL	Measurement of radioactivity	Marginal binding reported in microsomes without metabolic activation (–NADPH); no control without microsomes.	Guengerich et al. (1981)
Binding to calf thymus DNA	Reconstituted CYP system, 60 min, 37 °C	+/–	+	1 mM [2,3-^{14}C]AN; DNA 1.5 mg/mL	Measurement of radioactivity	Marginal binding reported without metabolic activation (-NADPH); no control without CYP.	
Binding to calf thymus DNA	37 °C, pH 7.7, 10 min to 4 h, rat liver microsomes (2 mg of protein/mL), 1.5 mg DNA/mL	+/–	+	1 mM [2,3-^{14}C]AN; DNA, 1.5 mg/mL	Measurement of radioactivity	Marginal binding reported in microsomes without metabolic activation (-NADPH), no control without microsome.	

Table 4.3 (continued)

End-point	Conditions	Results[a]		Concentration	Method of detection or characterization	Comments	Reference
		Without metabolic activation	With metabolic activation				
Binding to calf thymus DNA	37 °C, pH 7.7, 10 min to 4 h, rat liver microsomes (2 mg of protein/mL), 1.5 mg DNA/mL	+	NR	1 mM [2,3-^{14}C]CEO or 1 mM [1-^{14}C]CEO; DNA, 1.5 mg/mL	Measurement of radioactivity	Higher binding of [2,3-^{14}C]CEO compared with [1-^{14}C]CEO.	Guengerich et al. (1981) (cont.)
Binding to calf thymus DNA	30 min, 37 °C, with or without rat liver microsomes; Wistar (M)	(+/–)	(+/–)	8.3 and 16.5 mM [2,3-^{14}C]AN; DNA, 4 mg/mL	Measurement of radioactivity	Most of bound radioactivity removed after further purification of the DNA, pH not given.	Peter et al. (1983)
Binding to calf thymus DNA	37 °C, pH 10, 30 min, HLLO	NR	(+)	1 mM [^{14}C]AN; DNA, 4 mg/mL; HLLO 0.02–0.5 mg/mL	Measurement of radioactivity	Actual concentration of CEO not known; missing comparison with binding of AN without activation by HLLO; non-physiological pH.	Roy and Kulkarni (1999)
Binding to calf thymus DNA	37 °C, pH 7.0–7.5, 40 d	+	NR	DNA, 3 mg/mL, ~1.36 M AN	Paper chromatography, isolation, characterization by UV and MS	Total hydrolysis of adducted DNA yielded seven 2-cyanoethyl adducts with nucleobases; structure determination was not completely convincing.	Solomon et al. (1984)
Binding to calf thymus DNA	37 °C, pH s7.0–7.5, 3 h	+	NR	DNA, 3 mg/mL, ~1.36 M CEO	HPLC, UV	Hydrolysis of adducted DNA; five deoxynucleoside adducts identified by HPLC retention-time comparison.	Solomon et al. (1993)
Binding to yeast-soluble RNA	37 °C, pH 7.7, 10 min to 4 h, rat liver microsomes (protein, 2 mg/mL)	NR	+	1 mM [2,3-^{14}C]AN, DNA 1.5 mg/mL	Measurement of radioactivity	No control without microsomes.	Guengerich et al. (1981)

Table 4.3 (continued)

End-point	Conditions	Results[a]		Concentration	Method of detection or characterization	Comments	Reference
		Without metabolic activation	With metabolic activation				
Binding to yeast RNA	30 min, 37 °C, rat liver microsomes (protein, 1 mg/mL), Wistar (M)	+	+	16.5 mM [2,3-^{14}C]AN	Measurement of radioactivity	Most radioactivity bound at uracil; pH not reported.	Peter et al. (1983)
Binding to BSA	37 °C, pH 10, 15 min, HLLO	NR	+	1 mM [^{14}C]AN, DNA 4 mg/mL, HLLO 0.02–0.5 mg/mL	Measurement of radioactivity	Actual concentration of CEO not reported; missing comparison with binding of AN without activation by HLLO.	Roy and Kulkarni (1999)
Binding to Hb	Blood from F344 rats (M), 37 °C, 2–15 min	+	NR	0.1, 0.4, 1.0 and 2.0 mM [2,3-^{14}C]AN	Measurement of radioactivity	Kinetics of binding; globin isolated from erythrocytes.	Gargas et al. (1995)
Binding to Hb	Blood from F344 rats (M), 37 °C, 2–15 min	+	NR	0.1, 0.4, 1.0 and 2.0 mM [2,3-^{14}C]CEO	Measurement of radioactivity	Kinetics of binding; globin isolated from erythrocytes.	
Binding to rat liver proteins	S9 fraction of rat liver, Wistar (M), 4 °C or 37 °C, pH 7.4, 0–60 min	+	+	44 µM [3-^{14}C]AN; protein, 0.5–3 mg/assay	Measurement of radioactivity	Linear increase with protein incubation time and protein concentration; without metabolic activation means at 4 °C.	Duverger-Van Bogaert et al. (1982)
Binding to rat liver proteins	Rat liver cytosol (S/10), Wistar (M), 4 °C or 37 °C, pH 7.4	+	+	44 µM [3-^{14}C]AN; protein, 2 mg/assay	Measurement of radioactivity	Similar levels of binding with and without metabolic activation.	
Binding to rat liver proteins	Rat liver microsomes (P/4)	+	+	44 µM [3-^{14}C]AN; protein, 2 mg/assay	Measurement of radioactivity	Without metabolic activation means at 4 °C.	
Binding to BSA	37 °C, pH 7.4, 60 min	+	NR	44 µM [3-^{14}C]AN; protein, 2 mg/assay	Measurement of radioactivity	Single concentration.	
Binding to BSA	Rat liver microsomes, 10 min, 37 °C	+	+	1 mM [2,3-^{14}C]AN	Measurement of radioactivity	Without metabolic activation means microsomes (–NADPH).	Guengerich et al. (1981)
Binding to BSA	30 min, 37 °C, with or without rat liver microsomes; Wistar rat (M)	+	+	16.5 mM [2,3-^{14}C]AN	Measurement of radioactivity	Single concentration.	Peter et al. (1983)

Table 4.3 (continued)

End-point	Conditions	Results[a]		Concentration	Method of detection or characterization	Comments	Reference
		Without metabolic activation	With metabolic activation				
Binding to rabbit muscle GAPDH	25 and 37 °C, pH 7.4, 5–200 min	+	NR	50, 100, 200 or 400 µM AN	MALDI-TOF and ESI-MS/MS	AN selectively located in the active site of GAPDH at Cys-149.	Campian et al. (2002)
Binding to rat liver proteins	Rat liver microsomes, Wistar (M) with or without NADPH, 37 °C, pH 7.5, 5–60 min	+	(–)	9.1 mM [2,3-^{14}C]AN; protein, 2 mg/mL	Measurement of radioactivity	No increase in binding with NADPH-regenerating system compared with microsomes without NADPH-regenerating system.	Peter and Bolt (1981)
Binding to rat liver proteins	Rat liver cytosol, Wistar (M), 37 °C, pH 7.5, 60 min	+	NR	9.1 mM [2,3-^{14}C]AN; protein, 2 mg/mL	Measurement of radioactivity	Single concentration.	

AN, acrylonitrile; BSA, bovine serum albumin; CEO, cyanoethyl oxide; *N*3-CEO-dT, *N*3-(2-cyano-2-hydroxyethyl)-2′-deoxythymidine; CYP, cytochrome P450; Cys, cysteine; d, day(s); ESI-MS/MS, electrospray ionization-tandem mass spectrometry; F344, Fischer 344; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; h, hour(s); Hb, haemoglobin; HLLO, human lung lipoxygenase; HPLC, high-performance liquid chromatography; HPLC-UV, high-performance liquid chromatography-ultraviolet detection; M, male(s); MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; min, minute(s); MS, mass spectrometry; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NR, not reported; *N*7-OXE-Gua, *N*7-(2-oxoethyl)guanine; S9, 9000 × *g* supernatant; UV, ultraviolet.

[a] +, positive; +/–, equivocal; (+/–), equivocal in a study of limited quality; (+) or (–), positive or negative in a study of limited quality.

adduct. In addition, 2′-deoxyguanosine-5′-phosphate (dGMP) also yielded *N*7-(2-cyano-2-hydroxyethyl)-2′-deoxyguanosine-5′-phosphate (*N*7-CEO-dGMP) (Fig. 4.3). All deoxynucleotide adducts were isolated and identified by proton nuclear magnetic resonance (^{1}H-NMR), correlation spectroscopy two-dimensional nuclear magnetic resonance (COSY 2D-NMR), FAB-MS, and UV spectra. A plausible mechanism was proposed involving ring closure of these phosphodiester adducts and subsequent strand scission of the adducted DNA (Yates et al., 1994) (see Table 4.4).

Binding of acrylonitrile to proteins did not require metabolic activation, as documented in studies in vitro on rat blood (Gargas et al., 1995), rat liver proteins (Peter and Bolt, 1981; Duverger-Van Bogaert et al., 1982), bovine serum albumin (Guengerich et al., 1981; Duverger-Van Bogaert et al., 1982; Peter et al., 1983), and rabbit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Campian et al., 2002). In the latter case, acrylonitrile bound selectively to Cys-149 at the active site of GAPDH (Table 4.3). However, metabolic activation by rat liver microsomes (Guengerich et al., 1981; Duverger-Van Bogaert et al., 1982; Peter et al., 1983) but not by rat liver cytosol (Duverger-Van Bogaert et al., 1982) increased the amount of bound radiolabel.

Synopsis

[Acrylonitrile is an α,β-unsaturated nitrile that undergoes Michael addition reactions with various biologically important thiols and amines. It is also metabolically activated to a reactive epoxide, CEO.

The reactions of both acrylonitrile and CEO with GSH in vitro and in vivo have been described in Section 4.1. Although DNA adducts were not directly identified in exposed humans in vivo, and only one DNA adduct, *N*7-OXE-Gua, was detected at very low levels in rats, binding to DNA, RNA, and blood and tissue proteins was clearly demonstrated in rats. Moreover, several adducts were identified when acrylonitrile or CEO reacted with DNA in vitro. The Working Group noted that metabolic activation of acrylonitrile to CEO plays a crucial role in DNA adduct formation. Acrylonitrile appeared to be sufficiently reactive to bind directly to proteins and RNA (mainly at uracil), but binding to DNA proceeded slowly and was therefore more limited. The haemoglobin adduct of acrylonitrile, CEV, was found in humans exposed to acrylonitrile, but also, at much lower levels, in people with no apparent previous exposure to acrylonitrile. The binding of acrylonitrile to DNA in vitro required extremely high concentrations and long reaction time or metabolic activation, but positive binding results were consistently obtained with the metabolite CEO, whereas the binding of acrylonitrile to proteins showed consistently positive results with and without metabolic activation. Reactions with deoxynucleotides and deoxynucleosides confirmed the low reactivity of acrylonitrile and the much higher reactivity of CEO. The Working Group considered that acrylonitrile, on the basis of its structure and kinetics, is definitely electrophilic, although it remained unclear whether or not it could bind covalently to DNA at realistic human exposure levels.]

4.2.2 Is genotoxic

(a) Humans

(i) Exposed humans

See Table 4.5.

DNA damage, assessed by the comet assay, was increased in the spermatozoa of 30 chemical plant workers exposed to acrylonitrile at 0.8 mg/m^3 compared with 30 matched controls with no known exposure to acrylonitrile (Xu et al., 2003). This resulted in a significant ($P < 0.01$) increase in the rate of formation of nuclei with DNA strand breaks, and in the magnitude of fragmentation and tail length (Xu et al., 2003).

Table 4.4 End-points relevant to electrophilicity, nucleoside and nucleotide adducts, in acellular experimental systems exposed to acrylonitrile

End-points	Conditions	Adduct(s)	Method of detection or characterization	Comments	Reference
Adduct formation with nucleotide dT	150 mM [2,3-^{14}C]CEO, 10 mM dT, 37 °C, pH 7.4, 3 h	*N*3-CEO-dT, *N*3-DHE-dT	HPLC, ^{1}H-NMR, 2D NMR, and FAB-MS	Isolated by HPLC.	Yates et al. (1993)
Adduct formation with nucleoside dGMP	150 mM [2,3-^{14}C]CEO, 25 mM dGMP, 37 °C, pH 7.0–7.5, 3 h	*N*7-CEO-dGMP, CEO-dGMP	HPLC, ^{1}H-NMR, 2D NMR, and FAB-MS	Isolated by HPLC.	Yates et al. (1994)
Adduct formation with nucleoside dAMP	150 mM [2,3-^{14}C]CEO, 25 mM dAMP, 37 °C, pH 7.0–7.5, 3 h	CEO-dAMP	HPLC, ^{1}H-NMR, 2D NMR, and FAB-MS	Isolated by HPLC.	Yates et al. (1994)
Adduct formation with nucleoside dCMP	150 mM [2,3-^{14}C]CEO, 25 mM dCMP, 37 °C, pH 7.0–7.5, 3 h	CEO-dCMP	HPLC, ^{1}H-NMR, 2D NMR, and FAB-MS	Isolated by HPLC.	Yates et al. (1994)
Adduct formation with nucleoside dTMP	150 mM [2,3-^{14}C]CEO, 25 mM dTMP, 37 °C, pH 7.0–7.5, 3 h	CEO-dTMP	HPLC, ^{1}H-NMR, 2D NMR, and FAB-MS	Isolated by HPLC.	Yates et al. (1994)
Adduct formation with nucleotide dA	2M CEO, 20 mM dA, 37 °C, pH 7.0–7.5, 3 h	N^6-CEO-dA, *N*1, N^6-εdA	UV, ^{1}H-NMR, and MS	Isolated by paper chromatography and HPLC.	Solomon et al. (1993)
Adduct formation with nucleotide dC	2M CEO, 20 mM dC, 37 °C, pH 7.0–7.5, 3 h	*N*3-CEO-dU	UV, ^{1}H-NMR, and MS	Isolated by paper chromatography and HPLC.	Solomon et al. (1993)
Adduct formation with nucleotide dG	2M CEO, 20 mM dG, 37 °C, pH 7.0–7.5, 3 h	*N*7-OXE-Gua	UV, ^{1}H-NMR, and MS	Isolated by paper chromatography and HPLC.	Solomon et al. (1993)
Adduct formation with nucleotide dT	2M CEO, 20 mM dT, 37 °C, pH 7.0–7.5, 3 h	*N*3-OXE-dT	UV, ^{1}H-NMR, and MS	Isolated by paper chromatography and HPLC.	Solomon et al. (1993)
Adduct formation with nucleotide dA	Saturated aqueous AN, 37 °C, pH 5.0–7.0, at 4, 10, and 40 d	*N*1-CE-dA, N^6-CE-dA	UV, DCI MS, and EI MS	Isolated by paper chromatography and HPLC; no adducts detected after 4 d.	Solomon et al. (1984)
Adduct formation with nucleotide dC	Saturated aqueous AN, 37 °C, pH 5.0–7.0, at 4, 10, and 40 d	*N*3-CE-dC	UV, DCI MS, and EI MS	Isolated by paper chromatography and HPLC; no adducts detected after 4 d.	Solomon et al. (1984)
Adduct formation with nucleotide dG	Saturated aqueous AN, 37 °C, pH 5.0–7.0, at 4, 10, and 40 d	*N*7-AN-Gua, IRO-*N*7,*N*9-bisAN-Gua	UV, DCI MS, and EI MS	Isolated by paper chromatography and HPLC; no adducts detected after 4 d.	Solomon et al. (1984)

Table 4.4 (continued)

End-points	Conditions	Adduct(s)	Method of detection or characterization	Comments	Reference
Adduct formation with nucleotide dT	Saturated aqueous AN, 37 °C, Ph 5.0–7.0, at 4, 10 and 40 d	*N*3-AN-dT	UV, DCI MS, and EI MS	Isolated by paper chromatography and HPLC; no adducts detected after 4 d.	Solomon et al. (1984)

AN, acrylonitrile; *N*3-AN-dT, *N*3-(2-cyanoethyl)-2′-deoxythymidine; *N*7-AN-Gua, *N*7-(2-cyanoethyl)guanine; *N*1-CE-dA, *N*1-(2-carboxyethyl)-2′-deoxyadenosine; N^6-CE-dA, N^6-(2-carboxyethyl)-2′-deoxyadenosine; *N*3-CE-dC, N3-(2-carboxyethyl)-2′-deoxycytidine; CEO, cyanoethyl oxide; CEO-dGMP, CEO-dAMP, CEO-dCMP, and CEO-dTMP, 2-cyano-2-hydroxyethyl phosphodiester of dGMP, dAMP, dCMP, and dTMP, respectively; N^6-CEO-dA, N^6-(2-cyano-2-hydroxyethyl)-2′-deoxyadenosine; *N*7-CEO-dGMP, *N*7-(2-cyano-2-hydroxyethyl) 2′-deoxyguanosine-5′-phosphate; *N*3-CEO-dT, *N*3-(2-cyano-2-hydroxyethyl)thymidine; *N*3-CEO-dU, *N*3-(2-cyano-2-hydroxyethyl)-2′-deoxyuridine; dA, 2′-deoxyadenosine; *N*1,N^6-εdA, 1,N^6-etheno-2′-deoxyadenosine; dAMP, 2′-deoxyadenosine-5′-phosphate; d, day(s); dC, 2′-deoxycytosine; DCI, desorption chemical ionization; dCMP, 2′-deoxycytosine-5′-phosphate; dG, 2′-deoxyguanosine; dGMP, 2′-deoxyguanosine-5′-phosphate; *N*3-DHE-dT, *N*3-(2,2-dihydroxyethyl)thymidine; dT, 2′-deoxythymidine; dTMP, 2′-deoxythymidine-5′-phosphate; EI, electron ionization; FAB-MS, fast atom bombardment-mass spectrometry; h, hour(s); HPLC, high-performance liquid chromatography; IRO-*N*7,*N*9-bisAN-Gua, imidazole ring-opened *N*7,*N*9-bis(2-cyanoethyl)guanine; MS, mass spectrometry; ^{1}H-NMR, proton nuclear magnetic resonance; 2D NMR, two-dimensional nuclear magnetic resonance; *N*3-OXE-dT, *N*3-(2-oxoethyl)-2′-deoxythimidine; *N*7-OXE-Gua, *N*7-(2-oxoethyl)guanine; UV, ultraviolet.

Table 4.5 End-points relevant to genotoxicity in humans exposed to acrylonitrile

End-point	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
DNA strand breaks	Spermatozoa	Chemical plant Cross-sectional study	30 men exposed (0.8 mg/m^3) 30 controls	+ ↑ rate of comet nuclei, ↑ magnitude of damage ↑ tail length ($P < 0.01$)	Age, smoking, drinking, X-ray or chemotherapy exposure	Inadequate exposure characterization; followed WHO guidelines on experimental sperm evaluation.	Xu et al. (2003)
HPRT variant frequency	PBLs	Hungary, viscose rayon plant Cross-sectional study	13 fibre producers, 13 maintainers, 26 matched controls Month 0, 0–17.6 mg/m^3 peak AN Month 7, 0.3–5.1 mg/m^3 peak AN	(+) ($P < 0.05$)	Sex, age, smoking, drinking	Also evaluated exposures to 6 industrial controls (clerical staff); found smoking impact to be a significant covariate, measured co-exposures to DMF; end-point evaluated may be prone to false positives resulting from differences in basal proliferation and label incorporation.	Major et al. (1997)
Chromosomal aberrations	PBLs	Hungary, viscose rayon plant Cross-sectional study	13 fibre producers, 13 maintainers, 26 matched controls; Month 0, 0–17.6 mg/m^3 peak AN Month 7, 0.3–5.1 mg/m^3 peak AN	+ (total CA), ($P < 0.01$) + (exchanges) ($P < 0.05$)	Sex, age, smoking, drinking	Also evaluated exposures to six industrial controls (clerical staff); analysis of variance for various covariates, found that only for smoking was the impact significant; measured co-exposures to DMF. Effects increased when categorized by smoking status.	Major et al. (1998)
Chromosomal aberrations	PBLs	Czechia, India rubber-polymerization plant Cross-sectional study	39 men exposed (0.05–0.3 mg/m^3), 22 men exposed (0.05–0.7 mg/m^3); 49 controls from Prague	– (total% CA) ↑ % rcp: + ($P < 0.05$) ↑ % ins: + ($P < 0.05$) ↑ % chr 4: + ($P < 0.05$)	Age, smoking	Used FISH technique; inadequate exposure characterization; probable that data from 39/45 participants reported by Sram et al. (2004) were re-evaluated, but no information was provided.	Beskid et al. (2006)
Chromosomal aberrations	PBLs	Czechia, India rubber-polymerization plant Cross-sectional study	45 men exposed (0.05–0.3 mg/m^3); 23 controls from same region, and 33 controls from Prague	–	Smoking	Used conventional and FISH techniques; inadequate exposure characterization; unclear control for age; included chromatid exchange along with other effects.	Sram et al. (2004)

Table 4.5 (continued)

End-point	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
Chromosomal aberrations	Blood cells (probably PBLs)	Portugal, acrylic-textile plant Cross-sectional study	4 exposed polymer workers, 6 exposed maintainers, 5 controls (AN levels, NR)	(–) Polymer workers (+) Maintainers, counting breaks, but not gaps ($P = 0.003$)	Smoking	Inadequate exposure characterization in the reporting of cytogenic methodology, or rationale for evaluating subsample of each population (24 workers and 10 controls described as included).	Borba et al. (1996)
Chromosomal aberrations	PBLs	Germany, polymer plants Cross-sectional study	18 exposed workers (3.3–10.9 mg/m^3), 18 controls	–	Age	Inadequate reporting of cytogenic methodology; lack of information regarding control for smoking; probably co-exposure to other polymer chemicals.	Thiess and Fleig (1978)
Sister-chromatid exchange	Blood cells (probably PBLss)	Portugal, acrylic-textile plant Cross-sectional study	3 exposed polymer workers, 8 exposed maintainers, 4 controls (AN levels, NR)	(–) Polymer workers (–) Maintainers	Smoking	Inadequate exposure characterization in the reporting of cytogenic methodology, or rationale for evaluating subsample of each population (24 workers and 10 controls described as included).	Borba et al. (1996)
Sister-chromatid exchange	PBLs	Hungary, viscose rayon plant Cross-sectional study, two measures Observational	13 fibre producers, 13 maintainers, 26 matched controls Month 0, 0–17.6 mg/m^3 peak AN Month 7, 0.3–5.1 mg/m^3 peak AN	+ ($P < 0.01$)	Sex, age, smoking, drinking	Also evaluated exposures to six industrial controls (clerical staff); analysis of variance for various covariates, found that only for smoking was the impact significant; measured co-exposures to DMF. Effects increased when categorized by smoking status.	Major et al. (1998)
Aneuploidy	Spermatozoa	Chemical plant Cross-sectional study	9 men exposed (0.8 mg/m^3); number of controls NR (probably 7)	(+), ↑ sex chromosome disomy, most frequently XY ($P < 0.01$)	Age, smoking, drinking, X-ray or chemo exposure	Inadequate exposure characterization; no description of rationale for subselection of 9 participants and undescribed number of unexposed controls; followed WHO guidelines on experimental sperm evaluation.	Xu et al. (2003)

Table 4.5 (continued)

End-point	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
mtDNA deletion	Peripheral blood nucleated cells (probably lymphocytes)	Petrochemical plant workers, and university staff Cross-sectional study	47 plant workers (0.25 mg/m^3; range, 0–3.70 mg/m^3); 47 staff as controls (sex, NR, probably male)	(+) ($P < 0.05$)	Age	Inadequate exposure characterization; no consideration of co-exposures, confounders, or covariates besides age; inadequate description of mtDNA deletion assessment.	Ding et al. (2003)

AN, acrylonitrile; CA, chromosomal aberration; chr, chromosome; DMF, dimethylformamide; FISH, fluorescence in situ hybridization; HPRT, hypoxanthine-guanine phosphoribosyltransferase; ins, insertions; mtDNA, mitochondrial DNA; NR, not reported; PBL, peripheral blood lymphocyte; rcp, reciprocal translocations; WHO, World Health Organization.

[a] ↑, increase; +, positive; –, negative; (+) or (–), positive or negative in a study of limited quality.

In the same study, aneuploidy was also found to be increased in the spermatozoa of 9 of the 30 workers, with sex chromosome disomy being the most prevalent effect. The percentage of aneuploidy was 0.69% in the exposed group and 0.35% in the controls ($P < 0.01$) (Xu et al., 2003). [The Working Group noted that the exposure duration was < 2.8 years; in addition, the rationale for the subselection of nine exposed participants, and the number of unexposed controls were not reported. These might be a source of misclassification of the groups, thus limiting the informativeness of the study.]

Major et al. (1998) observed that in peripheral blood lymphocytes (PBLs) collected from 26 textile workers (13 fibre producers, 13 maintainers) exposed to acrylonitrile at peak concentrations ranging from 0 to 17. 6 mg/m^3 at a viscose rayon plant in Hungary, the frequencies of chromosomal aberration (CA) and of sister-chromatid exchange (SCE) were significantly higher ($P < 0.01$) than those observed in PBLs from 26 matched controls. The effects increased when the subgroups were re-evaluated by smoking status (Major et al., 1998). In addition, the frequency of CA was higher in PBLs from fibre producers than in PBLs from maintainers, and this was also consistent with the elevated estimates of personal exposure to acrylonitrile measured in the urine of fibre producers (Major et al., 1998). [The Working Group noted that worker exposures to dimethylformamide (DMF), a potentially confounding co-exposure, were also evaluated. Moreover, urinary concentrations were reported in units of milligrams of acrylonitrile per millimole of creatinine, and concentrations of acrylonitrile (when detected) ranged from 1.610 to 63.500 mg/mmol creatinine, which appears to be orders of magnitude higher than expected on the basis of ambient air concentrations. The Working Group noted that this was the result of a typographical error, i.e. "mg/mmol creatinine" was intended to read "µg/mmol creatinine". Furthermore, nondifferential exposure misclassification was very likely, thus the study may be of limited informativeness.] [The Working Group noted that the results on unscheduled DNA synthesis (UDS) from this study are discussed in Section 4.2.3.]

Increased variant frequencies in 6-thioguanine-resistant (6-TG) foci after lectin stimulation, presumed to be indicative of inactivating mutations in the hypoxanthine-guanine phosphoribosyl transferase (*HPRT*) gene, were found in PBLs from both groups of exposed workers, compared with controls ($P < 0.05$; Major et al., 1997). [The Working Group noted some limitations in this study. The authors used an autoradiographic readout of 6-TG resistance, presuming that labelled cells resulted from *HPRT*-deficient mutations conferring 6-TG resistance. This assay may be prone to false positives resulting from basal proliferation of PBLs, which was not controlled for. The labelling index (a measure of lectin-induced proliferation) was decreased in PBLs from both worker groups, compared with controls (not including the early industrial control group in which five out of six members reported health concerns, primarily infections). In addition, it was not clear to what extent this observation may confound the interpretation of *HPRT* variant frequency. Some of these concerns were discussed in a recent review by Albertini et al. (2023).]

Elevated frequencies of CA were not reported in PBLs from polymer-plant workers in two studies in Germany (Thiess and Fleig, 1978) and Czechia (Sram et al., 2004), and although CAs were elevated in a separate study in acrylic-textile plant workers in Portugal (in maintainers only, $P = 0.003$), this was not associated with significant elevations in the frequency of SCE (Borba et al., 1996). Borba et al. (1996) also assessed urine from these acrylonitrile-exposed textile plant workers and from controls for genotoxicity in the Ames TA98 strain in vitro; although no significant differences were reported between the exposure groups, significant differences ($P < 0.001$) in

urine mutagenic potency were reported between smokers and non-smokers. [The Working Group considered that the interpretation of the findings reported by Borba et al. (1996) was limited by the small sample size of the exposure groups, the inadequate characterization of exposure, the cytogenic methodology, and the rationale for evaluating a different subsample of each study population for each finding, which is consistent with comments made during the previous evaluation by the *IARC Monographs* (IARC, 1999).]

A follow-up study in 61 male workers involved in the polymerization of India rubber at a plant in Czechia also reported no significant increase in the overall frequency of CA; however, an extended analysis using fluorescence in situ hybridization (FISH) on chromosomes 1 and 4 revealed specific increases in the frequency of reciprocal translocations and insertions, as well as increased frequency of CA on chromosome 4 ($P < 0.05$) associated with decreased frequency of CA on chromosome 1 ($P < 0.001$) (Beskid et al., 2006). [The Working Group noted that interpretation of these results was limited by the lack of information regarding what might be selective reanalysis of some of the exposed workers (39/45) and possibly control samples (49/33) reported by Sram et al. (2004).]

The results of the analysis of background frequencies of CA in workers in the plant in Czechia confirmed that the frequencies for the study-specific non-exposed controls at the petrochemical site evaluated by Sram et al. (2004) and Beskid et al. (2006) were comparable to the national reference values determined for the same time frame (Sram et al., 2007). [The Working Group noted that the studies reported by Sram et al. (2004, 2007) and Beskid et al. (2006) shared several limitations and probably had nondifferential exposure misclassification and were therefore of limited informativeness.]

The effect of occupational acrylonitrile exposure on mitochondrial DNA (mtDNA) deletions was assessed in peripheral blood nucleated cells (probably PBLs) from 47 chemical industry workers (average workshop air acrylonitrile concentration, 0.25 mg/m^3; range, 0–3.70 mg/m^3) and 47 university staff members of similar age. Deletion fragments of 5.0 kb were detected in 8/47 of the workers, but in none of the staff controls ($P < 0.05$; Ding et al., 2003). [The Working Group considered that the interpretation of these results was limited by deficiencies in several elements of the study design and reporting, including incomplete exposure assessment, and lack of exposure characterization for the university staff referent group; insufficient detail provided regarding the assessment of mtDNA deletions, e.g. it was unclear whether several deletions were measured or only a single common deletion, and no LOD was reported; and lack of consideration of covariates beyond age, any other confounders, and appropriateness of university staff as a referent group for factory workers.]

In an environmental epidemiology study, birth records were reviewed for infants born to mothers living within 25 km of an acrylonitrile-using factory in Nyergesújfalu, Hungary. Surrogate markers for germline mutations included 15 "sentinel" congenital anomalies and Down syndrome. No environmental measurements of acrylonitrile were performed, no facility exposure estimates were reported, and there were no increases in observations of these abnormalities compared with numbers expected (Czeizel et al., 1999). [The Working Group noted that the interpretation of the results of this study was significantly limited by its ecological design, including the complete absence of exposure assessment, possible co-exposures to VCM from the factory, and lack of direct measures of genotoxicity. The study was considered not informative.]

(ii) Human cells in vitro

See Table 4.6.

Much of the available evidence pertaining to genotoxic effects of acrylonitrile in human cells

Table 4.6 End-points relevant to genotoxicity in human cells in vitro exposed to acrylonitrile

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
ssDNA strand breaks (alkaline elution)	Testicular cells (primary)	(–)	NT	1000 µM	No toxicity observed, 30 min exposure duration.	Bjørge et al. (1996)
ssDNA strand breaks (alkaline elution)	Hepatocytes (primary)	+	NT	1000 µM, D-R observed	No toxicity observed, 20 h exposure duration.	Robbiano et al. (1994)
ssDNA strand breaks (alkaline elution)	Bronchial epithelial cells (secondary)	+	NT	200 µM, D-R observed	20 h exposure duration.	Chang et al. (1990)
Gene mutation, plasmid with human *HRAS*	NIH/3T3 fibroblasts	CEO: (–)	NT	90 µM	Plasmid with human *HRAS* exposed to CEO, then transfected into NIH/3T3 cells; clones selected by resistance to growth and contact inhibition.	Yuan and Wong (1991)
Gene mutation, *HPRT* locus	Lymphoblastoid TK6 cells	CEO: +	NT	150 µM	Concurrent cytotoxicity, 2 h exposure then 9 d unexposed culture.	Recio et al. (1990)
Gene mutation, *HPRT* locus	Lymphoblastoid AHH-1 cells	+	NT	25 µg/mL (471 µM), evidence of D-R	Cytotoxicity at LEC, 28 h exposure.	Crespi et al. (1985)
Gene mutation, *TK* locus	Lymphoblastoid TK6 cells	(–)	+	HIC –S9, 20 µg/mL (377 µM) LEC +S9, 40 µg/mL (754 µM), no D-R observed	No cytotoxicity: –S9, 20 h exposure, lower doses evaluated; +S9, 3 h exposure.	Crespi et al. (1985)
Gene mutation, *TK* locus	Lymphoblastoid TK6 cells	AN: – CEO: +	AN: +/– CEO: NT	1400 µM 100 µM, D-R observed	AN effect only at toxic dose, and of a small magnitude compared with CEO, 2 h exposure then < 8 d unexposed culture.	Recio and Skopek (1988)
Chromosomal abnormalities	Lymphoblastoid TK6 cells	CEO: +	NT	150 µM	Only one dose evaluated; evaluated complete karyotypes of metaphase chromosomes from 8 tk_s mutants reported by Recio and Skopek (1988).	Kodama et al. (1989)
Chromosomal aberrations	PBLs (primary)	AN: – CEO: +	NT NT	100 µM 1 µM, D-R observed	Effects at subcytotoxic doses, 24 h exposure duration, limited reporting of cytogenetic methodology.	Cerna et al. (1981)

Table 4.6 (continued)

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Sister-chromatid exchange	PBLs (primary)	–	+	500 µM, no D-R observed	Effects at subcytotoxic doses, evaluated 20 metaphases/condition, 1 h exposure duration, 72 h unexposed culture duration.	Perocco et al. (1982)
Sister-chromatid exchange	PBLs (primary)	–	–	10 µg/mL (188 µM)	Only two doses evaluated; –S9, 48 h exposure; +S9, 1 h exposure, 24 h unexposed culture duration; evaluated 30–90 metaphases/condition.	Obe et al. (1985)
Sister-chromatid exchange	Bronchial epithelial cells (secondary)	+	NT	150 µM, D-R observed	Effects at subcytotoxic doses, 20 h exposure duration.	Chang et al. (1990)

AN, acrylonitrile; CEO, cyanoethyl oxide; D-R, dose–response relation; h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; min, minute(s); NT, not tested; PBL, peripheral blood lymphocyte; S9, 9000 × *g* supernatant, Aroclor-induced rat liver microsome fraction; ssDNA, single-strand DNA; tk_s, TK mutant colonies with a slow-growth phenotype.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (–), negative in a study of limited quality.

in vitro was summarized in the previous evaluation by the *IARC Monographs* (IARC, 1999).

Three separate studies investigating DNA single-strand breaks have been reported. A positive dose–response relation was reported with acrylonitrile at concentrations of ≥ 1000 μM in human primary hepatocytes (Robbiano et al., 1994), and increases were reported in secondary human bronchial epithelial cell cultures after 20 hours of exposure at ≥ 200 μM (Chang et al., 1990), but there was no significant increase in the frequency of DNA single-strand breaks in human primary testicular cells after 30 minutes of exposure at 1000 μM (Bjørge et al., 1996).

CA was evaluated in a single report, in which acrylonitrile at 100 μM gave negative results, but the putative metabolite, CEO (called glycidonitrile in this study), gave positive results in a dose–responsive manner at ≥ 1 μM in primary PBLs (Cerna et al., 1981). [The Working Group noted that, in the case of CEO, exogenous metabolic activation was not evaluated, and it was unclear whether the PBLs were metabolically competent.]

Chromosomal abnormalities (including partial arm deletions and duplications) determined by karyotype analysis of mutants in the lymphoblastoid TK6 cell line (developed by Recio and Skopek, 1988, and described below) were reported in four out of eight slow-growing thymidine kinase (TK) mutant (tk_s) colonies induced by exposure to 2-acyanoethylene oxide [CEO] at 150 μM, compared with none in eight spontaneous tk_s mutants (Kodama et al., 1989). SCE was not elevated in primary PBLs treated with CEO at concentrations of ≤ 188 μM in the presence or absence of S9 (Obe et al., 1985) and only increased in primary PBLs after 1 hour of exposure to 500 μM in the presence of metabolic activation (Perocco et al., 1982). SCE increased in a dose–responsive manner in secondary bronchial epithelial cell cultures at ≥ 150 μM after 20 hours of exposure (Chang et al., 1990).

A significantly increased frequency of gene mutation was reported at both the *TK* (Recio and Skopek, 1988) and *HPRT* loci (Recio et al.,1990) in the lymphoblastoid TK6 cell line after 2 hours of exposure to CEO at ≥ 100 μM, at the *TK* locus after 2 hours of exposure to acrylonitrile at 1400 μM in the presence of S9 (Recio and Skopek, 1988), and after 3 hours of exposure to acrylonitrile at 754 μM in the presence of S9 (Crespi et al., 1985). Mutations at the *HPRT* locus were also increased after 28 hours of exposure to acrylonitrile at ≥ 471 μM in AHH-1 lymphoblastoid cells, which retain mixed function oxygenase metabolic capability, unlike TK6 cells (Crespi et al., 1985).

When Recio et al. (1990) evaluated the DNA sequences of CEO-induced *HPRT* mutant genes, substitution mutations were observed in about half (19 out of 39), with 11 mutations at base pairs, eight at G:C, and two –1 frameshift mutations involving G:C base pairs. Likewise, approximately half of the *HPRT* cDNA (17 out of 39) from *HPRT* mutants exhibited the complete loss of one or more exons, which may have resulted from mutations affecting messenger RNA (mRNA) splicing (Recio et al., 1990).

In a novel procedure involving exposure of a plasmid containing the human *HRAS* proto-oncogene to CEO or other chemicals, followed by transfection into NIH/3T3 murine fibroblasts and morphological and microscopic evaluation of proliferation, concentrations of CEO at ≤ 90 μM gave rise to two clones selected by resistance to growth inhibition. However, these clones were scored as negative for the presence of *HRAS* mutations, since proliferation rates and serum dependence were similar to those of the negative (i.e. *HRAS* wildtype) controls; and because of the lack of changes in the size of *HRAS* gene restriction fragments by Southern blot, as was observed for compounds used as positive controls (Yuan and Wong, 1991).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.7.

The most informative and relevant studies on the genotoxicity of acrylonitrile in non-human mammalian species in vivo were reported by Walker et al. (2020a, b). In these experiments in mice and rats, the test article was acrylonitrile (purity, >99%, checked by GC; containing hydroquinone monoethyl ether (polymerization inhibitor) at 35–45 ppm). Acrylonitrile was administered either by gavage or in the drinking-water. [The Working Group noted that use of the latter route required some modelling of water consumption patterns in order to estimate actual exposures to acrylonitrile.] Multiple genotoxicity end-points were analysed, including mutation at the endogenous *Hprt* locus (mice and rats); mutation at the *lacZ* locus in transgenic mice (MutaMouse); mutational specificity (determined by DNA sequencing) in *hprt* mutants; DNA damage as measured by the comet assay; formation of specific DNA adducts, as measured by HPLC-MS or immunoassay; and micronucleus formation. Each exposure group contained 11–13 mice or 4–5 rats. [The Working Group noted that the investigators were highly experienced and that the experimental procedures and statistical analyses were very good or excellent. In addition, the Working Group noted that some negative results, such as the absence in mice of detectable acrylonitrile-induced *lacZ* mutants (the tissues studied were bone marrow, brain, lung, splenic T cells, and testis) or any increase in micronucleus formation, might reflect specific protocol choices rather than a true absence of effect.]

Statistically significant dose-dependent increases in mutation were observed with acrylonitrile in at least one system in each species. In splenic T lymphocytes of female F344 rats exposed to acrylonitrile via drinking-water for 4 weeks, *hprt* mutant frequencies rose monotonically with dose: for acrylonitrile concentrations of 0 (control), 33, 100, and 500 ppm in drinking-water, the mutant frequencies (mean ± SE, per 10^6 cells) were 2.6 ± 0.4, 3.4 ± 0.3, 5.1 ± 0.6, 11.1 ± 1.2, respectively, with the results for the two higher doses being statistically significant relative to the controls (Walker et al., 2020a). Groups of 11–13 female B6C3F_1 wildtype mice (age, 6 weeks) were treated with acrylonitrile at a dose of 0, 2.5, 10, or 20 mg/kg bw by gavage, 5 days per week, for 6 weeks. In splenic T lymphocytes from these mice, *hprt* mutant frequencies (mean ± SE, per 10^6 cells) rose monotonically with dose; at the highest dose (20 mg/kg bw), the frequency was 5.3 ± 2.3, which was statistically significantly higher than that in the zero-dose controls (1.7 ± 0.9) (Walker et al., 2020b). [The Working Group considered that these findings indicated that acrylonitrile is a mutagen in both rats and mice in vivo.]

Studies using the alkaline comet assay demonstrated that acrylonitrile induces hepatic DNA damage. Groups of 5 female B6C3F_1 mice were treated with acrylonitrile at a dose of 0, 2.5, 10, or 20 mg/kg bw by gavage, 5 days per week, for 6 weeks. DNA damage, as measured by the Olive tail moment comet assay parameter, was observed for the mouse liver cells (Walker et al., 2020b). The extent of damage increased monotonically and significantly with acrylonitrile dose, as follows: for doses of 0, 2.5, 10, and 20 mg/kg bw, the Olive tail moment was 2.4 ± 0.35, 2.9 ± 0.51, 3.8 ± 0.67, and 5.9 ± 0.71 (mean ± SE), respectively. An important role of CYP2E1 was also demonstrated in parallel studies in CYP2E1-null mice: no increase in DNA damage was observed at the higher dose of 60 mg/kg bw. Similar results were obtained in an analysis of forestomach cells: there was a dose-dependent increase in DNA damage in wildtype mice but not in CYP2E1-null mice.

DNA damage studies were also performed in ovary tissue. No dose-dependent increase was seen in the wildtype mice. In the CYP2E1-null mice, for doses of 0, 2.5, 10, 20, and 60 mg/kg bw,

Table 4.7 End-points relevant to genotoxicity in non-human mammalian systems in vivo exposed to acrylonitrile

End-point, assay	Species, strain, (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Gene mutation, *hprt* (6-thioguanine resistance)	Mouse, $B6C3F_1$ CYP2E1-WT (F)	Blood, spleen, forestomach, etc.	+	20 mg/kg per day	From age 6 wk; AN by gavage, 5 d/wk, for 6 wk; collection of tissues at 24 h after last dose	AN-dose-dependent increase in mutant frequency; approx. 3-fold increase (statistically significant vs control) at highest dose only, 20 mg/kg per day.	Walker et al. (2020b)
Gene mutation, *hprt* (6-thioguanine resistance)	Mouse, C57BL/6N CYP2E1-null (F)	Blood, spleen, forestomach, etc.	+	60 mg/kg per day	As above but tested at a higher AN dose than for the WT mice (60 vs 20 mg/kg per day); the 60 mg/kg per day dose is lethal to WT mice; collection of tissues at 24 h after last dose	No increase in mutant frequency at lower dose (20 mg/kg per day); approx. 3-fold increase (statistically significant increase vs control) at higher dose, 60 mg/kg per day.	Walker et al. (2020b)
Gene mutation, *hprt* (6-thioguanine resistance)	Mouse, *lacZ* transgenic MutaMouse (M)	Splenic T cells	+	500 ppm	From age 10–11 wk; AN in drinking-water, for 4 wk; collection of tissues 7 wk after exposure period	AN-dose-dependent increase in mutant frequency, approx. 3-fold increases (statistically significant vs control) at 500 and 750 ppm.	Walker et al. (2020b)
Gene mutation, *lacZ* (phage plaque growth on P-gal plates)	Mouse, *lacZ* transgenic MutaMouse (M)	Splenic T cells	–	750 ppm	From age 10–11 wk; AN in drinking-water, for 4 wk; collection of tissues 7 wk after exposure period	No significant differences between *lacZ* Mf values in control vs exposed mice in any tissue at any dose of AN.	Walker et al. (2020b)

Table 4.7 (continued)

End-point, assay	Species, strain, (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Gene mutation, *hprt* (6-thioguanine resistance); sequencing of *hprt* exon 3 to determine mutational specificity	Rat, F344 (F)	Thymus and spleen	+	100 ppm	From age 4 wk; AN in drinking-water; 0, 33, 100, or 500 ppm; for 4 wk; collection of tissues 2 wk after exposure period	Near-linear AN-dose-dependent increase in mutant frequency, from 2.6 (control) to 11.1 (500 ppm) × 10^{-6}.	Walker et al. (2020a)
DNA damage, alkaline comet assay	Mouse, $B6C3F_1$ (WT) Mouse, (CYP2E1-null) (F)	Ovary	–, Olive tail moment +, Olive tail moment	None 10 mg/kg	10, 20, 60 mg/kg per day by gavage, 5 d/wk for 6 wk	No increase with dose–response relation.	Walker et al. (2020b)
DNA damage, alkaline comet assay	Mouse, $B6C3F_1$ (WT) (F)	Liver	+, Olive tail moment	10 mg/kg	0, 2.5, 10, or 20 mg/kg per day, gavage, 5 d/wk for 6 wk	Dose-dependent increase.	Walker et al. (2020b)
DNA damage, alkaline comet assay	Mouse, (CYP2E1 null) (F)	Liver	–, Olive tail moment	60 mg/kg	Extended to 60 mg/kg per day, gavage, 5 d/wk for 6 wk		Walker et al. (2020b)
DNA damage, alkaline comet assay	Rat, Sprague-Dawley (M)	Liver	+	62.5 mg/kg	15.7, 31.3, 62.5 mg/kg per day, 3× at 3 h, 24 h, 48 h before tissue sampling	Dose-dependent increase in % tail DNA.	Nakagawa et al. (2015)
DNA damage, alkaline comet assay	Rat, Sprague-Dawley (M)	Glandular stomach	–	None	15.7, 31.3, 62.5 mg/kg per day, 3× at 3 h, 24 h, 48 h before tissue sampling	No increase in % tail DNA.	Nakagawa et al. (2015)
DNA damage, terminal transferase labelling	Rat, Sprague-Dawley (M)	Adrenal cortex	+, increase in DNA damage in labelled groups		0.05–0.15 mg/g bw via femoral vein	No details on the finding, only ++ scoring was reported.	Didenko et al. (1999)

Table 4.7 (continued)

End-point, assay	Species, strain, (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA fragmentation, sedimentation assay	Rat, Wistar, adult (M)	Plasma and brain	+	100 ppm	100 ppm in drinking-water and killed at day 14 and day 28	Significant increase DNA ratio in plasma and brain. Also, cotreatment with taurine. No change in DNA ratio in taurine-co-treated rats. Limitation of the study: condition of tissue collection not reported.	Mahalakshmi et al. (2003)
DNA fragment index, fluorometric assay	Rat, Sprague-Dawley (M)	Sperm	+	46 mg/kg	46 mg/kg, intragastric, 6 d/wk for 28 d	Increase in DNA fragment index: control, 2.50%; AN, 39.27%. Limitations of the study: single dose; as-made smears fixed in Carnoy solution and acridine orange; difficult to make quantitative comparison with other findings.	Shi et al. (2021)
DNA double-strand breaks, γH2AX foci	Mouse, Kunming, age 4 wk (F)	Oocytes	+ Control: 1.00 ± 1.09; high-dose AN: 7.48 ± 4.46; $P < 0.05$	20 mg/kg	5, 10, or 20 mg/kg per day, AN by gavage, 5 d/wk, for 6 wk; oocytes collected at termination	Large number of γH2AX foci in the nuclear region of oocytes.	Luo et al. (2022)
DNA damage, comet assay	Rat, Sprague-Dawley, age 6 wk (M)	Leukocytes and brain cortex	–	Not measured directly	0, 3, 30, 100, or 200 ppm AN in deionized drinking-water for 28 d; 100 nuclei measured per treatment	No significant increase in DNA damage in either tissue, at any dose.	Pu et al. (2009)

Table 4.7 (continued)

End-point, assay	Species, strain, (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA damage, fpg-modified comet assay			+	30 ppm (LEC)		Statistically significant monotonic dose-dependent increases were seen in both tissues.	
DNA damage, comet assay	Rat, F344 (F)	Leukocytes	–	100 ppm	100 ppm AN in deionized drinking-water for 28 d	One single dose.	Pu et al. (2016)
DNA damage, fpg-modified comet assay			+	100 ppm		One single dose.	
DNA damage, fpg-modified comet assay			+, after antioxidants treatment, lesser increase (compared with control)	100 ppm	100 ppm AN in deionized drinking-water for 28 d; vitamin E, green tea polyphenols, *N*-acetylcysteine, sodium selenite	Pretreatment with antioxidant-enriched diet.	

AN, acrylonitrile; bw, body weight; CYP, cytochrome P450; d, day(s); F, female; F344, Fischer 344; fpg, formamidopyrimidine-DNA glycosylase; h, hour(s); γH2AX, γ-H2A histone family member X; HID, highest ineffective dose; *hprt*, hypoxanthine-guanine phosphoribosyl transferase; LEC, lowest effective concentration; LED, lowest effective dose; M, male; Mf, mutation frequency; ppm, parts per million; wk, week(s); WT, wildtype; vs, versus.

[a] +, positive; –, negative.

the Olive tail moment was 1.3 ± 0.1, 3.5 ± 0.58, 2.1 ± 0.14, and 2.0 ± 0.20. Damage levels at all doses were elevated above those in the controls, but without a clear dose–response relation. The authors observed that the ovary data may reflect "an unusually low value and low variability in the control" (Walker et al., 2020b). [The Working Group noted that the mass of the mouse ovary is normally about 2 mg (Zheng et al., 2021) whereas the mass of the liver is about 2–3 g, so the analysis of the ovary tissue may have been difficult. There were very few published reports of in vivo comet assay studies on ovary tissues from mice or rats. On the basis of the above, the Working Group agreed with the authors' conclusions that exposure to acrylonitrile induces significant tissue-specific DNA damage and mutagenesis in wildtype mice and that acrylonitrile is a mutagen in rats in vivo.]

As a part of an international validation study of the in vivo rodent alkaline comet assay, Nakagawa et al. (2015) examined acrylonitrile and other agents under coded-test-chemical conditions. Male Sprague-Dawley rats were purchased at age 6 weeks and acclimated for 1 week before the start of dosing. At least 5 rats were used per dose group. Acrylonitrile (purity not reported; obtained by the coordinators of the international validation study, Uno et al., 2015). Acrylonitrile was administered by gavage ("orally … using stomach tubes and plastic syringes") three times, at 48, 24, and 3 hours, before tissue sampling. In the liver, the median percentage tail DNA was 0.9% in the negative control group and 0.6% in the group receiving acrylonitrile at the lowest tested dose (15.7 mg/kg per day) but increased with acrylonitrile dose to 1.7% and 2.1% at 31.3 and 62.5 mg/kg per day, respectively. There was a significant difference between the percentage tail DNA for the negative control group and that for the group treated with acrylonitrile (62.5 mg), and there was a significant dose-dependent increase in the percentage tail moment DNA. In contrast, no dose-dependent increase in DNA damage was observed in the glandular stomach cells (Nakagawa et al., 2015). [The Working Group considered that the study was well designed, with good replication and statistical analysis, and it provided strong evidence that orally administered acrylonitrile causes DNA damage in the rat liver.]

Genotoxic effects of acrylonitrile in mammals have also been reported in other studies.

Didenko et al. (1999) published a study on mechanisms of damage to the adrenal cortex caused by insults such as sepsis, ischaemia or reperfusion, and chemical toxicity. Acrylonitrile was used as an example of a chemical that causes adrenal toxicity (Szabo and Lippe, 1989). Acrylonitrile (purity, not reported; vehicle, 0.2% Tween 20) was administered to male Sprague-Dawley rats via the femoral vein, at doses 0.05–0.15 mg/g bw. Animals were euthanized after 8 or 16 hours. DNA was isolated from the adrenal cortex and DNA 3′ ends were labelled with terminal transferase. Acrylonitrile-induced DNA damage was observed (Didenko et al., 1999). [The Working Group noted some limitations to this study, such as that the DNA damage was scored as "++" without further quantitation. The actual dose of acrylonitrile was not indicated, other than "0.05–0.15 mg/g", and the number of animals tested (replicates) was not stated.]

However, UDS was not observed in hepatocytes or spermatocytes from male F344 rats exposed orally to acrylonitrile at 60 mg/kg per day for 5 days (Butterworth et al., 1992) (see also Section 4.2.3). Neither the mean net nuclear silver grain count (indicating [^{3}H]thymidine incorporation) nor the percentage of cells with five or more net nuclear silver grains (considered to be cells "in repair") was increased in the acrylonitrile-treated animals, relative to the untreated controls, in either of the tissues examined.

Two studies evaluated the potential of acrylonitrile to induce DNA fragmentation. Mahalakshmi et al. (2003) exposed groups of six male adult Wistar rats to acrylonitrile (source

and purity not stated) at a concentration of 100 ppm in drinking-water, and the animals (6 per treatment group) were euthanized after 14 or 28 days. On the basis of measurement of water consumption, the dose of acrylonitrile administered over the treatment period was estimated to be 8–10 mg/kg per day. Another group of rats was treated with acrylonitrile plus taurine. DNA fragmentation was measured in brain tissue homogenates. The method used was separation of supernatant (fragmented DNA) from pellet (intact DNA), trichloroacetic acid (TCA) precipitation, and quantitation of DNA by the diphenylamine colorimetric reaction. The percentage fragmentation was calculated from the ratio of supernatant DNA to total DNA. The authors stated that "The percentage of DNA fragmentation was significantly increased in plasma and brain of acrylonitrile-treated rats … [but] levels were near normal in taurine supplemented acrylonitrile-treated and control rats" (Mahalakshmi et al., 2003). [The Working Group considered that only one set of DNA fragmentation data was reported, apparently for brain tissue (in Table 2 of the paper), with percentage values (± SD) of 8.83 ± 2.04, 14.16 ± 2.48, and 16 ± 2.09 for the controls, and the acrylonitrile-treated groups treated for 14 days or 28 days, respectively. Furthermore, the Working Group noted that details on conditions for the preparation of the tissues were not reported. In addition, the Working Group considered that greater clarity was required regarding the methods used and the reporting of results.]

Shi et al. (2021) treated five groups of 12 adult male Sprague-Dawley rats with acrylonitrile (purity, 99%) at a dose of 46 mg/kg administered intragastrically 6 days per week for 28 days. After the rats were killed, sperm were recovered from the epididymis, and smears were fixed in Carnoy solution, stained with acridine orange, and analysed by fluorescence microscopy. The acridine orange nuclear fluorescence colour distinguishes mature (green fluorescence) from immature (orange fluorescence) spermatozoa, as explained below. The DNA fragment index, calculated as the percentage of spermatozoa exhibiting orange fluorescence divided by the total of those exhibiting orange or green fluorescence, increased from 2.50% in sperm from the control animals to 39.27% in sperm from the acrylonitrile-treated animals.

The basis of the analytical protocol used by Shi et al. was described in (Hoshi et al., 1996). In somatic cells, DNA is complexed with histones, but in mature spermatozoa, DNA is mainly complexed with small basic proteins called protamines. The redox state of the protamines shifts from reduced thiol to oxidized disulfide during spermatozoon maturation. In the mature sperm (containing disulfide-rich protamines), DNA is relatively resistant to denaturation. Acridine orange binds to the single-stranded, denatured DNA of the immature sperm as an aggregate that fluoresces orange but intercalates into the double-stranded DNA of the mature sperm as a monomer that fluoresces green. [The Working Group considered this study to be less informative than other genotoxicity studies, because the assay used is probably measuring sperm maturation rather than DNA damage per se. Therefore, there was no straightforward way to compare these data quantitatively with those from the other studies discussed here.]

Luo et al. (2022) exposed female Kunming mice, (age, 4 weeks) (randomly divided into four groups, 50 mice per group) to acrylonitrile (purity not reported) at a dose of 5, 10, or 20 mg/kg per day for 28 days, following the dosing protocol of Walker et al. (2020b). Oocytes were collected and immunostained for γH2AX, a marker of DNA double-strand breaks. The number of γH2AX foci increased very significantly from 1.00 ± 1.09 per oocyte in the control group to 7.48 ± 4.46 in the group at 20 mg/kg per day ($P < 0.05$). [The Working Group considered the findings relevant since they demonstrated that acrylonitrile induces DNA damage in mouse oocytes.]

Pu and colleagues published three studies (two in vivo and one in vitro) on acrylonitrile and DNA damage measured by the comet assay. In an in vivo study, male Sprague-Dawley rats (aged 6 weeks), were acclimated for 1 week before treatment with acrylonitrile (purity, >99%) at 0, 3, 30, 100, or 200 ppm in deionized drinking-water for 28 days (Pu et al., 2009). The comet assay was performed on samples of leukocytes and brain cortex (100 nuclei were measured per treatment). Two comet assay protocols were used, either with or without formamidopyrimidine-DNA glycosylase (fpg) treatment before electrophoresis. Fpg (also known as 8-oxoguanine DNA glycosylase) is a base-excision repair enzyme that converts many types of damaged bases into strand breaks (Azqueta et al., 2013). The results represent means ± standard deviation for three animals per dose. Significant increases in DNA damage were not seen in the standard comet assay, in either tissue, at any acrylonitrile dose. With the fpg-modified assay, dose-dependent monotonic increases in DNA damage were observed with acrylonitrile in both tissues (see also Section 4.2.4). In the leukocytes, the increases above the control value were statistically significant at 30, 100, and 200 ppm. In the brain, the increases above the control value were statistically significant at 100 and 200 ppm. [The Working Group noted that the group sizes were small, although statistically significant effects were observed.]

A paper by the same author described an in vivo study in female F344 rats (age, 6–8 weeks) acclimated for 1 week before treatment with acrylonitrile (purity not reported) at 100 ppm in deionized drinking-water for 28 days (Pu et al., 2016). Additional groups were fed antioxidant-enriched diets, starting 1 week before acrylonitrile administration, and continuing throughout treatment. As in the earlier study (Pu et al., 2009), acrylonitrile induced DNA damage in leukocytes when assessed with the fpg-modified comet assay but not with the standard comet assay. Olive tail moment measurements (means ± SD) were as follows: control animals, 0.43 ± 0.13; acrylonitrile-treated animals, 0.78 ± 0.12 (four animals per group; results for treated animals were statistically significantly different from those for controls). The antioxidant-enriched diets (containing vitamin E, green tea polyphenols, *N*-acetylcysteine, or sodium selenite) were significantly protective.

[The Working Group noted that these studies showed that acrylonitrile induced oxidative DNA damage, as measured by the fpg-modified comet assay, in rats in the in vivo studies described above and in the in vitro studies described below, and that the end-point was also relevant to the key characteristic of "induces oxidative stress" (see Section 4.2.4).]

(ii) Non-human mammalian systems in vitro

See Table 4.8.

Pu et al. (2006) described an in vitro study in DI TNC1 rat astrocyte cells treated with acrylonitrile (purity, >99%) for 24 hours. With the standard comet assay, no significant damage was induced by acrylonitrile (0–1.0 mM; three or four independent experiments; 100 nuclei measured per treatment). With the fpg-modified comet assay, a dose-dependent monotonic increase in damage was seen that was statistically significant only at 1.0 mM, the highest dose tested. Since some cytotoxicity was observed at 2.5 mM, the assay was not attempted at that concentration. [The Working Group noted that it would have been useful to test additional doses between 1.0 and 2.5 mM.] The DNA damage was reduced by co-incubation with the antioxidants α-tocopherol, trolox, or epigallocatechin-3 gallate (EGCG).

Robbiano et al. (1994) applied the alkaline filter-elution technique to study DNA damage induced by acrylonitrile in primary cultures of rat hepatocytes (and human hepatocytes; see Section 4.2.2(a)). Hepatocytes were isolated (by collagenase perfusion) from livers of male Sprague-Dawley rats and incubated in vitro

Table 4.8 End-points relevant to genotoxicity in non-human mammalian cells in vitro exposed to acrylonitrile

End-point, assay	Species, strain (sex), tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA damage, alkaline elution	Rat, Wistar (M), testicular cells	–		300 µM		Bjørge et al. (1996)
DNA damage, alkaline elution	Rat, Sprague-Dawley (M), primary hepatocyte cultures	+		5.6 mM		Robbiano et al. (1994)
DNA damage, comet assay	Rat, DI TNC1 astrocytes	–	NA	1 mM; dose range, 0–1.0 mM; 3 or 4 independent experiments; 100 nuclei measured per treatment		Pu et al. (2006)
DNA damage, Fpg-modified comet assay		+	NA		Cytotoxicity observed at 2.5 mM. End-point relevant to oxidative stress (KC5), see Section 4.2.4.	
DNA damage, Fpg-modified comet assay	Rat, DI TNC1 astrocytes	↓ DNA damage with antioxidants	NA		Co-incubation with antioxidants α-tocopherol, trolox, or EGCG. End-point relevant to oxidative stress (KC5), see Section 4.2.4.	
Oxidative DNA damage, 8-oxodG	Calf thymus DNA	+, ↑ 8-oxodG formed by H_2O_2 plus Cu^{2+}	NT	0.5% AN, approx. 100 mM	HPLC and electrochemical detection of 8-oxodG. End-point relevant to oxidative stress (KC5), see Section 4.2.4.	Murata et al. (2001)

AN, acrylonitrile; EGCG, epigallocatechin-3 gallate; fpg, formamidopyrimidine DNA glycosylase; HIC, highest ineffective concentration; H_2O_2, hydrogen peroxide; HPLC, high-performance liquid chromatography; KC5, key characteristic of carcinogens, "induces oxidative stress"; LEC, lowest effective concentration; M, male; NA, not available; NT, not tested; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine.

[a] ↑, increase; ↓, decrease; +, positive; –, negative.

with acrylonitrile (in Williams' Medium E, without serum) for 20 hours at 37 °C. The alkaline filter-elution technique (Ahnström, 1988) is performed by lysing cells on 2 μm-pore polycarbonate filters that impede DNA passage. Alkaline solution is pumped through the filter to elute the DNA. The rate of elution increases with damage (primarily single-strand breaks) that generates shorter fragments of DNA. [The Working Group noted that a positive result in alkaline filter-elution analysis is an indication of DNA damage, which may be repairable but which may result in genotoxicity.] Robbiano et al. (1994) reported statistically significant, acrylonitrile concentration-dependent (over the range 1.0–5.6 mM) increases in DNA damage in the hepatocytes.

Bjørge et al. (1996) applied the alkaline filter-elution method in a study in testicular cells isolated from mature male Wistar rats (and also in human testicular cells; see Section 4.2.2(a)). The cells were incubated in vitro with acrylonitrile for 30 minutes, at 32 °C, in HEPES buffer plus 1% bovine serum albumin. Using this method, Bjørge et al. did not observe any acrylonitrile-concentration-dependent DNA damage at concentrations of ≤ 300 μM, the highest acrylonitrile concentration tested. [The Working Group noted that the difference between the positive results of Robbiano et al. and the negative results of Bjørge et al. may be explained by the inability of the testicular cells to metabolize or activate acrylonitrile.]

Acrylonitrile, at concentrations of 12.5 nL/mL and higher (in the absence of S9), induced mutations conferring resistance to trifluorothymidine (*Tk* mutations) in mouse lymphoma L5178Y cells (Myhr et al., 1985).

(iii) Non-mammalian species

See Table 4.9.

Two studies demonstrated DNA damage in marine organisms exposed to acrylonitrile. Lin et al. (2018a) exposed juvenile marine flounder fish (*Paralichthys olivaceus*) to acrylonitrile (source and purity not reported) at concentrations of 0.1, 0.2, and 0.4 mg/L in seawater for 28 days. Zheng et al. (2018) exposed scallops (*Chlamys farreri*, a bivalve mollusc) to acrylonitrile (purity, > 99.0%, analytical grade; dissolved in < 0.001% v/v dimethyl sulfoxide, DMSO) at concentrations of 0.5, 2.0, or 5.0 mg/L in seawater and sampled the scallops at 0, 1, 4, 7, 10, and 14 days.

DNA was isolated from the liver and brain (flounder study) and from digestive glands (scallop study). In both studies, DNA damage was assessed by the alkaline unwinding assay for detection of strand breaks. Acrylonitrile concentration-dependent DNA damage was observed in flounder brain, but not in flounder liver, and in the scallop digestive glands. To explain the organ-specific DNA damage in the flounder study, Lin et al. (2018a) speculated that, "The central nervous system is particularly vulnerable to oxidative stress due to the high rate of O_2 utilization and the relatively poor concentrations of classical antioxidants and related enzymes". [The Working Group noted that both studies were reliable, having appropriate methods, replication, and statistical analysis.]

Several studies were available investigating gene mutations in various strains of *Salmonella typhimurium* (the Ames test); however, the most relevant were performed by Zeiger and Haworth (1985) and de Meester et al. (1978).

An Ames test using the preincubation method was reported by Zeiger and Haworth (1985). [The Working Group considered that the authors followed clearly described and appropriate protocols, including replication, statistical error analysis, coded scoring, and appropriate positive and negative controls. Both hamster and rat liver S9 were used, and S9 concentrations of 5%, 10%, and 30% were compared, which is an unusually thorough analysis. Monotonic positive dose–response relations were obtained.] The strongest positive response was obtained in strain TA1535 with 30% hamster liver S9. Revertant scores rose from 23 (no-mutagen control) to

Table 4.9 End-points relevant to genotoxicity in non-mammalian experimental systems exposed to acrylonitrile

Test system (species, strain)	End-point, assay	Results[a]			Concentration (LEC or HIC) Dose range	Reference
		Without metabolic activation	With metabolic activation	Activation system		
Fish (flounder) *Paralichthys olivaceus*, juvenile	DNA strand breaks, alkaline unwinding assay	+ in DNA from brain but not liver	NA	None	0.1, 0.2, and 0.4 mg/L, in seawater for 28 d.	Lin et al. (2018a)
Mollusc (scallop) *Chlamys farreri*	DNA strand breaks, alkaline unwinding assay	+ in DNA from digestive gland	NA	None	0.5, 2.0, and 5.0 mg/L, in seawater, and sampled scallops at 0, 1, 4, 7, 10, and 14 d.	Zheng et al. (2018)
Bacteria, *Salmonella typhimurium*, TA1535	Gene mutation (base substitution, at G:C)	–	+	5–30% Aroclor-induced male rat liver S9	0.1–6.66 mg per plate.	Zeiger and Haworth (1985)
Bacteria, *Salmonella typhimurium*, TA1535	(base substitution, at G:C)	–	+	5–30% Aroclor-induced hamster liver S9	0.1–6.66 mg per plate.	Zeiger and Haworth (1985)
Bacteria, *Salmonella typhimurium*, TA100	(base substitution, at G:C)	–	+[b]	5–30% Aroclor-induced male rat liver S9	0.1–6.66 mg per plate.	Zeiger and Haworth (1985)
Bacteria, *Salmonella typhimurium*, TA100	(base substitution, at G:C)	–	+	5–30% Aroclor-induced hamster liver S9	0.1–6.66 mg per plate.	Zeiger and Haworth (1985)
Bacteria, *Salmonella typhimurium*, TA98	Gene mutation (frameshift +1)	–	–	10% Aroclor-induced male rat or hamster liver S9	0.1–6.66 mg per plate	Zeiger and Haworth (1985)
Bacteria, *Salmonella typhimurium*, TA1538	(frameshift +1)	–	–	300 µg/mL Aroclor-induced mix S9	2.5–25 µg/mL	de Meester et al. (1978)
Bacteria, *Salmonella typhimurium*, TA1537	(frameshift –1)	–	–	300 µg/mL Aroclor-induced mix S9	2.5–2 µg/mL	de Meester et al. (1978)
Bacteria, *Salmonella typhimurium*, TA97	(frameshift –1)	–	–	10% Aroclor-induced male rat or hamster liver S9	0.1–6.66 mg per plate.	Zeiger and Haworth (1985)

d, day(s); G:C, guanosine:cytosine; HIC, highest ineffective concentration; LEC, lowest effective concentration; NA, not applicable; S9, 9000 × *g* supernatant.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative in a study of limited quality.

[b] The increase was not dose-related.

432 at the highest acrylonitrile dose (6.66 mg per plate). This highest dose is higher than the usual maximum dose tested (5 mg); however, even at a lower dose, 3.33 mg per plate, the yield was 364 revertants per plate, an increase of more than 15-fold, far higher than the usual criterion for a positive response, which is doubling of the revertant yield. [The Working Group noted that the results of the study showed clearly that acrylonitrile gives positive results in the Ames test. However, the dose ranges used were typically > 1 mg for the strongest responses, so the mutagenic effect was weak compared with potent mutagens, such as 2-aminoanthracene, which are active in the microgram range.]

In a preliminary study, de Meester et al. (1978) tested acrylonitrile in several strains in the Ames test. No mutagenicity was detected in any strain in the absence of S9 activation. When rat hepatic S9 prepared by various induction protocols (no induction; Arochlor 1254 induction; phenobarbital induction) was used, positive results were obtained in strains TA1530 and TA1535 but not in TA1537. Strains TA1530 and TA1535 bear the *hisG*46 target (the target sequence GGG is reverted by a G:C→A:T base substitution). Strain TA1537 bears the *hisC*3076 target (the target sequence GGGG is reverted by a 1-base deletion) (Levy et al., 2019).

Emmert et al. (2006) examined several toxicants or potential mutagens in a modified Ames test in strain YG7108pin3ERb$_5$. This strain is a derivative of *S. typhimurium* strain YG7108 (Yamada et al., 1995). Strain YG7108 was constructed by inactivating the genes encoding the two methylguanine-DNA methyltransferase DNA repair genes, *ada* and *ogt*, in strain TA1535, making the strain highly sensitive to alkylating agents. Strain YG7108pin3ERb5 incorporates a plasmid for expression of the recombinant human enzymes CYP2E1, CYP reductase, and cytochrome *b*5. This metabolically competent strain was designed for detection of small-molecule CYP2E1 substrates, such as dialkylnitrosamines, which may be activated to produce alkylating reactive intermediates. Acrylonitrile (purity, 99%; tested as an aqueous solution) was not mutagenic in this bacterial strain; however, it was cytotoxic over the dose range 0–5 mg per plate, as judged by a dose-dependent reduction in revertant yield, relative to zero-dose controls (Fig. 6 in Emmert et al., 2006). Specific toxicity experiments were not performed. Toxicity with acrylonitrile was not observed in the parental strain YG7108. [The Working Group noted that the contradiction of previous results (i.e. the positive results in the TA1535 strain, obtained by Zeiger and Haworth) may be explained by the protective effects of the highly concentrated S9 mix from hamsters that was used in the study by Zeiger and Haworth. The high protein content may have been protective by scavenging reactive, toxic intermediates, so that genotoxicity was not masked by strong toxicity (Emmert et al., 2006).]

The results of many earlier in vitro assays for genotoxicity in mammalian cells, the studies by de Meester et al. (1978), Zeiger and Haworth (1985), other studies using the Ames test; and the assays in various non-mammalian species, e.g. *Escherichia coli*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Tradescantia* were tabulated in *IARC Monographs* Volume 71 (IARC, 1999). [In the previous evaluation by the *IARC Monographs* programme, acrylonitrile was considered to be a bacterial mutagen, usually but not exclusively in the presence of metabolic activation. It was reported that urine samples from rats and mice exposed to acrylonitrile were mutagenic in the absence of metabolic activation; bile samples from rats were not. The findings in fungi were considered to be inconsistent ("variable"). The Working Group considered that the more recent studies complemented the substantial body of evidence reviewed previously (IARC, 1999) and demonstrated that acrylonitrile is a DNA-damaging mutagen requiring metabolic activation.]

Synopsis

[Overall, evidence from a few studies suggested that there is induction of chromosome-level mutations in humans exposed to acrylonitrile across different occupational sectors. There is evidence for similar effects in human cells, as well as for gene mutations in human lymphoblastoid cell lines. The available data also showed that acrylonitrile induces mutations in experimental systems ranging from bacteria to rodents. Acrylonitrile can cause DNA damage in rodents, both in vitro and in vivo (as observed by the comet assay), and especially oxidative damage (as revealed by fpg enzyme treatment) (see also Section 4.2.4). The Working Group noted that acrylonitrile typically requires metabolic activation by enzymatic or chemical systems in order to exhibit genotoxicity (see also Section 4.1 and 4.2.1).]

4.2.3 Alters DNA repair or causes genomic instability

Unscheduled DNA synthesis

Many studies have examined the induction of UDS by acrylonitrile in vitro or in vivo, in human cells, experimental animals, or animal cells. Several of these publications were previously reviewed in *IARC Monographs* Volume 71 (IARC, 1999). [The Working Group noted that the UDS protocol (Pimpley et al., 2020) measures the incorporation of radioactivity from [^{3}H]thymidine into DNA outside the DNA synthesis ("S") phase of the cell cycle. Such incorporation is attributable (mainly or entirely) to the activity of the nucleotide-excision repair (NER) system, which excises patches of DNA in which the presence of helix-distorting lesions has been recognized, followed by repair synthesis using the complementary strand as template. UDS can be measured by visual autoradiographic analysis ("grain counting") under the microscope, which is appropriate for in vitro studies, or by scintillation counting. Cells in S phase incorporate large amounts of [^{3}H]thymidine (in "scheduled", replicative DNA synthesis), so these cells must be eliminated from the analysis. In the grain-counting approach (analysis of individual cells), S-phase cells can be ignored by the scorer. In the scintillation counting approach (bulk analysis), interference from S-phase cells is avoided by treatment with hydroxyurea (blocking replication, via inhibition of ribonucleotide reductase), although incomplete blocking may distort the results. The UDS technique measures NER but does not distinguish between faithful (non-mutagenic) and error-prone (pro-mutagenic) repair. Other mechanisms of DNA repair, such as base-excision repair, are less well detected. Background rates of UDS limit the sensitivity of the assay. No molecular information is obtained, e.g. with regard to the chemical nature of the DNA damage or its mutagenic specificity. The use of UDS has declined as DNA sequencing-based technologies have been developed for the analysis of mutagenesis. In summary, induction of UDS is a correlate of DNA repair and genotoxicity but should not be equated with them.]

[The Working Group noted that knowledge gaps remain with regard to the structures, biological significance, and mechanisms of repair of acrylonitrile-derived covalent DNA adducts. NER is unlikely to be as important for acrylonitrile as it is for PAHs and aromatic amine carcinogens, which form bulky helix-distorting DNA lesions.]

With respect to UDS studies on the effects of acrylonitrile, two experimental designs can be distinguished. In one design, cells are exposed to acrylonitrile in vitro and then UDS activity (presumably reflecting repair of acrylonitrile-induced DNA damage) is measured. In another design, cells (e.g. blood cells) are obtained from acrylonitrile-exposed humans or animals, treated in vitro with a known DNA-damaging agent (usually ultraviolet light, UV), and then UDS activity is measured. The latter design

tests whether acrylonitrile exposure affects the activity of systems for the repair of UV-induced DNA damage.

(a) Humans

(i) Exposed humans

Major et al. (1998) studied a cohort of 26 male workers (13 "maintainers" and 13 "fiber producers"; mean age ± SE, 33 ± 1.1 years; accumulated exposures, 3–10 years) occupationally exposed to acrylonitrile and DMF in the textile (viscose rayon) industry in Hungary, comparing the workers with 26 "matched historical control subjects without known occupational exposure". Analyses were performed at three time points: November 1992 (time point 1); 7 months later (time point 2); and 20 months later (time point 3). However, not all analyses were performed on all participants at all time points. In particular, at time point 3, only 17 of the 26 exposed workers were studied, the nine excluded having "left the plant for undisclosed reasons".

In a subsequent study, Major et al. (1999) examined premature centromere division as a cytogenetic end-point in a broader study of workers occupationally exposed to possible genotoxic agents in various industrial settings, e.g. the rubber, petrochemical, and pharmaceutical industries. This 1999 publication included, inter alia, "18 subjects occupationally exposed to acrylonitrile and/or dimethylformamide in the polyacrylic textile industry". Presumably, these 18 individuals were a subset of the 26 workers studied in the 1998 publication (Major et al., 1998), since the data reported for the ambient air exposures to acrylonitrile in the two publications were identical.

Acrylonitrile and DMF exposures were assessed by measuring concentrations in factory air and in workers' urine using GC-FID. Air samples were collected at time points 1 and 2, whereas urine samples were collected at time point 1 only. The peak concentrations in the ambient air samples were reported as 0–17.6 mg/m^3 at time point 1 and 0.3–5.1 mg/m^3 at time point 2 for acrylonitrile, and 0.6–23.0 mg/m^3 at time point 1 and 3.5–22.8 mg/m^3 at time point 2 for DMF.

For the "fiber producer" workers, the reported urinary acrylonitrile concentrations, in units stated as "mg per mmol creatinine", were as high as 36.9 before and 63.5 after the work shifts (Major et al., 1998). For the "maintainers" workers, the corresponding values were much lower, only as high as 1.6 before and 2.9 after the work shifts. [The Working Group suggested that these units were probably micrograms per millimole creatinine, rather than milligrams per millimole, as stated.]

Blood samples were obtained and processed for the cytogenetic analysis of PBLs. Several cytogenetic effects, including CA and SCE (Major et al., 1998; as reported in Section 4.2.2), but not premature centromere divisions (Major et al., 1999), were seen at elevated frequencies in the group of exposed workers. For example, the mean values for CA in the exposed participants were significantly higher than those in the control participants at time point 1 – 3.00 ± 0.46% (exposed) versus 1.68 ± 0.32% (controls); $P < 0.01$ – increasing further at time points 2 (5.07 ± 0.65) and 3 (4.88 ± 0.61). The values were significantly higher among the "fiber producers" than among the "maintainers".

Ultraviolet light-induced unscheduled DNA synthesis (UV-UDS) was also examined in experiments in which the PBLs were irradiated (in Petri dishes) with UV light and then UDS was measured. Therefore, these experiments tested the possibility that activity with respect to the repair of UV-induced DNA damage was affected by exposure of the participants to acrylonitrile or DMF. The experiments did not test whether the exposures induced repair of DNA damage caused by acrylonitrile itself. For the control participants, mean UV-UDS activity was 6.9 ± 0.6 (relative units ± SE). For the exposed

workers, mean activity at time point 1 was not significantly different (7.2 ± 0.6) from that in the controls; however, mean activity at time point 2 (7 months) was significantly greater (10.2 ± 0.5, $P < 0.01$). Increased activity at time point 2 was observed both for the "maintainers" (10.7 ± 0.7) and the "fiber producers" (9.4 ± 0.7). UDS activity was not measured at time point 3 "due to technical reasons". [The Working Group noted that the increased UV-UDS activity observed in the experiments at time point 2 may be an anomaly, since it was not seen at time point 1 and, at time point 2, there was no significant difference between the results for the "maintainers" and the "fiber producers", despite much higher chemical exposures in the latter group.]

In the study by Major et al. (1998), some confounding factors were considered, including biological confounding factors (age, leukocyte count, haematocrit) and lifestyle-related factors (smoking and alcohol consumption). [The Working Group noted that interpretation of these results (which were not reviewed in Volume 71; IARC, 1999) should be placed in the context of the small sample size and the combined exposures with other chemicals, including DMF.]

(ii) Human cells in vitro

See Table 4.10.

Evidence for UDS after acrylonitrile treatment was available from four studies, with positive results reported at 0.5 M in primary PBLs (Perocco et al., 1982) and at 180–36 μM in HeLa cervical cancer cells (Rizzi et al., 1984), and negative results reported at 1 mM in secondary mammary epithelial cell cultures (Butterworth et al., 1992) and at 50 mM in HeLa cells (Martin and Campbell, 1985). [The Working Group noted that the effective dose of 0.5 M reported by Perocco et al. (1982) is a supraphysiological concentration that is likely to be of little to no relevance to the determination of potential cancer hazard. Furthermore, the findings of both Perocco et al. (1982) and Butterworth et al. (1992) were constrained by limitations in study conduct and/or reporting, including concurrent cytotoxicity, the limited range of doses evaluated, and the lack of metabolic competency. The results from Rizzi et al. (1984) were presented in summary form, which was insufficient to allow for evaluation of the study conduct and design.] Butterworth et al. (1992) also reported significant induction of UDS in cultured human mammary epithelial cells after exposure at 100 μM to CEO, the product of CYP2E1-catalysed metabolism of acrylonitrile.

The study by Martin and Campbell (1985) was included in the volume edited by Ashby et al. (1985), in which acrylonitrile was selected as one of the agents in a "basket" of 10 target compounds tested by the many groups participating in a collaborative study of short-term tests. In the Appendix of that volume, it was stated that the purity of the acrylonitrile test article was "99%", as confirmed by NMR and MS analysis. [The Working Group assumed that the acrylonitrile was of the highest purity commercially available; however, most of the individual papers from the volume either did not mention the source of the acrylonitrile or stated that it was obtained from John Ashby.]

(b) Experimental systems

See Table 4.11.

Six studies measured UDS in tissues of rats receiving acrylonitrile orally, mostly after a single dose (Hogy and Guengerich, 1986; Ahmed et al., 1992a, b, 1996; Butterworth et al., 1992; Abdel-Rahman et al., 1994).

Hogy and Guengerich (1986) observed UDS in the liver but not the brain of male F344 rats that received acrylonitrile, followed immediately with hydroxyurea and, later, with [^{3}H]thymidine (repeated doses). Using similar methods, the research group of Ahmed and colleagues reported acrylonitrile-induced UDS in the rat lung (Ahmed et al., 1992a), testis (Ahmed et al., 1992b), glandular stomach (Abdel-Rahman et al.,

Table 4.10 End-points relevant to the alteration of DNA repair or genomic instability in human cells in vitro exposed to acrylonitrile

End-point	Assay	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Unscheduled DNA synthesis	Liquid scintillation counting ([^{3}H]thymidine uptake)	Blood, lymphocytes from donors (n = 6). Primary cells	(+) in presence of S9 mix and hydroxyurea (10 mM)	500 mM, 4 h	AN purity, 99%. Supraphysiological concentration.	Perocco et al. (1982)
Unscheduled DNA synthesis	DNA repair assay. Autoradiography (nuclear grain count)	Normal mammary epithelial cells (HMEC). Secondary cells	(–)	1000 µM (HIC)	Cytotoxicity. Cells lack metabolic competence. The activated metabolite of AN, CEO, gave positive results.	Butterworth et al. (1992)
Unscheduled DNA synthesis	Liquid scintillation counting ([^{3}H]thymidine uptake)	Skin fibroblasts, HeLa cells	+ in the absence of S9 and presence of hydroxyurea	36 µM (LEC)	Only available as abstract.	Rizzi et al. (1984)
Unscheduled DNA synthesis	Liquid scintillation counting on extracted DNA ([^{3}H]thymidine uptake)	Skin fibroblasts, HeLa S3 cells	(–) in 2 of 3 assays with or without metabolic activation from rat liver S9	50 mM, 2.5 h	AN purity, assumed to be > 99%. Hydroxyurea was added in the flask (10 mM).	Martin and Campbell (1985)

AN, acrylonitrile; h, hour(s); CEO, cyanoethyl oxide; h, hours(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; S9, 9000 × g supernatant.
[a] +, positive; (+) or (–), positive/negative in a study of limited quality.

Table 4.11 End-points relevant to the alteration of DNA repair or genomic instability in non-human mammalian systems exposed to acrylonitrile

End-point	Assay	Species, strain (sex), or cell line	Tissue or cell line	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
In vivo								
Unscheduled DNA synthesis	Scintillometric method ([^{3}H] thymidine uptake)	Rat, F344 (M)	Liver	+	50 mg/kg bw	Gavage, single dose [^{3}H]thymidine (repeated doses)	Purity, > 99%, containing 35 ppm hydroquinone monomethyl ether. Treatment with hydroxyurea after AN by gavage.	Hogy and Guengerich (1986)
			Brain	–	None			
Unscheduled DNA synthesis	DNA repair assay, liquid scintillation ([^{3}H]thymidine uptake)	Rat, Sprague-Dawley (M)	Lung	+, ↑	46.5 mg/kg bw	Gavage, single dose Intraperitoneal dosing with [^{3}H]thymidine, 0.5, 6, or 24 h after AN administration	Treatment with hydroxyurea after AN by gavage.	Ahmed et al. (1992a)
Unscheduled DNA synthesis	DNA repair assay, liquid scintillation ([^{3}H]thymidine uptake)	Rat, Sprague-Dawley (M)	Testis	+, ↑	46.5 mg/kg bw	Gavage, single dose Intraperitoneal dosing with [^{3}H]thymidine, 0.5, 6, or 24 h after AN administration	Treatment with hydroxyurea after AN gavage.	Ahmed et al. (1992b)
Unscheduled DNA synthesis	DNA repair assay, liquid scintillation ([^{3}H]thymidine uptake)	Rat, Sprague-Dawley (M)	Pyloric stomach mucosa	+, ↑	4.6, 23, and 46 mg/kg bw	Gavage, single dose	Presence of 10 mM hydroxyurea in glass vials [^{3}H]thymidine at 10 µCi/mL in ex vivo.	Ahmed et al. (1996)
Unscheduled DNA synthesis	DNA repair assay. Liquid scintillation ([^{3}H]thymidine uptake)	Rat, Sprague-Dawley (M)	Glandular stomach mucosa	+, ↑	46.5 mg/kg bw	Gavage, single dose Intraperitoneal dosing with [^{3}H]thymidine, 0.5, 6, or 24 h after AN administration	Treatment with hydroxyurea after AN gavage.	Abdel-Rahman et al. (1994)
Unscheduled DNA synthesis	DNA repair assay, autoradiography (nuclear grain count).	Rat, F344 (M)	Liver and sperm	–	None 75 mg/kg, gavage, single dose 60 mg/kg bw, gavage, five doses			Butterworth et al. (1992)
In vitro								
Unscheduled DNA synthesis	DNA repair assay. Liquid scintillation counting	Rat, albino Holtzman (M)	Hepatocytes	(+)	1 mM		AN purity, assumed to be > 99%; 2 of 3 assays gave negative results; hydroxyurea was added.	Glauert et al. (1985)

Table 4.11 (continued)

End-point	Assay	Species, strain (sex), or cell line	Tissue or cell line	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Unscheduled DNA synthesis	DNA repair assay, autoradiography (nuclear grain count)	Rat, F344 (M)	Hepatocytes	–	None	10 mM Two independent hepatocyte preparations were tested, and 20 cells were counted per treatment	AN purity, assumed to be > 99%.	Probst and Hill (1985)
Unscheduled DNA synthesis	DNA repair assay, autoradiography (nuclear grain count)	Rat, F344 (M)	Hepatocytes	–	None	≤ 1 mM		Butterworth et al. (1992)

AN, acrylonitrile; bw, body weight; F344, Fischer 344; h, hour(s); HID, highest ineffective dose; LED, lowest effective dose (units as reported); M, male; ppm, parts per million.
[a] ↑, increase; ↓, decrease; +, positive; –, negative; (+), positive in a study of limited quality.

1994), and pyloric stomach mucosa (Ahmed et al., 1996). In the first publication, Ahmed et al. (1992a) found that replicative DNA synthesis in the lungs was significantly decreased and UDS was increased after acrylonitrile treatment. In contrast, UDS was not observed in hepatocytes and spermatocytes of rats exposed orally to acrylonitrile at 60 mg/kg per day for 5 days (Butterworth et al., 1992). The study by Didenko et al. (1999) is considered in Section 4.2.2(b).

The following two reports describing studies on UDS in vitro were also published in the volume edited by Ashby et al. (1985). Glauert et al. (1985) reported that UDS activity was statistically significantly increased (to 129 ± 4% of the control value, mean ± SE) with acrylonitrile at a concentration of 1 mM in cultured rat hepatocytes. The authors also described, in the Materials and Methods section, experiments in which hepatocytes were exposed to UV, presumably as a positive control; however, data for this experiment were not shown.

Probst and Hill (1985) reported negative results for UDS in cultured rat hepatocytes with acrylonitrile at concentrations of between 10 mM and 0.5 µM. [The Working Group assumed that the acrylonitrile was of the highest purity commercially available. In the "Summary report on the performance of the assays for DNA damage" (in Ashby et al., 1985), the positive UDS result reported by Glauert et al. was criticized by Williams, who noted that results for acrylonitrile were negative in two of the three UDS assays reported in the volume. Therefore, the positive result was evaluated as equivocal.] In addition, Butterworth et al. (1992) reported negative results for UDS induction in male F344 rat primary cultured hepatocytes, with acrylonitrile at concentrations of ≤ 1 mM.

Synopsis

[The Working Group noted that substantial knowledge gaps remain in our understanding of the repair of DNA damage that may result from exposure to acrylonitrile. There is some evidence for repair of acrylonitrile-induced DNA damage in human cells in vitro, but no studies have definitively demonstrated alterations in DNA repair of acrylonitrile-induced DNA damage in exposed humans. There is some evidence for acrylonitrile-induced UDS (possibly because of repair of acrylonitrile-induced DNA damage) in rats in vivo. There is equivocal evidence for acrylonitrile-induced UDS in rat hepatocytes in vitro; both positive and negative results have been reported. As reported by Walker et al. (2020a), acrylonitrile – mainly via the reactivity of its metabolite CEO – can bind to DNA, but little is known about the structures of and mutagenic mechanisms for the resulting adducts. Specific mechanisms for the repair of acrylonitrile-derived DNA damage remain unclear. Most published studies of DNA repair after acrylonitrile exposure have relied on techniques (e.g. UDS) that can indicate that DNA repair has taken place, but that do not give detailed insight into the repair mechanisms. The inconsistencies in the UDS literature may reflect the numerous variables involved in, for example, study design, types of exposure, and performance of the assays used. Overall, the published studies provide some evidence for the hypothesis that acrylonitrile exposure causes DNA damage that triggers DNA repair activities.]

4.2.4 Induces oxidative stress

(a) Humans

(i) Exposed humans

See Table 4.12.

Oxidative stress induced by acrylonitrile in exposed humans was investigated in a limited number of studies. These included workers exposed during acrylonitrile production, smokers exposed to acrylonitrile via cigarette smoke, and humans affected by wood smoke.

Table 4.12 End-points relevant to oxidative stress in humans exposed to acrylonitrile

End-point	Assay	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
MDA	TBARS	Blood, erythrocytes	Portugal Case–control	10 exposed, 20 non-exposed	↑ MDA ($P < 0.001$)	Smoking, alcohol, X-ray exposure	Small sample size; non-specificity of the TBARS analysis.	Borba et al. (1996)
8-OHdG	ELISA	Urine	Guatemala Nested cohort study	23 women	No correlation between 8-OHdG and AN metabolites	Sociodemographic characteristics, cooking fuel	Small sample size; no control group; no information on duration, exposure levels, or latency; no adjustment made for co-exposures.	Weinstein et al. (2017)
8-OHdG	LC-MS/MS	Urine	Taiwan, China Cross-sectional study	853 young adults	AN metabolites associated with 8-OHdG in urine	Age, gender, smoking, alcohol, BMI	A potential role of other cigarette-smoke components; no information on duration, exposure levels, latency, or presence of other co-exposures; no adjustment for co-exposures; no controls.	Lin et al. (2018b)
8-OHdG, MDA	HPLC, TBARS	Urine	China Cross-sectional study	13 participants (exposure experiment) + 259 children (questionnaires)	AN metabolites associated with 8-OHdG and MDA in urine	Healthy volunteers	A potential role of other cigarette-smoke components; no control group; no information on exposure duration, exposure level, or latency; non-specificity of the TBARS analysis.	Kuang et al. (2022)

AN, acrylonitrile; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; TBARS, thiobarbituric acid-reactive substances.

[a] ↑, increase.

In a case–control study, lipid peroxidation (assessed as levels of malondialdehyde, MDA, in erythrocytes) was increased in workers occupationally exposed to acrylonitrile when compared with the controls (administrative workers from the same plant) (Borba et al., 1996). No co-exposure in the exposed group was noted. [The Working Group noted that the study group was very small (10 exposed participants), which limited the statistical power of the data. The Working Group also noted that the thiobarbituric acid-reactive substances (TBARS) assay was used to assess lipid peroxidation and concluded that these results might not be accurate because of the known non-specificity of the TBARS analysis.]

In a nested cohort study, 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels in urine were not correlated with particulate matter, PAHs, or acrylonitrile metabolites associated with wood smoke exposure (Weinstein et al., 2017). [The Working Group noted that this was a small study (23 participants in total, no controls included), which was designed to investigate the effect of wood smoke, a complex mixture. Results were reported for a specific biomarker of acrylonitrile exposure; however, no adjustment for co-exposures was made.]

In a cross-sectional study, urinary levels of 8-OHdG were associated with those of acrylonitrile metabolite CEMA, suggesting a role for acrylonitrile in the induction of oxidative DNA damage (Lin et al., 2018b). [The Working Group noted that this study had several limitations: no specific source of acrylonitrile was identified, direct effects of acrylonitrile were not assessed, no adjustment for co-exposures was made, and no control participants were involved.]

Acrylonitrile metabolites, and other metabolites of volatile organic compounds (VOCs), were detected in the urine, in a study on passive smoking in a group of 13 participants (seven adults, six children) and follow-up in a group of 259 children for whom questionnaire data were collected. The metabolites were associated with DNA damage (8-OHdG) and lipid peroxidation (MDA in urine) (Kuang et al., 2022). [The Working Group noted that this study suggested that acrylonitrile induced oxidative stress indirectly, since the agent was a part of a complex mixture of VOCs present in secondhand smoke. No adjustments for co-exposures were made. The Working Group also noted the non-specificity of the TBARS analysis for measuring lipid peroxidation.]

(ii) Human cells in vitro

See Table 4.13.

Two studies in human astrocyte and endometrial carcinoma cell lines suggested a role for acrylonitrile in the induction of oxidative stress in vitro on the basis of the formation of 8-OHdG and generation of reactive oxygen species (ROS). [The Working Group noted that the end-points 8-OHdG induction, ROS generation, and decrease in GSH levels were detected with acrylonitrile at high concentrations (above 50 μM) and not consistently in astrocytes (Jacob and Ahmed, 2003), whereas ROS production was noted at low, environmentally relevant doses in endometrial carcinoma cells (Kim et al., 2018).]

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.14.

Numerous studies were performed in rodents to assess various markers of oxidative stress induced by acrylonitrile. Thus, the effect was observed in, for example, the liver/gastric system in general, testis sperm, brain, and blood, using standardized biomarkers, e.g. ROS, GSH, MDA, and antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPx; and 8-OHdG). Most of the studies were performed in rats treated with acrylonitrile at doses of ≤ 150 mg/kg (intraperitoneal administration) or ≤ 500 ppm (in drinking-water); other species included mice, hamsters, and guinea-pigs,

Table 4.13 End-points relevant to oxidative stress in human cells in vitro exposed to acrylonitrile

End-point	Assay	Tissue, cell line	Results[a]	Concentration	Experiment conditions	Comments	Reference
8-OHdG	HPLC (8-oxodG)	Human astrocytes	↑	100 µM	25–400 µM	High AN concentrations with limited relevance to real-world exposure.	Jacob and Ahmed (2003)
ROS			↑				
GSH			↓				
GSSG			↑				
CAT			↑				
ROS (superoxide)	DCFH-DA	Human endometrial carcinoma cells	↑	10^{-9} M	10^{-9} and 10^{-11} M	Environmentally relevant doses.	Kim et al. (2018)

AN, acrylonitrile; CAT, catalase; DCFH-DA, 2′,7′-dichlorodihydrofluorescein diacetate; GSH, reduced glutathione; GSSG, oxidized glutathione; HPLC, high-performance liquid chromatography; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; ROS, reactive oxygen species.
[a]↑, increase; ↓, decrease.

Table 4.14 End-points relevant to oxidative stress in non-human mammalian systems in vivo exposed to acrylonitrile

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dose regimen	Comments	Reference
Lipid peroxidation (MDA, conjugated dienes)	Rat, Sprague-Dawley (F)	Adrenal gland, stomach, brain, and duodenal mucosa	↑	150 mg/kg	150 mg/kg, i.v. exposure up to 90 min; conjugated dienes in liver microsomes increased after AN injection, no effect in other tissues	Lipid peroxidation may not be involved in pathogenesis of AN-associated adrenal injury, but it may occur in other tissues as a result of AN exposure. Extremely high-dose exposure scenario.	Silver and Szabo (1982)
Lipid peroxidation (ethane in exhaled air)	Rat, Sprague-Dawley (M)	Liver	↑	10 mg/kg	10–100 mg/kg (i.p.) (acute exposure); 5, 10, 40 mg/kg (subacute exposure)	The study suggested that AN causes lipid peroxidation in the liver.	Ivanov et al. (1989)
Lipid peroxidation (MDA)	Rat, Sprague-Dawley (M)	Brain Liver	↑ No change	200 ppm	0–200 ppm; 14, 28, 90 d; effects in the brain only, after 14 d		Jiang et al. (1998)
Lipid peroxidation (TBARS)	Rat, Sprague-Dawley (M)	Brain	No changes	300 ppm	3, 30, 300 ppm (day 22); in water	The results were not clear and interpretation was misleading. The findings for 8-oxodG formation in the same study are reported below.	Whysner et al. (1998)
Lipid peroxidation (TBARS, lipid peroxides)	Rat, Wistar (M)	Blood, brain	↑	100 ppm	100 ppm in water; 14 and 28 d	AN induced lipid peroxidation, and decreased enzyme activity and levels of non-enzymatic antioxidants.	Mahalakshmi et al. (2003)
Lipid peroxidation (TBARS)	Rat, Wistar (M)	Heart, cerebral cortex	↑	200 ppm	200 ppm in water; 14 d	The study confirmed induction of lipid peroxidation by AN in various rat tissues.	Nagasawa et al. (2003)
Lipid peroxidation (MDA)	Rat (M)	Testis	↑	30 mg/kg bw	7.5, 15, 30 mg/kg bw (i.p.); 4, 8, 13 wk; increase after 13 wk, as well as 2 wk after treatment	The study confirmed that AN induces oxidative damage in testis in rats, but only on the level of lipid peroxidation.	Huang et al. (2005)
Lipid peroxidation	Rat, Swiss albino (M)	Brain	↑	50 mg/kg	50 mg/kg bw, 28 d (oraladministration)	Confirmation of oxidative damage to the brain by AN; the effects were reduced by the antioxidant hesperidin.	El-Sayed et al. (2008)

Table 4.14 (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dose regimen	Comments	Reference
Lipid peroxidation	Rat, Sprague-Dawley (M)	Brain (various regions)	↑	25 mg/kg bw	25, 50, 75 mg/kg bw (i.p.); effects mostly in cortex	Different effects of AN in various brain regions; a study limitation was the low number of oxidative stress markers.	Rongzhu et al. (2009)
Lipid peroxidation (MDA)	Rat, Sprague-Dawley (M)	Brain, liver	↑	50 mg/kg bw	50 mg/kg bw (i.p.)	Oxidative damage induced in the brain and liver of rats was confirmed; it was reduced by curcumin.	Guangwei et al. (2010)
Lipid peroxidation (MDA)	Rat, albino (M)	Liver	↑	50 mg/kg	50 mg/kg per day (p.o.), daily for 5 wk	The study supported the role of AN in oxidative damage to liver tissue.	Abo-Salem et al. (2011)
Lipid peroxidation (TBARS)	Rat, Sprague-Dawley (M)	Stomach	↑	10 mg/kg	Single dose of 25 mg/kg (oral dose), 1, 2, 4, 6 and 12-h treatment; dose–response: 10, 25 or 50 mg/kg, 2-h treatment	The study supported the role of AN in oxidative damage to the gastric system.	Al-Abbasi (2012)
Lipid peroxidation (MDA)	Rat, Sprague-Dawley (M)	Testis	↑	50 mg/kg bw	50 mg/kg bw per day (gavage), 90 d	Induction of oxidative stress by AN and mechanisms of toxicity in testis (apoptosis).	Dang et al. (2017a)
Lipid peroxidation (MDA)	Rat, Sprague-Dawley (M)	Testis, sperm	↑	50 mg/kg bw	50 mg/kg bw per day (gavage), 6 d/wk, 12 wk	A mechanistic study showing mechanisms of AN toxicity in sperm.	Dang et al. (2017b)
Lipid peroxidation (MDA)	Rat, Sprague-Dawley (M)	Brain	↑	46 mg/kg bw	46 mg/kg bw per day, 28 d, gavage	Identification of protective effects of apigenin in the brains of rats treated with AN; it has value as a mechanistic study.	Zhao et al. (2019)
Lipid peroxidation (MDA)	Rat, Wistar (M)	Stomach	↑	30 mg/kg bw	30 mg/kg bw, 3 h treatment		Hamdy et al. (2012)
Lipid peroxidation (MDA)	Rat, Sprague-Dawley (F)	Brain	No changes	NA	100 ppm in water, 28 d	A study showing convincing results on oxidative damage induction by AN in the brain cells, but the link with astrocytoma was indirect.	Pu et al. (2016)
ROS	Rat, Sprague-Dawley (M)	Brain	↑	5 ppm	14, 28, 90 d; effects in the brain only		Jiang et al. (1998)
		Liver	No changes				

Table 4.14 (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dose regimen	Comments	Reference
ROS (superoxide)	Rat, Sprague-Dawley (M)	Stomach	↑	10 mg/kg bw	Single dose, 25 mg/kg bw (oral dose), treatment for 1, 2, 4, 6, and 12 h; dose–response study, 10, 25, and 50 mg/kg bw, treatment for 2 h	The study supported the role of AN in oxidative damage to the gastric system.	Al-Abbasi (2012)
ROS	Rat, Sprague-Dawley (M)	Sperm	↑	50 mg/kg bw	50 mg/kg bw per day (gavage), 6 d/wk, 12 wk	A mechanistic study showing mechanisms of AN toxicity in sperm.	Dang et al. (2017b)
ROS	Rat, Sprague-Dawley (M)	Brain, blood	↑	30 ppm	3, 30, 100, 200 ppm AN in water; 28 d	AN did not induce DNA breaks but caused oxidative stress in leukocytes and brain – convincing results suggested suitability of blood as a surrogate tissue for the brain.	Pu et al. (2009)
GSH	Rat, Sprague-Dawley (F)	Liver, lung, kidney, adrenal tissue	↓	20 ppm	150 mg/kg, i.v.; exposure up to 90 min, 20, 100, 500 ppm in drinking-water	A comparison of acute and chronic effects of AN in various tissues – differences between both types of exposure.	Szabo et al. (1977)
GSH	Rat, Sprague-Dawley (M)	Brain Liver	↓ No changes	50 ppm	0–200 ppm; 14, 28, 90 d; effects after 14 d only		Jiang et al. (1998)
GSH	Rat, Sprague-Dawley (M)	Brain, liver, forestomach	↑	300 ppm	3, 30, 300 ppm (day 22); 1–100 ppm (3, 10, 31, 94 d); in water; positive results in forestomach, day 22		Whysner et al. (1998)
GSH	Rat, Wistar (M)	Blood, brain	↓	100 ppm	100 ppm in water; 14 and 28 d	AN induced lipid peroxidation, and decreased enzyme activity and levels of non-enzymatic antioxidants.	Mahalakshmi et al. (2003)

Table 4.14 (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dose regimen	Comments	Reference
GSH	Rat (M)	Testes	↑	15 mg/kg bw	7.5, 15, 30 mg/kg bw (i.p.); 4, 8, 13 wk; increase after 4 wk, followed by decrease at other time points	The study confirmed that AN induces oxidative damage in the testis in rats, but only on the level of lipid peroxidation.	Huang et al. (2005)
GSH	Rat, Swiss albino (M)	Brain	↓	50 mg/kg bw	50 mg/kg bw per day (oral), 28 d	Confirmation of oxidative damage to the brain by AN; the effects were reduced by antioxidant hesperidin.	El-Sayed et al. (2008)
GSH/GSSG	Rat, Sprague-Dawley (M)	Brain, blood	↓	30 ppm	3, 30, 100, 200 ppm, in water; 28 d	AN does not induce DNA breaks but causes oxidative stress in leukocytes and brain – convincing results suggesting suitability of blood as a surrogate tissue for the brain damage.	Pu et al. (2009)
GSH	Rat, Sprague-Dawley (M)	Brain (various regions)	↓	50 mg/kg bw	25, 50, 75 mg/kg bw (i.p.); effects mostly in cortex and cerebellum	Different effects of AN in various brain regions; a limitation was the limited number of oxidative stress markers.	Rongzhu et al. (2009)
GSH	Rat, Sprague-Dawley (M)	Brain, liver	↓	50 mg/kg bw	50 mg/kg bw (i.p.); significant decrease in liver only	Oxidative damage induced in the brain and liver of rats was confirmed; it was reduced by curcumin.	Guangwei et al. (2010)
GSH	Rat, albino (M)	Liver	↓	50 mg/kg bw	50 mg/kg bw per day (p.o.), daily for 5 wk	The study supported the role of AN in oxidative damage to liver tissue.	Abo-Salem et al. (2011)
GSH	Rat, Sprague-Dawley (M)	Stomach	↓	10 mg/kg	Single dose: 25 mg/kg (oral), treatment for 1, 2, 4, 6, and 12 h; dose–response study: 10, 25, or 50 mg/kg, treatment for 2 h	The study supported the role of AN in oxidative damage to the gastric system.	Al-Abbasi (2012)
GSH	Rat, Wistar (M)	Stomach	↓	30 mg/kg bw	30 mg/kg bw, treatment for 3 h		Hamdy et al. (2012)

Table 4.14 (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dose regimen	Comments	Reference
GSH/GSSG	Rat, Sprague-Dawley (M)	Testis	↓	50 mg/kg	50 mg/kg per day (gavage), 90 d	Induction of oxidative stress by AN and mechanisms of toxicity in testis (apoptosis); a limitation was that the study was in rats.	Dang et al. (2017a)
GSH/GSSG	Rat, Sprague-Dawley (M)	Testis	↓	50 mg/kg	50 mg/kg per day (gavage), 6 d/wk, 12 wk	A mechanistic study showing mechanisms of AN toxicity in sperm, limited by the fact that it was performed in rats.	Dang et al. (2017b)
GSH	Rat, Sprague-Dawley (M)	Brain	No change	46 mg/kg bw	46 mg/kg bw per day, 28 d, gavage	Identification of protective effects of apigenin in the brains of rats treated with AN; it has value as a mechanistic study.	Zhao et al. (2019)
8-OHdG	Rat, Sprague-Dawley (M)	Brain	↑	50–200 ppm	0–200 ppm; 14, 28, and 90 d	Chronic exposure to AN causes carcinogenesis in the rat brain; liver is not a target for AN-induced carcinogenesis.	Jiang et al. (1998)
		Liver	No change			Lack of effects in the liver may be due to its high antioxidant capacity r.	
8-OHdG	Rat, Sprague-Dawley (M)	Brain	↑	30 ppm	3, 30, 300 ppm (day 22); 1–100 ppm (3, 10, 31, 94 d); in water; positive results in the brain and liver, day 22	Induction of 8-oxodG in the rat brain after AN exposure, but no lipid peroxidation – the results were not clear and their interpretation was somewhat misleading: 8-oxodG formation does not suggest epigenetic change.	Whysner et al. (1998)
		Liver	↑				
		Forestomach	No change				
Oxidative DNA damage	Rat, Sprague-Dawley (M)	Brain, blood	↑	100 ppm	3, 30, 100, 200 ppm, in water; 28 d	AN did not induce DNA breaks but caused oxidative stress in leukocytes and brain – convincing results suggesting suitability of blood as a surrogate tissue for the brain damage.	Pu et al. (2009)
Oxidative DNA damage	Rat, Sprague-Dawley (F)	Brain	↑	100 ppm	100 ppm, in water, 28 d	A study showing convincing results on oxidative damage induction by AN in the brain cells, but the link with astrocytoma was indirect.	Pu et al. (2016)
Oxidative DNA damage	Rat, Sprague-Dawley and F344 (F)	Brain,	↑	100 ppm	100 ppm, 28 d, drinking-water	Possible role of oxidative DNA damage induced by AN in the brain but not in Zymbal gland.	Williams et al. (2017)

Table 4.14 (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dose regimen	Comments	Reference
Oxidative DNA damage (cont.)		Zymbal gland	No change	100 ppm	100 ppm, 28 d, drinking-water;		
CAT	Rat, Sprague-Dawley (M)	Liver, brain	↓	5 ppm	0–200 ppm; 14, 28, 90 d; effects in the brain only; after 14 d also ↑ in the liver	Chronic AN exposure causes carcinogenesis in the rat brain; liver is not a target for AN-induced carcinogenesis; lack of effects in the liver may be due to its high antioxidant capacity.	Jiang et al. (1998)
SOD	Rat, Sprague-Dawley (M)	Liver, brain	↓	5 ppm	0–200 ppm; 14, 28, 90 d; effects in the brain only	Chronic AN exposure causes carcinogenesis in the rat brain; liver is not a target for AN-induced carcinogenesis; lack of effects in the liver may be due to its high antioxidant capacity.	Jiang et al. (1998)
CAT, GPx	Rat, Sprague-Dawley (M)	Brain	No change	300 ppm	3, 30, 300 ppm (day 22), in water; positive results in brain and liver, day 22		Whysner et al. (1998)
CAT, SOD, GPx	Rat, Wistar (M)	Blood, brain	↓	100 ppm	100 ppm in water; 14 and 28 d	AN induced lipid peroxidation, and decreased enzyme activity and levels of non-enzymatic antioxidants.	Mahalakshmi et al. (2003)
SOD, GPx	Rat (M)	Testes	↑	15 mg/kg bw	7.5, 15, 30 mg/kg bw (i.p.); 4, 8, 13 wk; GPx after 4 wk (30 mg/kg), remained 2 wk after treatment; SOD after 8 wk (15 and 30 mg/kg)	The study confirmed that AN induces oxidative damage in the testis in rats, but only on the level of lipid peroxidation.	Huang et al. (2005)
CAT, SOD, GPx	Rat, Swiss albino (M)	Brain	↓	50 mg/kg bw	50 mg/kg bw per day (oral); 28 d	Confirmation of oxidative damage to the brain by AN; the effects were reduced by the antioxidant hesperidin.	El-Sayed et al. (2008)
SOD, GPx	Rat, Sprague-Dawley (M)	Brain (various regions)	↓	25 mg/kg bw	25, 50, 75 mg/kg bw (i.p.)	Different effects of AN in various brain regions; a limitation was the limited number of oxidative stress markers.	Rongzhu et al. (2009)

Table 4.14 (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dose regimen	Comments	Reference
CAT, SOD	Rat, Sprague-Dawley (M)	Brain, liver	↓	50 mg/kg bw	50 mg/kg bw (i.p.); decrease for CAT only	Induction of oxidative damage in the brain and liver of rats was confirmed; it was reduced by curcumin.	Guangwei et al. (2010)
SOD, GPx	Rat, albino (M)	Liver	↓	50 mg/kg bw	50 mg/kg bw per day (p.o.), daily for 5 wk	The study supported the role of AN in oxidative damage to liver tissue.	Abo-Salem et al. (2011)
CAT, SOD, GPx	Rat, Wistar (M)	Stomach	↓	30 mg/kg bw	30 mg/kg bw, treatment for 3 h		Hamdy et al. (2012)
SOD	Rat, Sprague-Dawley (M)	Testis	No change	50 mg/kg bw	50 mg/kg bw per day (gavage), 90 d	Induction of oxidative stress by AN and mechanisms of toxicity in testis (apoptosis).	Dang et al. (2017a)
SOD, GPx	Rat, Sprague-Dawley (M)	Testis	No change	50 mg/kg bw	50 mg/kg bw per day (gavage), 6 d/wk, 12 wk	A mechanistic study showing mechanisms of AN toxicity in sperm.	Dang et al. (2017b)
SOD	Rat, Sprague-Dawley (M)	Brain	↓	46 mg/kg	46 mg/kg bw per day, 28 d, gavage; decrease for GPx only	Identification of protective effects of apigenin in the brains of rats treated with AN; it has value as a mechanistic study.	Zhao et al. (2019)
GPx			No change				
MPO	Rat, Wistar (M)	Gastric	↑	30 mg/kg bw	30 mg/kg bw		Hamdy et al. (2012)
XO	Rat, Sprague-Dawley (M)	Gastric	↑	25 mg/kg	25 mg/kg	The study supported the role of AN in oxidative damage to the gastric system and provided mechanistic evidence for the role of XO.	Al-Abbasi (2012)
GSH	Rat, Sprague-Dawley; mouse, CD-1; hamster, Syrian golden (M, F)	Brain, lung, liver, kidney	↓, most pronounced in rats	75 mg/kg	75 mg/kg	AN affects GSH levels; these levels might be responsible for tissue- and species-specific differences in sensitivity to AN exposure.	Cote et al. (1984)

Table 4.14 (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dose regimen	Comments	Reference
GSH	Mouse, GP-20-(F); rat, Sprague-Dawley (M); hamster (F); guinea-pig (M)	Liver	↓, most pronounced in mice, least in rats	20–80 mg/kg bw	20–80 mg/kg bw	Species differences in response to AN treatment, which might be related to differences in metabolic formation and inactivation of the active intermediate.	Vainio and Mäkinen (1977)
ROS	Mouse (F)	Ovary	↑	5, 10, 20 mg/kg bw	5, 10, 20 mg/kg bw per day for 28 d	The study suggested reproductive toxicity of AN and identified its mechanisms.	Luo et al. (2022)

AN, acrylonitrile; bw, body weight; CAT, catalase; d, day(s); F, female; F344, Fischer 344; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; h, hour(s); HID, highest ineffective dose; i.p., intraperitoneal; i.v., intravenous; LED, lowest effective dose; M, male; MDA, malondialdehyde; min, minute(s); MPO, myeloperoxidase; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; p.o., per os; ppm parts per million; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; wk, week(s); XO, xanthine oxidase.

[a]↑, increase; ↓, decrease.

which were treated with doses of ≤80 mg/kg (Vainio and Mäkinen, 1977). In rats, acrylonitrile treatment increased lipid peroxidation levels (mostly analysed as MDA or TBARS) (Silver and Szabo, 1982; Ivanov et al., 1989; Jiang et al., 1998; Whysner et al., 1998; Mahalakshmi et al., 2003; Nagasawa et al., 2003; Huang et al., 2005; El-Sayed et al., 2008; Rongzhu et al., 2009; Guangwei et al., 2010; Abo-Salem et al., 2011; Al-Abbasi, 2012; Dang et al., 2017a, b; Zhao et al., 2019), superoxide or other ROS production (Jiang et al., 1998; Pu et al., 2009; Al-Abbasi, 2012; Dang et al., 2017b), DNA oxidation (8-OHdG) (Jiang et al., 1998; Whysner et al., 1998; Pu et al., 2009, 2016; Williams et al., 2017), and activities of xanthine oxidase (Al-Abbasi, 2012) and myeloperoxidase (Hamdy et al., 2012). Decreased levels of GSH (Szabo et al., 1977; Cote et al., 1984; Jiang et al., 1998; Whysner et al., 1998; Mahalakshmi et al., 2003; Huang et al., 2005; El-Sayed et al., 2008; Pu et al., 2009; Rongzhu et al., 2009; Guangwei et al., 2010; Abo-Salem et al., 2011; Hamdy et al., 2012; Dang et al., 2017a, b; Zhao et al., 2019) and decreased activities of SOD, CAT, or GPx (Jiang et al., 1998; Whysner et al., 1998; Mahalakshmi et al., 2003; Huang et al., 2005; El-Sayed et al., 2008; Guangwei et al., 2010; Abo-Salem et al., 2011; Hamdy et al., 2012; Dang et al., 2017a, b; Zhao et al., 2019) were also observed.

(ii) Experimental systems in vitro

See Table 4.15.

Experimental studies in vitro were performed in blood (macrophages), nervous system (microglia, astrocytes), hepatic, or embryonic cells. Depending on the cell type, the tested concentrations ranged from hundreds of nanomoles to tens of micromoles.

In rat macrophages, acrylonitrile induced the production of H_2O_2, which was reduced by antioxidants (the enzymes SOD and CAT, and mannitol) (Bhooma and Venkataprasad, 1997). In rat erythrocytes, acrylonitrile induced lipid peroxidation (Farooqui et al., 1990). This was also observed in rat microglia and astrocytes (Caito et al., 2013). In rat astrocytes, acrylonitrile induced oxidative DNA damage (Pu et al., 2006); in rat glial cells, it induced DNA oxidation (8-OHdG) and a decrease in GSH levels and CAT and SOD activities (Kamendulis et al., 1999a). In rat hepatocytes, acrylonitrile caused a dose-dependent decrease in GSH levels (Zitting and Heinonen, 1980; Kamendulis et al., 1999b). In Syrian hamster embryo cells, acrylonitrile induced ROS generation and 8-oxodG formation; levels of GSH and activities of CAT and SOD were decreased (Zhang et al., 2000, 2002).

With the aim of "clarifying the mechanisms of oxidative DNA damage induced by acrylonitrile", Murata et al. (2001) (see also Section 4.2.2) used HPLC combined with electrochemical detection to measure the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) (oxidative damage) in calf thymus DNA. The 8-oxodG content was measured in DNA treated (in buffer, 37 °C for 30 minutes) with various concentrations of hydrogen peroxide (H_2O_2) plus cupric ions (Cu^{2+}) (20 μM), either in the absence or presence of acrylonitrile (see Table 4.8). The concentrations of acrylonitrile tested were up to "0.5%", which would be approximately 100 mM. The presence of acrylonitrile increased the amount of 8-oxodG formed by the H_2O_2 plus Cu^{2+} system in a concentration-dependent manner.

Synopsis

[Overall, there were very few studies in exposed humans and human cell lines. In exposed humans, the data suggested an association between acrylonitrile exposure and lipid peroxidation and DNA damage. However, the four available studies in exposed humans showed several limitations, including co-exposure to complex mixtures, no adjustment for co-exposures, and little detail given regarding the exposure assessment. Data on the pro-oxidative properties of acrylonitrile in human cell lines were inconsistent; however, there were

Table 4.15 End-points relevant to oxidative stress in experimental systems in vitro exposed to acrylonitrile

End-points	Assay	Tissue, cell line	Results[a]	Concentration	Experiment conditions	Comments	Reference
SOD, CAT	Pick and Keisari method	Rat macrophages	↑	10 µM	200 nm to 20 µM; SOD, CAT prevented toxicity caused by AN	AN may cause damage to lung macrophages.	Bhooma and Venkataprasad (1997)
Lipid peroxidation	Conjugated dienes	Rat erythrocytes	↑	10 mM	10 mM, 25 mM	AN induced haemoglobin degradation and lipid peroxidation in erythrocytes; this affects membrane structure and inhibits ATPase activity.	Farooqui et al. (1990)
F_2-isoprostanes	GC-MS	Rat primary astrocytes, microglia	↑	0.75 mM	0.1, 0.5, 0.75, 1 mM; response for microglia only	A study suggesting neurotoxic effects of AN, primarily caused by toxicity in microglia.	Caito et al. (2013)
GSH	HPLC	Rat primary astrocytes, microglia	↑	0.5 mM	0.1, 0.5, 0.75, 1 mM	A study suggesting neurotoxic effects of AN, primarily caused by toxicity in microglia.	Caito et al. (2013)
8-OHdG	HPLC	Rat hepatocytes Glial cells	No change ↑	0.01 mM	Treatment for 1, 4, and 24 h; concentrations, 0.01, 0.1, and 1 mM; response for astrocytes only		Kamendulis et al. (1999a)
MDA	HPLC	Rat hepatocytes Glial cells	No change ↑	0.01 mM	Treatment for 1, 4, and 24 h; concentrations, 0.01, 0.1, and 1 mM; response for astrocytes only		Kamendulis et al. (1999a)
Hydroxyl radicals	HPLC-EC	Rat hepatocytes Glial cells	No change ↑	0.01 mM	Treatment for 1, 4, and 24 h; concentrations, 0.01, 0.1, and 1 mM; response for astrocytes only		Kamendulis et al. (1999a)

Table 4.15 (continued)

End-points	Assay	Tissue, cell line	Results[a]	Concentration	Experiment conditions	Comments	Reference
GSH	HPLC-EC	Rat hepatocytes Glial cells	No change ↓	0.1 mM	Treatment for 1, 4, and 24 h; concentrations, 0.01, 0.1, and 1 mM; response for astrocytes only		Kamendulis et al. (1999a)
CAT, SOD, GPx	Reaction of cell extracts with appropriate substrates	Rat hepatocytes Glial cells	No change ↑	0.1 mM	Treatment for 1, 4, and 24 h; concentrations, 0.01, 0.1, and 1 mM; response for astrocytes and SOD only		Kamendulis et al. (1999a)
GSH	HPLC-EC	Rat astrocytes Hepatocytes	↓ ↑	0.1 mM	0.01, 0.1, 1 mmol/L; 4–48 h		Kamendulis et al. (1999b)
Oxidative DNA damage	Comet assay	Rat astrocytes	↓	1 mM	0.005–1 mM, 24 h	DNA oxidation is the major negative impact of AN, metabolism by CYP enzymes required, GSH levels contribute to the resulting effect.	Pu et al. (2006)
GSH	The method of Saville	Rat hepatocytes	↓	0.05 mM	0.05–1 mM, for up to 120 min	A dose-dependent decrease.	Zitting and Heinonen (1980)
8-OHdG	HPLC-EC	Syrian hamster embryo cells	↑	75 µg/mL	12.5–75 µg/mL; for up to 7 d	AN causes cell transformation in which oxidative DNA damage plays a role.	Zhang et al. (2000)
ROS	HPLC-EC	Syrian hamster embryo cells	↑	25 µg/mL	25, 50, 75 µg/mL; for 4, 24, 48 h	Oxidative stress induced by AN was temporary, involved a decrease in antioxidants and increase in XO activity mediated by AN metabolism.	Zhang et al. (2002)
GSH	HPLC-EC	Syrian hamster embryo cells	↓	25 µg/mL	25, 50, 75 µg/mL; for 4, 24, 48 h; effect after 4 h only	Oxidative stress induced by AN was temporary, involved a decrease in antioxidants and increase in XO activity mediated by AN metabolism.	Zhang et al. (2002)

Table 4.15 (continued)

End-points	Assay	Tissue, cell line	Results[a]	Concentration	Experiment conditions	Comments	Reference
CAT, SOD XO	Reaction of cell extracts with appropriate substrates	Syrian hamster embryo cells	↓ ↑	50 µg/mL	25, 50, 75 µg/mL AN; 4, 24, 48 h; CAT: decrease (4 h) followed by increase; XO: increase after 24 and 48 h	Oxidative stress induced by AN was temporary, involved a decrease in antioxidants and increase in XO activity mediated by AN metabolism.	Zhang et al. (2002)

AN, acrylonitrile; ATPase, adenosine triphosphatase; CAT, catalase; CYP, cytochrome P450; d, day(s); EC, electrochemical detection; GC-MS, gas chromatography-mass spectrometry; GPx, glutathione peroxidase; GSH, glutathione; h, hour(s); HPLC, high-performance liquid chromatography; MDA, malondialdehyde; min, minute(s); 8-OHdG, 8-hydroxy-2′-deoxyguanosine; ROS, reactive oxygen species; SOD, superoxide dismutase; XO, xanthine oxidase.

[a]↑, increase; ↓, decrease.

studies supporting the hypothesis that acrylonitrile induces oxidative stress in experimental systems. On the basis of the above data, there was evidence that acrylonitrile affects oxidative status and induces oxidative damage in vivo. Similarly, in studies in non-human mammalian systems in vitro, the results confirmed the effects of acrylonitrile on oxidative stress, particularly in lung macrophages and nerve cells.]

4.2.5 Induces chronic inflammation

(a) Humans

Exposed humans

See Table 4.16.

Exposure to acrylonitrile increased the levels of inflammation end-points measured in the blood of 82 workers from two chemical facilities (Cave et al., 2011). These workers handling elastomer/polymer substances were assumed to be exposed to acrylonitrile, 1,3-butadiene, and styrene. Quantification by ELISA showed elevations in serum concentrations of cytokeratin 18 (CK-18), tumour necrosis factor alpha (TNFα), interleukins IL-6 and IL-8, and monocyte chemoattractant protein (MCP-1). [The Working Group noted that, although semiquantitative levels of acrylonitrile were developed from work histories, the direct association between exposure and inflammatory markers was not considered in multivariate regression models. Results were reported for all workers, who were also exposed to styrene, butadiene, and other chemicals. In addition, personal history of cigarette smoking was not reported.] In studies of 663 community residents in six neighbourhoods in Louisville, Kentucky, USA, serum elevations in alkaline phosphatase (ALP), considered to reflect inflammatory injury to the liver, correlated with levels of the urinary metabolite CEMA, measured using ultraperformance LC-MS (Wahlang et al., 2022, 2023). All analytes were normalized to urinary creatinine levels to adjust for dilution. Serum concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) showed no correlation with the urinary metabolite. [The Working Group noted that the limitations of these studies included a lack of information about the timing of urine collection relative to serum measurements and the observed positive associations between ALP and several other VOCs, with a moderate positive correlation for CEMA, which reflects only current exposure. The Working Group noted that a strength of these studies was the measurement of 16 urinary metabolites of VOCs, although no adjustments were made.]

(b) Experimental systems

Non-human mammals in vivo

See Table 4.17.

A study by the NTP (2001) showed histopathological evidence of chronic gastrointestinal inflammation in B6C3F$_1$ mice (male and female) exposed to acrylonitrile (at 0–60 mg/kg bw per day) by gavage 5 days per week for 14 weeks. Chronic inflammation was observed in the forestomach of females dosed with acrylonitrile at 40 mg/kg bw per day.

Supplementation of drinking-water with acrylonitrile at 100 ppm (provided ad libitum) increased the expression of inflammation mediators in the brain tissue of female F344 rats (Pu et al., 2016). The expression of TNFα and IL-1β was quantified using RT-PCR. [The Working Group noted that the paper did not provide information on whether the increase was statistically significant.]

In mice exposed to acrylonitrile by gavage at a dose of 5, 10, or 20 mg/kg bw per day for 28 days, histopathological evidence identified an increase in the proportion of atretic follicles, together with a significant decrease in the number of preovulatory follicles, as evidence of inflammation. Elevations in the expression of TNFα and IL-1β after acrylonitrile exposure

Table 4.16 End-points relevant to chronic inflammation in humans exposed to acrylonitrile

End-points	Location Setting and study design	Biosample type	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
CK-18 TNFα IL-6 IL-8 MCP-1	USA, Kentucky Chemical plant Cross-sectional study AN-butadiene-styrene elastomer/ polymer workers in two companies exposed from what appeared to be since the mid-1970s	Serum	Chemical workers (82 workers in two facilities); exposure characterized based on employment records	↑ ↑ ↑ ↑ ↑	Also evaluated 1,3-butadiene and styrene exposure levels. No indication whether carcinogenic pigments or dyes were present but was possible. No adjustments made.	Limitations: Small sample size; use of air measurements not described; unclear if historical changes were considered; No information on exposure levels or latency; exposure group may be heterogeneously exposed; no adjustments made for co-exposures; no control group. Strengths: Employer work histories form the basis of job information; cumulative exposure was estimated; dermal exposure considered; substantial exposure duration; two major occupational carcinogens evaluated. Nondifferential exposure, but differential was possible. Limited informativeness.	Cave et al. (2011)

Table 4.16 (continued)

End-points	Location Setting and study design	Biosample type	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
ALP	USA, Kentucky HEAL study Cross-sectional study	Blood	Urine levels of AN metabolite 663 participants	↑	15 other VOCs analysed. No adjustments made	Limitations: CYMA (also referred as CEMA) is a metabolite, with levels reflecting exposure in hours, but generally not longer-term; single exposure group; no information on duration, exposure levels, latency, co-exposures; qualitative analysis of entire population; no adjustments made for co-exposures; no controls. Strengths: CEMA is a highly specific and sensitive biomarker for AN that reflects all short-term exposure routes; moderate-sized study; analysis used continuous variables in regression analyses; measured several other co-exposures. Nondifferential misclassification. Limited informativeness.	Wahlang et al. (2022)

Table 4.16 (continued)

End-points	Location Setting and study design	Biosample type	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
ALP	HEAL study	Blood	Urine levels of AN metabolite 663 participants	↑	15 other VOCs analysed. No adjustments made.	Limitations: CEMA is a metabolite with levels reflecting exposure in hours, but generally not longer-term; single exposure group; no indication of duration, exposure levels, latency, co-exposures identified; qualitative analysis of entire population; no adjustments made for co-exposures; no controls. Strengths: CEMA is a highly specific and sensitive biomarker for AN that reflects all exposure routes over a work shift; moderate-sized study; analysis used continuous variables in regression analyses; measured several other co-exposures. Nondifferential misclassification. Limited informativeness.	Wahlang et al. (2023)

ALP, alkaline phosphatase; AN, acrylonitrile; CEMA, *N*-acetyl-*S*-(2-cyanoethyl)-L-cysteine (the authors referred to this metabolite as CYMA, but for consistency, it is referred to as CEMA here and elsewhere); CK, cytokeratin; HEAL, Health, Environment and Action in Louisville; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; TNFα, tumour necrosis factor alpha; USA, United States of America; VOC, volatile organic compound.

[a] ↑, increase.

Table 4.17 End-points relevant to chronic inflammation in non-human mammalian systems in vivo exposed to acrylonitrile

End-point	Assay	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Chronic active inflammation	Histopathology analysis	Mouse, $B6C3F_1$ (F)	Forestomach	↑	40 mg/kg	Gavage, 20, 40 or 60 mg/kg, 5 d/wk for 14 wk	No inflammation was observed in the carcinogenicity study after 2-yr exposure.	NTP (2001)
TNFα, IL-1β	RT-PCR	Rat, F344 (F)	Brain	↑	100 ppm, in drinking-water	100 ppm, in drinking-water, for 28 d while being fed ad libitum with NIH07 diet		Pu et al. (2016)
TNFα, IL-1β	Western blot assay	Mouse, Kunming (F)	Ovarian follicular cells	↑	5 mg/kg	Gavage, 5, 10, and 20 mg/kg bw, for 28 d Dose–response relation observed		Luo et al. (2022)

bw, body weight; d, day(s); F, female; F344, Fischer 344; HID, highest ineffective dose; IL, interleukin; LED, lowest effective dose; ppm, parts per million; RT-PCR, reverse transcription-polymerase chain reaction; TNFα, tumour necrosis factor alpha; wk, week(s); yr, year(s).

[a] ↑, increase.

were demonstrated using western blot analysis (Luo et al., 2022).

Synopsis

[Overall, the available studies associating acrylonitrile with chronic inflammation in exposed humans had some limitations. Some evidence of induction of inflammation was reported in studies in experimental systems in vitro and in vivo. The Working Group noted that levels of mediators of inflammation were increased in the blood and other tissues of experimental animals, suggesting potential involvement of leukocytes and lymphocytes.]

4.2.6 Is immunosuppressive

(a) Humans

(i) Exposed humans

See Table 4.18.

Three cross-sectional, observational studies have investigated end-points that reflect immunosuppression.

Changes in T-cell populations were observed in workers at a worsted and cloth factory assumed to be exposed to polyacrylonitrile dust (n = 103) compared with the control group (female health administration workers, n = 20) (Samarova, 1993). Significant reductions in the T-lymphocyte population and in the subpopulation of T-helper cells were observed among those who had worked at the factory for > 1 year in the spinning and roving shops. [The Working Group noted that the workers were exposed at very high levels of dust (> 10 000 µg/m^3 at some sites), and that the exposure was to polyacrylonitrile dust, not acrylonitrile. Any possible relationship between polyacrylonitrile dust and acrylonitrile exposure was not well described in the study. The study populations (both exposed and unexposed) varied substantially in number according to the specific end-points measured. The demographics and quantification of exposures were incomplete; the sex of the workers was uncertain but the controls were female. In addition, disparities were noted in the age ranges, with 78.5–80.0% of the workers being aged 20–39 years and almost half having worked for 6–12 years, whereas the control group was dominated by women aged 40–49 years and older (52%) who had worked for > 10 years (42.9%). It was unclear why results were provided for trainees (≤ 1 year) and non-trainees (> 1 year), with almost half having worked for 6–12 years.]

In a second study, in workers (n = 132) assumed to be exposed to chemicals, including acrylonitrile, during the manufacture of "synthetic fur" in a factory located in the area contaminated by radioactivity from the Chernobyl accident, there was a significant decrease in the total number of T lymphocytes and an increase in T-suppressor cells at a 2-year follow-up (Grebeniuk et al., 1999). There was also a tendency towards an increase in the T-helper cell population. The combined long-term effects of radiation and chemical exposure induced an alteration in humoral immunity, increasing the B lymphocyte population, decreasing the concentrations of serum immunoglobulins (IgM, IgG, and IgA), and increasing serum IgE. In addition, women in the knitting workshop (where the acrylonitrile concentration was significantly below the OEL) showed a markedly lower absolute absorption of microbes by peripheral blood leukocytes with a decrease in the digestion index and phagocytic index of neutrophils at all time points (< 2 years, 2–5 years, > 5 years), and a reduction in phagocytic activity only for 2–5 years of exposure. Women in the finishing workshop (where the acrylonitrile concentration was around the OEL) showed a significant reduction in absolute absorption rate, phagocytic activity, and digestion index during the first 5 years; after 5 years, the decrease in phagocytic activity and index was not statistically significant (Grebeniuk et al., 1999). [The Working Group noted that demographic information was not available for

Table 4.18 End-points relevant to immunosuppression in humans exposed to acrylonitrile

End-points	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
Lymphocyte count	Blood	Semey (previously called Semipalatinsk), Kazakhstan Occupational exposure in Semipalatinsk worsted and cloth company Cross-sectional, observational study	Two exposed groups: > 1 yr[a] and ≤ 1 yr. [a] around 50% worked for 6–12 yr Occupational exposure to polyacrylonitrile dust in combing, carding, roving and spinning shop was, respectively: 3.2 ± 0.5, 2.1 ± 0.8, 12.0 ± 3.9, 8.0 ± 1.5 mg/m^3 at old machine, and 2.9 ± 0.5, 4.7 ± 2.3, 5.2 ± 0.3 and 2.6 ± 0.5 mg/m^3 at new machines, which exceeded the AN dust OEL (5 mg/m^3) by up to 2.4 times 103 workers in the worsted and cloth company and 20 controls working in health administration	↓ in T lymphocytes (%) ($P < 0.05$) in roving workers exposed for > 1 yr compared with control group; ↓ in T-helper cells (%) ($P < 0.05$) in roving and spinning workers exposed for > 1 yr compared with workers exposed for ≤ 1 yr; ↓ in T lymphocytes (%) ($P < 0.05$) in spinning workers exposed for > 1 yr compared with workers exposed for ≤ 1 yr; ↓ in T-suppressor cells (%) ($P < 0.05$) in roving workers exposed for ≤ 1 yr compared with workers exposed for > 1 yr; ↓ in T-suppressor cells (%) ($P < 0.05$) in twist-wind workers exposed for > 1 yr compared with workers exposed for ≤ 1 yr	NR	Group controls were all female workers. Age of workers exposed to AN, 20–39 yr (78.5–80.0%). Missing details on the assay used, and covariates, and on exposure (AN exposure assumed).	Samarova (1993)

Table 4.18 (continued)

End-points	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
Lymphocyte count; IgM, IgG, IgA concentrations; neutrophil granulocyte phagocytic activity	Blood	Near Chernobyl, Ukraine, Occupational exposure at a factory manufacturing "synthetic fur" Cross-sectional, observational study	Exposure level to AN not measured. Exposure to radiation: ^{137}Cs was 5–15 K_i/km^2 132 female workers exposed to AN, 92 controls not exposed to low-dose radiation and intensity	↓ Total number of T lymphocytes, IgM, IgG, IgA, ↑ T-suppressor cells, B lymphocytes, IgE Knitting workers: ↓ absolute absorption of microbes by peripheral blood leukocytes, digestion index, phagocytic index of neutrophils (< 2 yr; 2–5 yr; > 5 yr); ↓ phagocytic activity at 2–5 yr Finishing workers: ↓ phagocytic activity and index of neutrophils (%), digestion index absolute absorption rate, phagocytic activity and index (< 5 yr)	NR	Missing details on the assay used, study design, covariates, exposure (AN exposure assumed). Co-exposure to radiation and chemical factors at low intensity contribute to the resulting immunosuppressive effects.	Grebeniuk et al. (1999)

Table 4.18 (continued)

End-points	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
Lymphocyte and other leukocyte count (neutrophils, eosinophils, and monocytes)	Blood	Italy, Occupational exposure at a polyacrylonitrile factory Cross-sectional, observational study	Exposure below the AN threshold of 2 ppm 218 exposed workers, 200 not exposed (controls). Mean age of 38 yr for both groups. Work seniority for exposed group was 6.54 yr and for the not exposed was 6.70 yr Sampling before work shift (at fasting)	↓ lymphocytes ↑ neutrophils ($P < 0.001$) No changes in leukocytes, eosinophils, and monocytes	Past and present history of diseases and/or disorders of the haematopoietic system, acute or chronic inflammation, treatment, other recreational exposures acting on blood count, smoke, alcohol, age, work seniority	Potential co-exposure with other substances at high doses and with possible haematotoxic action, such as methacrylate, sodium metabisulfite, sodium nitrite, sulfuric acid, and soda. Haematological electronic analyser.	Caciari et al. (2014)

AN, acrylonitrile; Cs, caesium; Ig, immunoglobulin; NR, not reported; OEL, occupational exposure limit; ppm, parts per million; yr, year(s).
[a]↑, increase; ↓, decrease.

either the workers or the controls. The immunosuppressive effect observed was attributed to the combination of the effects of low-intensity radiation and the chemicals used in fur production. In addition, there was not a clear relationship for any end-point that reflected exposure to acrylonitrile and duration of employment in the knitting and finishing workshops.]

In a third study, a potential immunosuppressive effect was observed in workers exposed to a low dose of polyacrylonitrile (Caciari et al., 2014). Peripheral blood cell counts indicated reductions in lymphocytes and increases in neutrophils in blood samples collected from exposed workers (n = 218) compared with counts from unexposed workers (n = 200). The haemoglobin concentration and cell counts for erythrocytes, leukocytes, eosinophils, and monocytes were not significantly different. [The Working Group noted that, in addition to acrylonitrile, the workers were potentially exposed to other substances, including methacrylate, various additives, and auxiliaries.]

(ii) Human cells in vitro

Polymorphonuclear leukocytes isolated from 24 healthy non-smokers (aged 26–60 years) and exposed in vitro to acrylonitrile at 3, 12, 18, 24, 36, and 72 mM for 20 minutes exhibited changes in chemotaxis as shown using a Boyden chamber (Monaco et al., 1995). A dose-dependent decrease in chemotaxis, with a 50% inhibition, was evident at 15 mM and was not explained by any increase in cell mortality. [The Working Group noted that investigation using a Boyden chamber assay is difficult and time-consuming. In addition, the Working Group noted that the acrylonitrile doses employed were high.]

In an investigation in Jurkat cell lines exposed to acrylonitrile at several doses (0, 20, 100, and 500 µM for 0–24 hours), potential immunotoxicity was evidenced by damage to lipid raft structures (essential for T-cell activation), resulting in Bcl10 protein and lipid raft separation (Li et al., 2014a). [The Working Group noted that direct end-points of immunosuppression were not quantified.]

(b) Experimental systems

See Table 4.19.

(i) Non-human mammals in vivo

In CD-1 mice, acrylonitrile (2.7 mg/kg bw per day, equivalent to 10% of the median lethal dose, LD_{50}) administered once daily by mouth for 5, 10, and 15 days demonstrated immunosuppression in vivo (Hamada et al., 1998). Immunosuppression was observed both locally and systemically after 5 days, and effects increased up to 15 days after dosing. Decreased levels of antibody (IgA)-producing cells in the small intestine and diminished uptake of radiolabelled thymidine in splenocytes after mitogen stimulation (with concanavalin A, lipopolysaccharide, and phytohaemagglutinin) were included among the measured end-points.

Subcutaneous exposure of non-inbred Wistar mice to acrylonitrile at 18 mg/kg (equivalent to 0.5 LD_{50}, the LD_{50} being 36 mg/kg for this mouse strain) did not affect immunosuppression measured as animal mortality (i.e. resistance to experimental infection; the median effective dose, ED_{50}, after *E. coli* injection) (Zabrodsky and Romashchenko, 1998). In non-inbred Wistar rats, end-points of humoral and cellular immunity at 24 hours could be affected by acrylonitrile at a subcutaneous dose of 37.5 mg/kg (or 0.5 LD_{50}, the LD_{50} being 75 mg/kg for this rat strain) (Zabrodsky and Romashchenko, 1998). These included the numbers of cells forming antibodies to sheep erythrocytes or spleen-independent antigens, delayed hypersensitivity reaction (increased paw weight), antibody-dependent cellular cytotoxicity, and macrophage-induced humoral immune response. [The Working Group noted that the acrylonitrile dose used reflected the original purpose of the study, which was to assess the efficacy of the antidote sodium

Table 4.19 End-points relevant to immunosuppression in non-human mammalian systems in vivo exposed to acrylonitrile

End-point	Assay	Species, strain (sex), or cell line	Tissue	Results[a]	Dose	Route, duration, dosing regimen	Comments	Reference
IgA-producing cells	IHC	Mouse, CD-1 (M)	Small intestine	↓ in duodenum, jejunum after treatment for 5, 10, and 15 d ↓ in ileum after treatment for 10 and 15 d	2.7 mg/kg bw per day (= 10% LD_{50})	Gavage, for 5, 10, and 15 d	Animals were killed 24 h after the last dose.	Hamada et al. (1998)
Lymphocyte proliferation	[^{3}H]thymidine uptake		Spleen	↓	Splenocytes from the orally treated mice were stimulated with mitogens in vitro (concanavalin, phytohaemaglutinin, or lipopolysaccharide)			
Resistance to *E. coli* infection	Average-effective lifetime	Mouse, Wistar		↓ ET_{50}	18 mg/kg bw (= 0.5 LD_{50}, 36 ± 4 mg/kg bw *E. coli* suspension was injected intraperitoneally 24 h after AN	Subcutaneous		Zabrodsky and Romashchenko (1998)
	Animal lethality, ED_{50}			No change				
Antibody production	Antibody-producing cells	Rat, Wistar	Spleen	↓ number of (thymus-dependent) antibody-forming cells to sheep erythrocytes, or thymus-independent antigens ↓ antibody-dependent cellular cytotoxicity (%)	37.5 mg/kg (= 0.5 LD_{50}, 75 ± 7 mg/kg bw) *E. coli* suspension was injected intraperitoneally 24 h after AN	Subcutaneous		
	Spectrophotometric			↓ macrophage-induced humoral immune response	After immunization with sheep erythrocytes (10^8)			
DHR	Weight measurement		Paw	↓				

Table 4.19 (continued)

End-point	Assay	Species, strain (sex), or cell line	Tissue	Results[a]	Dose	Route, duration, dosing regimen	Comments	Reference
Antibody production		Mouse, CBA	Spleen	↓ number of antibody-producing cells	14 mg/kg bw (= 0.5 LD_{50}, 28 mg/kg bw)	Subcutaneous, single dose	Missing details of assay.	Zabrodskii et al. (2000)
DHR	Weight measurement		Paw	↓				
Antibody production		Rat, random-bred (M)	Spleen	↓ number of (thymus-dependent) antibody-forming cells to sheep erythrocytes, or thymus-independent antigens	68 mg/kg bw (= 0.8 LD_{50}, 85 ± 5 mg/kg bw)	Gavage, single dose, i.p. immunization	Missing details of assay.	Zabrodskii and Germanchuk (2000)
Delayed hypersensitivity reaction	Weight measurement		Paw	↓				
Activity of NK cells (cytotoxicity)	Spectrophotometric		Spleen	↓ number of intact target cells 24 h after poisoning (% cytotoxicity)				
Activity of K cells (cytotoxicity)	Spectrophotometric		Splenocytes	↓ antibody-dependent cell cytotoxicity 5 d after immunization with sheep erythrocytes (% cytotoxicity)				
Lymphocyte and leukocyte count	Haematology analyser, microscopy	Mouse, $B6C3F_1$ (M and F)	Blood	↓	40 mg/kg bw (F) and 20 mg/kg bw (M)	At 0, 5, 10, 20, 40, and 60 mg/kg bw, gavage, 5 d/wk for 14 wk		NTP (2001)
Lymphocyte count	NR	Rat, Wistar (M)	Thymus	↓	60 mg/kg bw (= 0.8 LD_{50}, 75 mg/kg bw)	Subcutaneous, single dose	After 24 h treatment. Missing details of assay.	Zabrodskii et al. (2002)
NK cell activity, cytotoxicity (%)	Spectrophotometric		Spleen	↓ after 5 d after immunization with sheep erythrocytes				

Table 4.19 (continued)

End-point	Assay	Species, strain (sex), or cell line	Tissue	Results[a]	Dose	Route, duration, dosing regimen	Comments	Reference
Antibody production			Spleen	↓ antibodies to immunization (sheep erythrocytes), antibody-producing cells against thymus-dependent antigens (sheep erythrocytes) and against independent antigens 5 d after immunization			There was a decrease in antibody-dependent cell cytotoxicity (%).	Zabrodskii et al. (2002) (cont.)
DHR	Weight measurement		Paw	↓ increase in paw weight (%), 24 h after immunization with sheep erythrocytes by injection in hindlimb				
NK cell activity	Spectrophotometric	Mouse, mongrel (M)	Blood	↓	1 LD_{50}	Subcutaneous, single dose	T-activin at 5 µg/kg restored the activity of NK cells.	Zabrodskiĭ et al. (2003)
Lymphocyte count	Flow cytometry	Rat, Sprague-Dawley (F and M)	Blood	↓ CD3, CD4, and CD4/CD8 ratio at 20 mg/kg; ↓ CD4/CD8 at 5, 10 mg/kg; and ↑ CD8 at all doses (F) ↓ CD3 at 5 mg/kg, and CD4; ↓ CD4/CD8 ratio at 20 mg/kg and ↑ CD8 at 20 mg/kg (M)	5, 10, and 20 mg/kg	Gavage, daily, for 5 d for 13 wk		Li et al. (2014b)
			Spleen	↓ CD4 and CD4/CD8, ↑ CD8 at 20 mg/kg (F) ↓ CD4, CD4/CD8, and ↑ CD8 at 20 mg/kg (M)				

Table 4.19 (continued)

End-point	Assay	Species, strain (sex), or cell line	Tissue	Results[a]	Dose	Route, duration, dosing regimen	Comments	Reference
IL-1β and TNFα mRNA expression	RT-PCR		Spleen	↓ IL-1β at all doses (5,10, 20 mg/kg) and TNFα at 10 and 20 mg/kg (F) ↓ IL-1β and TNFα at 5 and 10 mg/kg (M)				Li et al. (2014b) (cont.)
TLR expression (innate immunity)	Western blot		Spleen	↓ TLR4 at 5 mg/kg (F) and at 20 mg/kg (M)				

AN, acrylonitrile; bw, body weight; CD, cluster of differentiation; d, day(s); DHR, delayed hypersensitivity reaction; ED_{50}, median effective dose; ET_{50}, median effective time that causes 50% mortality; F, female; h, hour(s); Ig, immunoglobulin; IHC, immunohistochemistry; IL, interleukin; i.p., intraperitoneal; K, killer; LD_{50}, median lethal dose; M, male; NK, natural killer; NR, not reported; RT-PCR, reverse transcription-polymerase chain reaction; TLR, toll-like receptor; TNFα, tumour necrosis factor alpha; wk, week(s).

[a] ↑, increase; ↓, decrease.

thiosulfate on the development of post-intoxication immunodeficiency after acute acrylonitrile poisoning.]

In CBA mice, acrylonitrile, injected subcutaneously at a dose of 14 mg/kg (or 0.5 LD_{50}, the LD_{50} being 28 mg/kg in this strain), suppressed the cell-mediated and humoral immune response. Decreases in the number of antibody-producing cells in the spleen and number of α-naphthyl butyrate esterase-positive cells (in the spleen and popliteal lymph nodes) were observed (Zabrodsky et al., 2000). CBA mice treated subcutaneously with acrylonitrile at 14 mg/kg also demonstrated changes in delayed-type hypersensitivity (Zabrodskii et al., 2000). Acrylonitrile decreased weight gain in the foot (an end-point reflecting the delayed hypersensitivity response and immunosuppression) after transfer of spleen cells. Numbers of antibody-producing cells in the spleen decreased after acrylonitrile treatment. [The Working Group noted that the dose of acrylonitrile used in the study was too high to assess the efficacy of compounds intended to reverse an acute intoxication with acrylonitrile.]

In "random bred" rats, the same investigators observed suppression of the cell-mediated and humoral immune response in vivo after administration of acrylonitrile as a single dose at 68 mg/kg (0.8 LD_{50}, the LD_{50} being 85 mg/kg in the "random bred" strain) into the stomach (Zabrodskii and Germanchuk, 2000). The humoral immune response to thymus-dependent (sheep erythrocytes) and thymus-independent antigens was evaluated by the number of antibody-producing cells, which was decreased in the spleen. The cellular immune response was evaluated by measuring spleen natural killer (NK) and killer (K) cell activities, which both decreased ($P < 0.05$). [The Working Group noted that the acrylonitrile dose used was high.]

In studies by the National Toxicology Program (NTP) in male and female $B6C3F_1$ mice, immunosuppression in vivo was demonstrated after administration of acrylonitrile at doses of 0, 5, 10, 20, 40, and 60 mg/kg bw per day by gavage 5 days per week for 14 weeks (NTP, 2001). Blood counts showed a decreased lymphocyte count in females at 40 mg/kg and in males at 20 mg/kg bw per day (NTP, 2001). [The Working Group noted that the doses of 40 and 60 mg/kg bw per day had a significant impact on mortality in both female and male mice so immunosuppression must have been observed at the highest doses that allowed animal survival.]

Immunotoxicity in vivo was demonstrated in a study in Wistar rats injected subcutaneously with acrylonitrile at 60 mg/kg (or 0.8 LD_{50}, the LD_{50} being 75 mg/kg) (Zabrodskii et al., 2002). Acrylonitrile decreased titres of antibodies to sheep erythrocytes and antibody-producing cells. The T-cell count in the thymus, natural cytotoxicity, antibody-dependent cell cytotoxicity, and the delayed-type hypersensitivity response were all decreased by acrylonitrile. In addition, the plasma concentration of corticosterone decreased after the administration of acrylonitrile. Suppression of both cellular and humoral immune responses was noted. [The Working Group noted that the acrylonitrile dose used was particularly high.] A decrease in the activity of NK cells in male mice after acute intoxication with acrylonitrile at the LD_{50} dose was reported (Zabrodskiĭ et al., 2003).

Female and male Sprague-Dawley rats exposed to acrylonitrile at 0, 5, 10, and 20 mg/kg by gavage (once per day, 5 days per week, for 13 weeks) showed changes in lymphocyte populations in the blood and spleen (by flow cytometry), toll-like receptor (TLR) expression in spleen cells (by western blot assay), and IL-1β and TNFα expression in spleen cells (by RT-PCR) (Li et al., 2014b). Relative to the control group, female rats exposed to acrylonitrile demonstrated decreased numbers of blood CD3 and CD4 cells, and CD4/CD8 cells in the group at 20 mg/kg; decreased blood CD4/CD8 cells in the groups at 5, 10, and 20 mg/kg; and increased blood CD8 cells in the groups at 5, 10, and 20 mg/kg. In male rats exposed

to acrylonitrile, numbers of blood CD3 cells were decreased in the group at 5 mg/kg; blood CD4 cells and CD4/CD8 cells were decreased in the group at 20 mg/kg; and blood CD8 cells increased in the group at 20 mg/kg. In females, acrylonitrile exposure induced a decrease in spleen CD4 and CD4/CD8 cells, whereas CD8 cells increased in the group at 20 mg/kg. In males, exposure to acrylonitrile was associated with decreased spleen CD4 cells and CD4/CD8 cells in the group at 20 mg/kg.

[The Working Group noted that TLRs (a family of pattern-recognition receptors expressed in the innate immune system) selectively recognize pathogen-associated molecular patterns carried by invading pathogens, mediating the capture of various pathogens by dendritic cells, inducing macrophage activation, and participating in innate immunity and adaptive immunity. TLRs are expressed on the surface of T cells and directly regulate T-cell function. Compared with the control group, TLR4 mRNA expression by splenic cells in female rats at 5 mg/kg decreased, whereas TLR4 mRNA expression decreased in male rats at 20 mg/kg. Compared with the control group, TLR4 protein expression levels in spleen cells decreased in females at 5 mg/kg and in males at 20 mg/kg. TLR4 regulates the expression of pro-inflammatory factors IL-1β and TNFα, and changes in their expression can be related to TLR4. Compared with the control group, IL-1β mRNA expression in spleen cells decreased in female rats in the groups at 5, 10, and 20 mg/kg, whereas TNFα mRNA expression decreased at 10 and 20 mg/kg. In the male rats, both IL-1β and TNFα mRNA expression decreased in the groups at 5 and 10 mg/kg.]

(ii) Non-human mammalian systems in vitro

One study in vitro was available to the Working Group. In splenocytes from C57/B16 mice, exposure to acrylonitrile decreased the proliferation of T cells and B cells after stimulation with concanavalin A and lipopolysaccharide (Poirier et al., 2002).

Synopsis

[The Working Group considered some exposures to acrylonitrile, including a polyacrylonitrile, in humans in specific occupational settings, and in rodents treated via subcutaneous administration or orally via gavage or in drinking-water. There was evidence that exposure to acrylonitrile can alter total lymphocyte counts as well as the number of T-cell and B-cell subpopulations. Immunoglobulins, phagocytic activity, and both the humoral immune and delayed-type hypersensitivity responses were altered in exposed humans and in experimental systems in vivo. In addition, acrylonitrile was shown to alter the function of neutrophils.]

4.2.7 Modulates receptor-mediated effects

(a) Humans

Exposed humans

See Table 4.20.

In an interventional occupational study by Ivănescu et al. (1990), 297 adult male workers (aged 19–40 years), who worked directly with or around a source of acrylonitrile at a chemical plant, were categorized on the basis of different sampling time; three different groups were sampled in three different years. The workers were exposed to acrylonitrile for periods ranging from 6 months to 10 years of work history. The control group of 145 men aged 17–49 years included non-exposed residents of another region, as well as different workers exposed to other contaminants. Testosterone was measured at the different sampling points, and mean testosterone levels and standard deviation were compared across groups. Mean serum testosterone levels in workers exposed to acrylonitrile ranged from 3.5 to 4.1 ng/mL at the three time points, which was lower than levels in blood

Table 4.20 End-points relevant to receptor-mediated effects in humans exposed to acrylonitrile

End-point	Assay	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
Testosterone levels	Radioimmunoassay	Blood (serum)	NR Longitudinal, interventional	Working history of 6 mo to 10 yr with AN exposure for 297 workers; 145 controls	↓ serum testosterone with longer work history, compared with controls ($P < 0.05$)	Location	No objective exposure assessment for AN and lack of adjustment for important confounders.	Ivănescu et al. (1990)
Thyroid volume	Ultrasound	Thyroid	Russian Federation Case–control study	Working history of 13.1 ± 5.1 yr for 18 workers; control group of 6 unexposed administrative workers	Diffuse changes and nodular masses		Ambient AN was confirmed among exposed workers, but no confounder control was done in analyses. Small sample size limited the power of the statistical tests.	Ivashova et al. (2016)
TPO	Enzyme immunoassay	Blood			↑ TPO ($P \leq 0.05$)			
TSH					↑ TSH ($P < 0.001$)			
T4					No changes in T4			
Thyroid enlargement	Clinical examination	Thyroid	Russian Federation, factory manufacturing AN Case–control study	Working history: two groups for men (< 10 yr or > 10 yr) and women (< 8 yr or > 8 yr); 123 workers exposed to AN; 66 controls	23.6% of AN workers had diffuse thyroid enlargement, and only 6% of controls	Controls matched on age, sex, and physical development, but no statistical adjustment for covariates		Goncharova et al. (1984)
TSH	Radioimmunoassay	Blood			↑ TSH			
T4, FT4, T3					↓ T4, FT4, T3			

AN, acrylonitrile; FT4, free thyroxine; mo, month(s); NR, not reported; T3, triiodothyronine; T4, thyroxine; TPO, thyroid peroxidase; TSH, thyroid-stimulating hormone; yr, year(s).
[a] ↓, decrease; ↑, increase.

donors and in other chemical workers (range of means, 5.4–7.3 ng/mL). A subset of 15 and 50 workers exposed to acrylonitrile was followed up longitudinally with samples taken 1 year apart. There were no significant changes in testosterone levels between the sampling points. [The Working Group noted that confounders were not taken into account in the statistical analyses or matching in the study design phase, and participants were not grouped by acrylonitrile exposure, which limited interpretation. In addition, it was not known whether workers engaged in night shift work, which could influence hormone levels.]

In a case–control study on occupational exposure, Ivashova et al. (2016) reported that 18 workers involved in rubber production were exposed to ambient acrylonitrile at higher levels (range, 0.01–0.15 mg/m^3) than were control administrative workers (0.0005 mg/m^3). Ultrasound measurement of the thyroid revealed diffuse changes and nodular masses in the majority (72%) of exposed workers, as well as higher levels of antibodies to thyroid peroxidase (TPO) and higher levels of thyroid-stimulating hormone (TSH) among exposed workers compared with controls. [The Working Group noted that the workers were also exposed to other chemicals, as well as noise, but these confounders were not considered in the analysis. In addition, the small sample size limited the power of the statistical tests.]

In another case–control study on occupational exposure, Goncharova et al. (1984) compared 123 workers at a production factory who were exposed to acrylonitrile with 66 control workers, examining associations with thyroid structure and function as well as cortisol levels. Clinical examination revealed that diffuse thyroid enlargement was present in 23.6% of exposed workers but only 6% of control workers. Significant increases in levels of TSH and decreases in thyroxine (T4), free thyroxine (FT4), and triiodothyronine (T3) in acrylonitrile-production workers were observed in men working for > 10 and in women working for > 8 years, in comparison with the controls. The increase in TSH and decrease in T4 were also observed in men with a working history of ≤ 10 years. [The Working Group noted that the control group did not differ greatly from the exposed workers in terms of age or sex and were matched on the duration of work history; however, other confounders were not considered in the statistical analyses.]

(b) Experimental systems

Non-human mammals in vivo

See Table 4.21.

In an experimental model in Sprague-Dawley-derived female rats, Szabo et al. (1984) administered acrylonitrile at a concentration of 0, 0.0001% (1 ppm), 0.002%, 0.01%, 0.05%, or 0.2% in drinking-water or by daily gavage for 7, 21 or 60 days. The exposed treatment group experienced time- and dose-dependent decreases in plasma corticosterone levels, and aldosterone was also decreased in groups at higher doses and prolonged times of exposure. The younger rats were also seen to be more susceptible than adult rats. Plasma corticosterone was the hormone that was most sensitive to the route (gavage versus drinking-water) and timing of the administration of acrylonitrile.

Nilsen et al. (1980) treated male Sprague-Dawley rats by intraperitoneal injections with acrylonitrile (33 mg/kg) on three consecutive days. Decreases in both serum corticosterone and prolactin and increases in follicle-stimulating hormone (FSH) levels were reported in exposed rats compared with controls. No effect was observed on levels of luteinizing hormone.

Another animal experiment in male and female Sprague-Dawley rats was conducted by Nemec et al. (2008). In this study, groups of 25 males and 25 females of the F_0 and F_1 parental generation were exposed to acrylonitrile vapour

Table 4.21 End-points relevant to receptor-mediated effects in non-human mammalian systems exposed to acrylonitrile

End-point	Assay	Species, strain (sex)	Tissue	Results[a]	Dose	Route, duration, dosing regimen	Comments	Reference
Corticosterone	Immunoassay	Rats, Sprague-Dawley (F)	Plasma	↓ at 0.05% at 21 d; at 0.01% and 0.05% at 60 d (AN in-drinking-water); ↓ at 0.0001 and 0.01% at 7 d and at all doses at 21 or 60 d (gavage)	0, 0.0001% (1 ppm), 0.002%, 0.01%, 0.05% or 0.2%	Drinking-water, or same concentration via daily gavage, for 7, 21, or 60 d; n = 3–5 in each group	Purity, NR.	Szabo et al. (1984)
Aldosterone	Immunoassay			↓ at 0.0001, 0.01, and 0.05% at 21 d (gavage); at 0.05% at 60 d (drinking-water)				
LH	Radioimmunoassay	Rat, Sprague-Dawley (M)	Plasma	No change	33 mg/kg	Intraperitoneal injection, daily for 3 d, killed 24 h after last treatment. n = 4 in each group	Purity, NR.	Nilsen et al. (1980)
FSH				↑				
Prolactin				↓				
Corticosterone				↓				
Cholinesterase	Spectrophotometric method	Rat, Sprague-Dawley (M and F)	Plasma	↓ in females at 90 ppm (F_0) or at 15 ppm (F_1)	AN vapour at 0, 5, 15, 45 ppm (F_1 and F_0), and 90 ppm (F_0)	Ambient, 6 h daily 7 d/wk for 10 wk before mating	Purity, NR.	Nemec et al. (2008)

AN, acrylonitrile; d, day(s); F, female; F_0, first generation; F_1, second generation; FSH, follicle-stimulating hormone; h, hour(s); LH, luteinizing hormone; M, male; NR, not reported; ppm, parts per million; wk, week(s).

[a] ↑, increase; ↓, decrease.

at concentrations of 0, 5, 15, 45 (two offspring generations) and 90 ppm (one offspring generation) for 6 hours daily. In this study, a 40% reduction in plasma cholinesterase activity was observed in F_0 females at 90 ppm compared with both experimental controls and historical controls for the laboratory. However, there was no significant decrease in erythrocyte cholinesterase activity, which might be more clinically relevant for neurotransmitter signalling.

Synopsis

[The Working Group noted that exposure to acrylonitrile may influence hormone levels in humans and in rodents in experimental systems. The studies in humans were restricted in methodology to case–control designs and had limited control for confounders, but two studies showed increases in levels of TSH. Corticosterone was reported to be reduced in two independent studies in Sprague-Dawley rats.]

4.2.8 Causes immortalization

(a) Humans

(i) Exposed humans

Alterations in biomarkers of cell-cycle regulation involved in cellular senescence, including p53 and pRB/$p16^{INK4a}$ (Reddel, 2000), can provide insight into the cellular mechanisms that may lead to escape from senescence, and potentially to immortalization (Smith et al., 2020). In a small cross-sectional study, plasma protein levels of p53 and $p21^{WAF1}$ (a downstream mediator of p53-induced cell-cycle arrest) were measured in samples from workers at a petrochemical facility with reported exposure to acrylonitrile ranging from 0.05 to 0.3 mg/m³ (n = 49) and from workers at a nearby building company in the same region (n = 24) with no measured exposure to acrylonitrile. No significant differences in p53 and $p21^{WAF1}$ expression were found, and no correlations in plasma levels of p53 and $p21^{WAF1}$ protein (measured by ELISA assay) were detected (Rössner et al., 2002) [The Working Group noted that age and sex were the only covariates controlled in the predominantly male cohort.] [The Working Group noted that genetic polymorphisms in GST (*GSTM1* and *GSTT1*) and epoxide (epoxide hydrolase, *EPHX1*, *-2*, *-3*, and *-4*) metabolic enzymes were also evaluated. No significant effects on p53 or $p21^{WAF1}$ expression related to the *GSTM1* or *GSTT1* genotype were found in either group. The *EPHX* genotype had no effect on p53 or $p21^{WAF1}$ expression in the acrylonitrile-exposed group, although the EPHX high-activity genotype had significantly higher expression of $p21^{WAF1}$ when compared with other EPHX genotypes in the control group. The expression of p53 was significantly lower in smokers than in non-smokers among acrylonitrile-exposed workers (Rössner et al., 2002).]

[The Working Group noted that this study had several limitations, including the small sample size given common variance in p53, lack of methodological details regarding the reported exposure of the acrylonitrile-exposed workers, lack of exposure assessment in the controls, and the higher prevalence of smoking in the exposed workers than in the unexposed. Nondifferential exposure misclassification was expected. The study was of limited informativeness.]

(ii) Human cells in vitro

See Table 4.22.

In the study in acrylonitrile-exposed workers described above, Rössner et al. (2002) additionally evaluated the effect of acrylonitrile exposure in cultured embryonic lung fibroblasts in vitro and reported that acrylonitrile at concentrations of 0.30–1 mM increased protein expression of both p53 and $p21^{WAF1}$, and that concentrations of the two proteins were strongly correlated (r = 0.87, P = *0.006*) in cells treated with acrylonitrile at 0.05–1 mM, unlike the results reported for exposed workers. In human primary cord blood cells exposed to acrylonitrile at 0.8 and

Table 4.22 End-points relevant to immortalization in human cells in vitro exposed to acrylonitrile

End-point	Assay	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
p53 and $p21^{WAF1}$ protein expression	ELISA and western blot	Cultured embryonic lung fibroblasts	↑; significant correlation between p53 and $p21^{WAF1}$ protein levels ($r = 0.87$, $P = 0.006$)	0.30 mM, 24 h	Effect at subcytotoxic doses.	Rössner et al. (2002)
p53 and p21 mRNA expression	Semiquantitative RT-PCR	Primary cord blood cells	↑	0.8 mM, 15 h	Only assessed by semiquantitative RT-PCR.	Diodovich et al. (2005)
p53 and p21 protein expression	Western blot	Human choriocarcinoma cancer cells, JEG-3 and BeWo	↑	0.00001 mM, 48 h	D-R effect, uncertainty regarding doses evaluated.	Kim and Choi (2020)
Anchorage-independent growth	Morphological evaluation in soft agar	Cultured neonatal foreskin fibroblasts	+	0.1 µg/mL, for 24 h, then 10–14 d unexposed culture	Effect at subcytotoxic doses; panel evaluated; small magnitude, no D-R.	Kurian et al. (1990)

d, day(s); D-R, dose–response relation; ELISA, enzyme-linked immunosorbent assay; h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; mRNA, messenger RNA, RT-PCR, reverse transcriptase-polymerase chain reaction.

[a] +, positive; ↑, increase.

3.2 mM for 6, 24, or 48 hours, upregulation of mRNA levels of the cell-cycle arrest genes p53 and $p21^{WAF1}$ (measured by semiquantitative PCR) was described (Diodovich et al., 2005). [The Working Group noted that the study did not include loading controls, and that levels of p53 and $p21^{WAF1}$ were evaluated semiquantitatively.] Protein expression of p53 and $p21^{WAF1}$ in JEG-3 and BeWo human choriocarcinoma cancer cell lines increased after exposure to acrylonitrile at ≥ 10 nM for 24 hours (Kim and Choi, 2020). [The Working Group noted that this study had several limitations, including that the doses used appeared to be very low in comparison with those in other studies evaluating the impact on cell-cycle regulation after exposure to acrylonitrile.]

In a separate study, cultured neonatal foreskin fibroblasts exposed to acrylonitrile at concentrations of ≥ 0.1 μg/mL exhibited an increased capacity for growth on soft agar without excess cytotoxicity, which is an early indication of anchorage-independent growth and potential transformation in human fibroblasts; however, the magnitude of the change was small, and no dose–response relation was apparent (Kurian et al., 1990).

(b) Experimental systems

See Table 4.23.

[The Working Group considered that the capacity for escape from various mechanisms of proliferation restriction, both internal and external, is one component of functional immortalization (Reddel, 2000). Indeed, assays for cellular transformation in vitro were designed in part to provide insight into neoplastic potential by evaluating the effect of external controls on proliferation in vitro in order to predict potential activity in experimental systems in vivo (OECD, 2007).]

In four studies, morphological transformation was assessed using the Syrian hamster embryo cell transformation assay (i.e. assessing microscopic changes in colony growth morphology, reviewed in OECD, 2015; LeBoeuf et al., 1999); positive results were reported after 6–7 days of exposure at subcytotoxic concentrations ranging from 0.01 to 50 μg/mL (Parent and Casto, 1979; Barrett and Lamb, 1985; Sanner and Rivedal, 1985; Zhang et al., 2000), as well as 8- and 9-fold induction of SA7 viral foci formation at 100 and 200 μg/mL, respectively, when acrylonitrile treatment was started 5 hours after viral infection (Parent and Casto, 1979). Notably, Zhang et al. (2000) reported that 1 day of acrylonitrile treatment yielded negative results, whereas 7 days induced morphological transformation that could be inhibited by co-incubation with the antioxidants α-tocopherol (50–150 μM) or epigallocathechin-3 gallate (EGCG) (5 μM).

Morphological transformation was also assessed in experiments in mouse C3H 10T1/2, NIH/3T3, or BALB/c 3T3 clone 1–13 fibroblasts; positive results were reported with acrylonitrile at subcytotoxic concentrations ranging from 6.3 to 16 μg/mL (Lawrence and McGregor, 1985; Matthews et al., 1985; Banerjee and Segal, 1986). [The Working Group noted that only two investigators assessed the requirement for metabolic activation by either S9 fraction (Lawrence and McGregor, 1985) or co-culture with irradiated rat liver cells induced by 12-*O*-tetradecenoylphorbol-13-acetate (TPA) (Matthews et al., 1985); although in both studies the presence of metabolic activation was required for positive results with acrylonitrile treatment, it was not clear whether this was a consistent and reproducible observation across murine fibroblasts.]

Transformed cloned C3H 10T1/2 and NIH/3T3 fibroblasts exposed to acrylonitrile were able to grow when isolated and transplanted into soft agar, unlike unexposed control fibroblasts (Banerjee and Segal, 1986). Matthews et al. (1985) also reported that exposure to acrylonitrile at concentrations starting at 0.76 mM (about 40 μg/mL) could induce the formation of BALB/c 3T3 fibroblast colonies that were resistant to subsequent exposure to ouabain (2 mM); this

Table 4.23 End-points relevant to immortalization in non-human mammalian systems exposed to acrylonitrile

End-point	Assay	Species, strain (sex) or cell line	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
In vitro								
Morphological transformation	SHE transformation in vitro	Hamster, SHE	Embryo	+	0.01 µg/mL	7 d exposure	Effect at subcytotoxic[b] doses, panel evaluated; purity, NR, no D-R.	Barrett and Lamb (1985)
Morphological transformation	SHE transformation in vitro	Hamster, SHE	Embryo	+	2 µg/mL	6 d exposure	Effect at subcytotoxic[b] doses, panel evaluated; purity, NR, no D-R.	Sanner and Rivedal (1985)
Morphological transformation	SHE transformation in vitro	Hamster, SHE	Embryo	+	50 µg/mL	6 d exposure	Effect at subcytotoxic[b] doses, positive control; no D-R.	Parent and Casto (1979)
Morphological transformation	SHE transformation in vitro	Hamster, SHE	Embryo	+ Transformation inhibited by co-incubation with α-tocopherol or EGCG	50 µg/mL	7 d exposure	Effect at subcytotoxic[b] doses, D-R, positive control, B[*a*]P; impact of antioxidants on positive control was not evaluated.	Zhang et al. (2000)
				–	75 µg/mL	1 d exposure, 6 d unexposed culture	Positive control, B[*a*]P.	Zhang et al. (2000)
Morphological transformation	Evaluation of type II or III foci formation in vitro	Mouse, C3H 10T1/2	Fibroblast	+	6.3 µg/mL	2 d exposure, 42 d unexposed culture	Effect at subcytotoxic[b] doses; inverse D-R with increasing toxicity.	Banerjee and Segal (1986)
Morphological transformation	Evaluation of basophilic, piled, and invasive foci in vitro	Mouse, NIH/3T3	Fibroblast	+	12.5 µg/mL	2 d exposure, 21 d unexposed culture	Effect at subcytotoxic doses, D-R.	Banerjee and Segal (1986)
Morphological transformation	Evaluation of type II or III foci formation in vitro	Mouse, C3H 10T1/2	Fibroblast	(+) with S9	16 µg/mL	1 d exposure, 56 d unexposed culture	Panel evaluated; cytotoxicity data, NR; no clearly positive results for positive controls with S9.	Lawrence and McGregor (1985)
				– without S9	40 µg/mL			

Table 4.23 (continued)

End-point	Assay	Species, strain (sex) or cell line	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Morphological transformation	Evaluation of colony transformation in vitro	Mouse, BALB/c 3T3, clone 1-13	Fibroblast	+ with RLC	0.13 mM (6.8 µg/mL)	3 d exposure, 24 d unexposed culture	Effect at subcytotoxic[b] doses, panel evaluated; purity, NR; no D-R; TPA added with RLC.	Matthews et al. (1985)
				– without RLC	0.28 mM			
	Ouabain-resistant colony formation in vitro			+ with S9	0.76 mM (40.3 µg/mL)	4-h exposure, 5–6 d unexposed culture, 15 d exposure to 2 mM ouabain	Effect at subcytotoxic[b] doses, panel evaluated; purity NR, no D-R.	
				NR without S9	NR			
Anchorage-independent growth	Morphological evaluation in soft agar, in vitro	Mouse, C3H 10T1/2	Fibroblast	+	NR	7–14 d after isolation of transformed foci and serial passaging	Unexposed (control) cells did not survive culture.	Banerjee and Segal (1986)
		Mouse NIH/3T3	Fibroblast	+	NR			
Induction of viral transformation	Simian adenovirus (SA7)-transformed foci formation	Hamster, SHE	Embryo	(+)	100 µg/mL	5 h after SA7 infection 18 h before SA7 infection	Effect at cytotoxic doses, unclear exposure durations for timing groups, no D-R.	Parent and Casto (1979)
				+/–	25 or 100 µg/mL			

B[*a*]P, benzo[*a*]pyrene; d, day(s); D-R, dose–response relation; EGCG, epigallocatechin-3 gallate; h, hour(s); HID, highest ineffective dose; LED, lowest effective dose; NR, not reported; RLC, co-culture with irradiated but metabolically functional liver cells from male F344 rats; S9, 9000 × *g* supernatant; SHE, Syrian hamster embryo; TPA, 12-*O*-tetradecenoylphorbol-13-acetate.

[a] ↓, decrease; ↑, increase; +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative in a study of limited quality.

[b] The mention "subcytotoxic doses" indicates that results were observed in conditions of < 50% cytotoxicity, considered to be the acceptable limit for transformation assays.

was hypothesized to arise from a mutation in the gene controlling the synthesis of cell membrane Na^+/K^+ adenosine triphosphatase.

Synopsis

[Overall, acrylonitrile induced anchorage-independent growth in human neonatal foreskin fibroblasts in one study and increased biomarkers of senescence in human cells in three other studies in vitro. There is additional evidence from several studies reporting morphological transformation in Syrian hamster embryo cells, as well as morphological transformation and anchorage-independent growth in murine fibroblasts.]

4.2.9 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

(i) Exposed humans

See Table 4.24.

In an occupational study (also described in previous Section 4.2.8), Rössner et al. (2002) measured p53 and $p21^{WAF1}$ protein levels in blood plasma samples from acrylonitrile-exposed workers in the petrochemical industry and unexposed controls; no significant differences in levels of p53 or $p21^{WAF1}$ were found. [The Working Group noted that this study was limited in sample size, that the exposure assessment was limited to area samples and protein levels at a single point in time, and that there was a lack of confounder adjustment or matching.]

In a cross-sectional epidemiological study of 603 participants conducted by Riggs et al. (2022) the CEMA (also known as CYMA) metabolite of acrylonitrile was inversely associated with circulating subtypes of angiogenic cells measured in the peripheral blood ($CD45^{dim}/CD146^+/CD34^+/AC133^+$ cells and $CD45^+/CD146^+/AC133^+$). The results of this study suggested that exposure to acrylonitrile suppresses several subtypes of angiogenic cells. These cells are important because they assist in the repair of the endothelium after injury and are hypothesized to be a good biomarker of vascular function (Hill et al., 2003). [The Working Group noted that the comparisons were cross-sectional, using a metabolite of acrylonitrile.]

(ii) Human cells in vitro

See Table 4.25.

In the study (Rössner et al., 2002) described above and in Section 4.2.8, the protein expression of both p53 and $p21^{WAF1}$, measured in cultured embryonic lung fibroblasts after treatment for 24 hours with acrylonitrile at concentrations ranging from 0.3 to 1 mM, increased in parallel and was strongly correlated between the two proteins.

In a study by Diodovich et al. (2005), human cord blood cells were exposed to acrylonitrile at 0.8 and 3.2 mM for 6, 24, or 48 hours to assess apoptosis, cell proliferation, and the expression of cell cycle-related proteins and selected cytokines/receptor genes and oncogenes. Of these cells, a small fraction are capable of self-renewal and differentiation into committed progenitors of the different myeloid and lymphoid compartments. After acrylonitrile treatment, significant inhibition of colony-forming units and no changes in apoptosis by flow cytometry were reported. Downregulation of the anti-apoptosis gene Bcl2 and activation of p53, $p21^{WAF1}$, and Bax genes, measured by semiquantitative PCR or western blot, were described. [The Working Group noted that this study showed that acrylonitrile can interfere with bone marrow cell differentiation. However, the study had some limitations, including that the experimental paradigm did not include loading controls and that p53 and $p21^{WAF1}$ were evaluated semiquantitatively; thus, the results for apoptosis are equivocal.]

In an in vitro model, Kim et al. (2018) tested acrylonitrile in a human endometrial adenocarcinoma cell line. Treatment with acrylonitrile

Table 4.24 End-points relevant to altered cell proliferation, cell death, or nutrient supply in humans exposed to acrylonitrile

End-point	Assay	Biosample type	Location Setting and study design	Exposure level No. of study participants	Response[a] (significance)	Covariates controlled	Comments	Reference
p53 and $p21^{WAF1}$ protein levels	ELISA assay	Blood (plasma)	Czechia Case–control study	49 workers exposed at 0.05–0.3 mg/m^3 and 24 unexposed controls	No change	Smoking	Small sample size and only testing exposed vs unexposed not controlling for other covariates. No details were reported on plant operations, duration, latency, dermal exposures or presence of other occupational carcinogens. Measurements results were provided but were area samples.	Rössner et al. (2002)
15 angiogenic cells	Flow cytometry	Blood	USA, Louisville, Kentucky Cross-sectional study	603 participants and 0 controls	↓ $CD45^{dim}$/ CD146+/ CD34+/ AC133+ cells and CD45+/ CD146+/ AC133 cells	Smoking	Cross-sectional study but relatively large sample size and urinary measures of exposure of CEMA (AN metabolite).	Riggs et al. (2022)

AN, acrylonitrile; CD, cluster of differentiation; CEMA, *N*-acetyl-*S*-(2-cyanoethyl)cysteine; ELISA, enzyme-linked immunosorbent assay; USA, United States of America.
[a] ↓, decrease.

Table 4.25 End-points relevant to altered cell proliferation, cell death, or nutrient supply in human cells in vitro exposed to acrylonitrile

End-points	Assay	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
p53 and $p21^{WAF1}$ protein expression	ELISA and western blot	Cultured embryonic lung fibroblasts	↑; significant correlation between p53 and $p21^{WAF1}$ protein levels ($r = 0.87$, $P = 0.006$)	0.30 mM, 24 h	Effect at doses below cytotoxicity (that is, 0.5 mM).	Rössner et al. (2002)
Granulocyte–macrophage colony-forming unit	Microscope examination and counting	Primary cord blood cells	Inhibited growth	0.8 mM		Diodovich et al. (2005)
Colony-forming unit-erythroid	Microscope examination and counting		Inhibited growth	3.2 mM		
Apoptosis	Flow cytometry		No changes	0.8 mM for 6, 24 and 48 h		
p53 and $p21^{WAF1}$ expression	Semiquantitative RT-PCR		↑	0.8 mM, 15 h	Only assessed by semiquantitative RT-PCR.	
Bcl2	Western blot		↓ at 48 h	0.8 mM for 6, 24 and 48 h	No loading control.	
Bax			↑ at all time points			
Cyclin D	Western blot	Endometrial adenocarcinoma cell	↑ at 10^{-11} M	10^{-11} M and 10^{-9} M for 48 h	Dose-dependent effects were reported.	Kim et al. (2018)
Cyclin E			↑ at 10^{-9} M			
$p21^{WAF1}$ and p27			↓	10^{-11} M and 10^{-9} M for 48 h	Dose-dependent effects were reported.	
Cyclin D	Western blot	Choriocarcinoma cancer cells, JEG-3 and BeWo	↓	10^{-8} M (BeWo) and 10^{-6} M (JEG-3) for 48 h	Dose-dependent effects were reported for all end-points listed.	Kim and Choi (2020)
Cyclin E			↓	10^{-8} M (JEG-3 and BeWo) for 48 h		
$p21^{WAF1}$ and p27			↑	10^{-8} M (JEG-3 and BeWo) for 48 h		
p53, Bax, caspase-3			↑	10^{-8} M (JEG-3 and BeWo) for 48 h, except for p53 at 10^{-6} M in JEG-3		

Table 4.25 (continued)

End-points	Assay	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Bcl-xl			↓	10^{-8} M (JEG-3 and BeWo) for 48 h		Kim and Choi (2020) (cont.)
Apoptosis	TUNEL assay		↑	10^{-8} M (BeWo) and 10^{-6} M (JEG-3) for 48 h		
Cell viability	EZ-cytox cell viability		↓	10^{-9} M (BeWo) and 10^{-8} M (JEG-3) for 6 d		

d, day(s); ELISA, enzyme-linked immunosorbent assay; h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; RT-PCR, reverse transcriptase-polymerase chain reaction; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling.

[a] ↑, increase; ↓, decrease.

for 6 days at a concentration ranging from 10^{-11} to 10^{-6} M increased cell viability. At 10^{-11} M and 10^{-9} M, acrylonitrile changed the gene expression of cell cycle-related genes (increases in cyclin D and cyclin E, and decreases in $p21^{WAF1}$ and p27) in a dose-dependent fashion; both concentrations (10^{-11} M and 10^{-9} M) also increased the migration/invasion capacity of these cells compared with that of the negative control vehicle, DMSO, and this was accompanied by stimulation of the epithelial–mesenchymal transition process. [The Working Group noted that the concentrations used were extremely low and that the methodology and data reporting might not be reliable.]

In another study, Kim and Choi (2020) assessed the cytotoxic effects of acrylonitrile and reported suppression of cell viability and increases in apoptosis in JEG-3 and BeWo human choriocarcinoma cancer cell lines. This was linked to increasing expression of cell-cycle arrest genes, p53 and $p21^{WAF1}$. Apoptosis was identified by increased expression of proteins related to ROS-mediated endoplasmic reticulum stress. A dose-dependent effect was observed for cell viability with acrylonitrile in the range of 10^{-9} to 10^{-6}M for 6 days. [The Working Group considered the measurement of cell cycle-related proteins in choriocarcinoma cells to be difficult to interpret.]

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.26.

Multiple studies have investigated the effect of acrylonitrile on cell proliferation, death, and/or nutrient supply in vivo. Szabo et al. (1984) investigated the subacute and chronic effects of ingested acrylonitrile on the adrenal glands, stomach, and duodenum in Sprague-Dawley rats. Daily doses caused adrenal enlargement and hyperplasia of the zona fasciculata of the adrenal cortex. Acrylonitrile-related changes in the stomach were also documented, including hyperplasia and elevated levels of non-protein sulfhydryls, which resembled preneoplastic alterations. In another study (Van de Zande et al., 1986), a single dose of acrylonitrile at 4 mg/kg bw injected intraperitoneally in male Wistar-derived rats caused an increase in activity of hepatic ornithine decarboxylase (ODC), a key enzyme involved in cell growth, differentiation, and tumour development in the liver.

In a study investigating covalent interactions with pulmonary DNA, Ahmed et al. (1992a) (also described in Section 4.2.3) exposed Sprague-Dawley rats to acrylonitrile as a single dose at 46.5 mg/kg bw by gavage. The lungs of treated animals showed moderate to marked hyperplasia of club cells (previously referred to as "Clara" cells) in the bronchioles. Ghanayem et al. (1997) exposed male F344 rats to acrylonitrile at doses ranging from 0.05 to 0.15 mg/kg bw and reported a dose-dependent increase in epithelial cell proliferation and an increase in the forestomach squamous mucosa (hyperplasia) and thickness. Didenko at al. (1999) administered a single dose of acrylonitrile at 0.05–0.15 mg/g bw via the femoral vein to rats that were then euthanized after 8 or 16 hours. Qualitative increases in DNA damage in the adrenal cortex as well as increases in $p21^{WAF1}$ mRNA and apoptosis were qualitatively reported. The NTP conducted a large, comprehensive study of the toxicological profile of acrylonitrile in 2001 (NTP, 2001) in mice exposed orally at several doses for 14 weeks or 2 years (as described in Section 3.1). The authors of this study concluded that there was clear evidence of carcinogenic activity of acrylonitrile in $B6C3F_1$ mice in the 2-year exposure groups. However, non-neoplastic lesions, such as epithelial hyperplasia of the forestomach and Harderian gland in males and of the forestomach in females, occurred in mice exposed to acrylonitrile for 2 years. In addition, after 14 weeks of dosing of acrylonitrile at 40 mg/kg bw per day, hyperplasia increased significantly in the forestomach of female mice. [The Working Group

Table 4.26 End-points relevant to alterations in cell proliferation, cell death, or nutrient supply in non-human mammalian systems in vivo exposed to acrylonitrile

End-point	Assay	Species, strain (sex) or cell line	Tissue	Results[a]	Dose	Route, duration, dosing regimen	Comments	Reference
Hyperplasia	Histology	Rat, Sprague-Dawley (F)	Adrenal gland	+	0.05% (500 ppm) or 0.2% (2000 ppm)	Drinking-water, 3 wk		Szabo et al. (1984)
			Adrenal gland	+	1, 20, 100, 500, and 2000 ppm	Drinking-water or gavage, 60 d		
			Stomach mucosa	+	100 or 500 ppm	Drinking-water for 21 d or 60 d		
Hepatic ODC	Radioassay using a scintillation counter	Rat, Wistar (M)	Liver	↑	4 mg/kg bw	i.p., single injection		Van de Zande et al. (1986)
Hyperplasia	Histopathological examination	Rat, Sprague-Dawley (M)	Lung (club cells)	+	46.5 mg/kg bw after 24 h	Gavage, single dose		Ahmed et al. (1992a)
Epithelial cell proliferation	BrdU IHC	Rat, F344 (M)	Forestomach	↑ thickness ↑ hyperplasia	0.43 mmol/kg bw per day (thickness) 0.22 mmol/kg bw per day (hyperplasia)	Gavage daily for 6 wk		Ghanayem et al. (1997)
$p21^{WAF1}$ expression and apoptosis	$p21^{WAF1}$ by northern blot; apoptosis by microscopy (ligation-mediated labelling of cells with DSBs)	Rat, Sprague-Dawley (M)	Adrenal tissue	↑ $p21^{WAF1}$ mRNA and apoptosis	0.05–0.15 mg/kg bw	Femoral vein injection, single dose		Didenko at al. (1999)

Table 4.26 (continued)

End-point	Assay	Species, strain (sex) or cell line	Tissue	Results[a]	Dose	Route, duration, dosing regimen	Comments	Reference
Hyperplasia	Histopathology	Mouse, $B6C3F_{11}$ (M and F)	Forestomach	+	20 mg/kg bw per day (F) 10 mg/kg bw per day (M)	Gavage daily 5 d/wk for 2 yr	Dose-related increases in the incidence of non-neoplastic forestomach lesions, including focal epithelial hyperplasia; these increases were statistically significant at 20 mg/kg bw (M and F)	NTP, 2001
			Harderian gland	+	10 mg/kg bw per day in males			
		Mouse, $B6C3F_{11}$ (F)		+	40 mg/kg bw per day	5 d/wk for 14 wk		
Hyperplasia	Histopathology	Rat, Sprague-Dawley (M and F)	Forestomach	+	35 ppm (F) 100 ppm (M)	Drinking-water, for 2 yr	Hyperplasia at 35, 100, and 300 ppm (F), and at 100 and 300 ppm (M).	Quast (2002)
Focal gliosis			Brain	+	35 ppm (F only)	+ also at 100 ppm (F only)	Perivascular cuffing.	

Table 4.26 (continued)

End-point	Assay	Species, strain (sex) or cell line	Tissue	Results[a]	Dose	Route, duration, dosing regimen	Comments	Reference
Hyperplasia	Histopathology	Rat, Spartan Sprague-Dawley (M and F)	Kidney	↑ at 12 mo (killed) at 10 mg/kg or 100 ppm (F) ↑ at 18 mo at 100 ppm (M)	0.1 or 10 mg/kg bw per day by intubation 1 or 100 ppm in drinking-water	Daily intubation for 7 d/wk, 0.1 or 10 mg/kg bw per day Continuously via drinking-water until euthanasia, 1 or 100 ppm Animals were killed at 6, 12, 18 mo in both studies. Study termination at 20 mo for intubated animals, and at 22 mo, or 19 mo for surviving males and females, respectively, in the drinking-water study		Johannsen and Levinskas (2002b)
			Forestomach	↑ incidence and/or severity of squamous cell hyperplasia at 10 mg/kg bw and 100 ppm in AN-treated rats			Squamous cell hyperplasia of the epithelium of the forestomach was seen both in treated and control animals. Forestomach hyperplasia was more pronounced and correlated with a much higher incidence of forestomach tumours (see Section 3) and was identified earlier (12 mo) in the intubation study.	
Hyperplasia	Histopathology	Rat, F344 (M and F)	Forestomach	↑ squamous cell hyperplasia at 3, 10, and 30 ppm	3 ppm	Drinking-water, 1, 3, 10, 30 or 100 ppm Study termination (F, ≥ 23 mo; M, 26 mo)		Johannsen and Levinskas (2002a)
Apoptotic changes	TUNEL fluorescence staining with cell counting	Rat, clean-grade Sprague-Dawley (M and F)	Brain (nerve cells)	↓	10 mg/kg bw per day	Gavage daily, 10 and 20 mg/kg bw per day, 5 d/wk for 13 wk	Dose–response effect.	Gu et al. (2021)

Table 4.26 (continued)

End-point	Assay	Species, strain (sex) or cell line	Tissue	Results[a]	Dose	Route, duration, dosing regimen	Comments	Reference
Apoptosis	TUNEL fluorescence staining with cell counting	Rat, Sprague-Dawley (M)	Testis	↑	46 mg/kg bw per day	Gastric administration 6 d/wk for 4 wk		Shi et al. (2021)

AN, acrylonitrile; BrdU, bromodeoxyuridine; bw, body weight; d, day(s); DSBs, DNA double-strand breaks; F, female; F344, Fischer 344; h, hour(s); IHC, immunohistochemistry; i.p., intraperitoneal; M, male; mo, month(s); ODC, ornithine decarboxylase; ppm, parts per million; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling; wk, week(s); yr, year(s).

[a] ↑, increase; ↓, decrease; +, positive;.

noted that chronic exposure (2 years) to acrylonitrile induced the formation of non-neoplastic lesions in male and female B6C3F$_1$ mice.]

Quast (2002) exposed 48 male and 48 female rats to acrylonitrile for 2 years (see Section 3.2). Multiple non-neoplastic and neoplastic lesions were found at an increased and/or decreased rate in several tissues in both sexes at all treatment levels. A significant increase in the incidence of hyperplasia and hyperkeratosis of the forestomach was reported across all doses in females, and at the two highest doses in male rats. A significant increase in the incidence of focal gliosis and perivascular cuffing in the brain of female rats was observed at doses of 35 and 100 ppm. In a separate study of long-term toxicity, Johannsen and Levinskas (2002b) exposed 200 rats to acrylonitrile at a dose of 0.1 or 10 mg/kg bw per day via intubation or 1 or 100 ppm via drinking-water for their lifetime. Acrylonitrile, via both routes of exposure, increased the incidence of palpable masses of the head and the non-glandular stomach and of the mammary region in female rats. An increase in the incidence of renal transitional cell hyperplasia was observed after 12 months in females at the higher dose and after 18 months in males at the higher dose administered via intubation. In addition, acrylonitrile at 10 mg/kg bw per day increased the severity of squamous cell hyperplasia of the forestomach, irrespective of route of exposure. [The Working Group noted that an increase in the incidence of forestomach tumours was reported in rats (see Section 3.2).]

In another study, Johannsen and Levinskas (2002b) exposed F344 rats via drinking-water for 2 years; they observed an increase in the incidence of hyperplasia or hyperkeratosis in squamous cells of the forestomach in both males and females at a dose of 3 ppm and higher (see Section 3.2).

Gu et al. (2021) exposed rats to acrylonitrile at several doses (0, 5, 10, and 20 mg/kg bw per day) by gavage for 13 weeks to study effects on apoptosis in brain cells. Rats exposed to acrylonitrile at 10 and 20 mg/kg bw per day exhibited a lower rate of apoptosis and a lower number of apoptotic cells than did rats in the control group. [The Working Group noted that the findings suggested that acrylonitrile has anti-apoptotic effects in cerebral cells.]

Shi et al. (2021) investigated the potential of apigenin, a natural flavone, to mitigate acrylonitrile-induced apoptosis in sperm and testis in male Sprague-Dawley rats. The results showed that apigenin reduced the testicular apoptosis induced by acrylonitrile. Apigenin mitigated oxidative stress and inhibited mitochondria-mediated testicular apoptosis associated with acrylonitrile exposure (see Section 4.2.4). [The Working Group noted that, in contrast to those in the central nervous systems, the findings indicated that acrylonitrile has the potential to induce oxidative stress and apoptosis in the testis, and also that the effects could be reversed by treatment with flavone.]

(ii) Non-human mammalian systems in vitro

Poirier et al. (2002) reported inhibition of T-lymphocyte and B-lymphocyte cell proliferation in vitro without any effect on cell viability after treatment with acrylonitrile. Kamendulis et al. (1999b) observed a dose-dependent inhibition of gap junctional intercellular communication in rat astrocytes but not in cultured hepatocytes exposed to acrylonitrile. The effect was reversed upon removal of acrylonitrile or treatment with antioxidants such as vitamin E, highlighting the role of ROS in the toxic effect (see Section 4.2.4).

Synopsis

[There was a limited number of studies reporting effects of acrylonitrile on cell proliferation or apoptosis in exposed humans. An increase in the incidence of hyperplasia with acrylonitrile at several concentrations was observed in experimental systems. In rodents, exposure to

acrylonitrile for up to 2 years induced hyperplasia, mostly in the forestomach of mice and rats and consistently in the two species.]

4.3 Evaluation of high-throughput in vitro toxicity screening data

The analysis of the in vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 136 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). Acrylonitrile was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2022).

The results of ToxCast/Tox21 high-throughput screening are presented based on the assays that have been mapped to the key characteristics (Reisfeld et al., 2022). The detailed results are available in the supplementary information for the present volume (Annex 4, Supplementary material for Section 4.3, Evaluation of high-throughput in vitro toxicity screening data, online only, available from: https://publications.iarc.who.int/646). Here, for brevity, assays for which there was a positive "hit call" for acrylonitrile are referred to as "active" assays.

There was only one active assay (without any caution flags) for acrylonitrile, namely, ATG_ERE_CIS_up, a cell-based, multiplexed-readout assay in HepG2 (a human liver cell line), with measurements taken at 24 hours after chemical dosing in a 24-well plate, and this assay mapped to key characteristic 8, "modulates receptor-mediated effects". Overall, this one active result was considered uninformative, given the large number of assays that were inactive. In summary, there was a lack of evidence to support multiple characteristics for acrylonitrile.

4.4 Other relevant evidence

Recent efforts to understand the health effects of population exposures to ambient or occupational mixtures of VOCs have involved the assessment of panels of urinary VOC metabolites including at least one potential metabolite of acrylonitrile. In independent evaluations of US population exposure data sets collected as part of the National Health and Nutrition Examination Survey (NHANES), one urinary metabolite specific to acrylonitrile (CEMA) was initially positively associated with measures of obesity, although this association disappeared in models fully adjusting for covariates (Lei et al., 2023). However, CEMA was significantly associated with changes in ALP (Wahlang et al., 2022) or in several serum biomarkers of liver injury (Liu et al., 2023), and although contributing to a slight but significant increase in the risk of non-alcoholic fatty liver disease, this comprised ≤ 1% of the total VOC effect in a mixtures analysis model (Liu et al., 2023). When urinary VOC metabolites in salon workers were assessed for a potential association with changes in the nasal microbiome, levels of *Staphylococcus* species were dissimilar in salon workers compared with office workers, and diversity levels differed between salon worker groups; this was significantly associated with a urinary biomarker common to both acrylonitrile and vinyl chloride exposure (HEMA) (Dalton et al., 2022).

Much of the information on general worker health in the context of chronic exposure to acrylonitrile comes from occupational studies in workers in China and Japan (reviewed in Fritz and Luke, 2015). Increases in the occurrence of self-reported symptoms such as headache, poor memory, dizziness, and a choking feeling in the chest have been reported in workers exposed to acrylonitrile at area concentrations

of 0.4–30 mg/m³ (Kaneko and Omae, 1992; Muto et al., 1992; Chen et al., 2000; Dong et al., 2000; as reviewed in Fritz and Luke, 2015). When neurobehaviour was examined in depth using a WHO-recommended test battery in workers at an acrylic fibre plant compared with control factory workers not exposed to acrylonitrile, significant deficits in tests for negative mood (e.g. anger, confusion, depression, fatigue, and tension), attention and response speed, auditory memory, and motor steadiness were associated with acrylonitrile exposures of 0.2–2 mg/m³ (Rongzhu et al., 2005). Neurotoxicity in the form of decrements in peripheral nerve function has also been reported in male Sprague-Dawley rats after 6, 9, or 12 weeks of oral exposure (gavage) to acrylonitrile at 50 mg/kg day, and after 12–24 weeks of 6-hour inhalation exposures at > 25 ppm (54 mg/m³); no effects were observed after oral exposure to four other unsaturated aliphatic nitriles (Gagnaire et al., 1998). Allergic contact dermatitis was confirmed by patch testing in five employees (four laboratory technicians and one maintenance worker) with unquantified exposure at an acrylonitrile-producing plant, one of whom also experienced paraesthesia (Bakker et al., 1991), also reported in a laboratory technician at a synthetic fibre company (Chu and Sun, 2001). Skin sensitization was confirmed in the guinea-pig maximization test and there was a dose-dependent increase in positive reactions with acrylonitrile at concentrations of 0.2–1.0% (in water) (Bakker et al., 1991).

Adverse effects on reproduction have been noted in male and female workers exposed to acrylonitrile as well as in female spouses of male workers. In female workers and spouses, increases in birth defects, stillbirths, premature delivery, sterility, and pregnancy complications have been reported in association with acrylonitrile at work-area concentrations ranging from 0.9 to 93 mg/m³ (reviewed in Fritz and Luke, 2015). In a separate cross-sectional study comparing workers exposed to acrylonitrile at a Chinese chemical plant with controls from other industries, female workers exposed to acrylonitrile had higher rates of both premature birth and post-term delivery, as well as increased rates of pregnancy complications, including infertility, threatened miscarriage, spontaneous abortion, unspecified birth defects, hyperemesis gravidarum, and anaemia associated with average work-area concentrations of 16.4 mg/m³ (range, 0–152.9 mg/m³, with 93% of measurements > 2 mg/m³) (Xiao et al., 2001). Significantly higher rates of menstrual cycle irregularities were also reported in female workers exposed to acrylonitrile, including abnormal menstruation cycle and duration, increased blood volume, and increased occurrence of dysmenorrhoea (Xiao et al., 2001). [The Working Group noted several limitations in the reporting and conduct of the study by Xiao et al. (2001), including the use of a retrospective questionnaire covering an unknown period of time potentially preceding workplace exposures, lack of control for other confounding factors aside from age and history of abnormal reproduction, and lack of discussion of potential co-exposures in the acrylonitrile-plant workers or other potential differences in workplace exposures between the exposed and control workers.]

In an earlier cross-sectional study in 475 female workers exposed to acrylonitrile employed at a Chinese rubber factory, Wu et al. (1995) reported an increased incidence of pregnancy-associated pernicious vomiting and anaemia, preterm delivery, and birth defects, and a decrease in the incidence of low birth weights, compared with the 527 controls. All female workers were exposed for ≥ 1 year to acrylonitrile at concentrations described as generally exceeding 2 mg/m³, with annual averages of ≤ 92 mg/m³. Furthermore, logistic regression analysis of numerous covariates revealed that illness, drug use, and exposure to X-rays during pregnancy were associated with increased rates of preterm birth. [The Working Group noted several limitations in the reporting and conduct

of the study by Wu et al. (1995), including the use of a retrospective questionnaire covering an unknown period of time potentially preceding workplace exposures, lack of discussion of potential co-exposures in the acrylonitrile-plant workers or other potential differences in workplace conditions between the exposed and control workers, and lack of description of the method of statistical analysis or significance for outcome comparisons. Although numerous confounding factors were evaluated by logistic regression, consideration of socioeconomic differences or nutrition were not clearly included and were noted by the authors as possibly explanatory for the decreased incidence in low birth weights in the acrylonitrile-exposed workers. It was unclear whether the workers or work location(s) described in Wu et al. (1995) were similar to those reported by Xiao et al. (2001).]

In male workers from an acrylonitrile plant, decreased sperm count and increased sperm abnormalities were reported after < 3 years of exposure at 0.8 mg/m^3 (Xu et al., 2003). In an environmental epidemiological study investigating children born to mothers living within 25 km of an acrylonitrile-using factory in Hungary, the reported rate of undescended testis was nearly 10 times the expected rate, and it decreased with increasing distance from the factory (Czeizel et al., 1999). This preceded a technological change at the factory, which involved control of acrylonitrile exposure; after implementation of this change, no births of boys with undescended testis were recorded in this region. [The Working Group noted that the study by Xu et al. (2003) was likely to be affected by nondifferential exposure misclassification, and that Czeizel et al. (1999) employed an ecological study design that had various limitations; these considerations limited the informativeness of both studies.]

Measures of comparable reproductive or developmental end-points were not significantly increased in several multigenerational studies of reproductive toxicity in Sprague-Dawley rats treated with acrylonitrile via oral administration (drinking-water) at ≤ 500 ppm (approximately 37 mg/kg bw per day in males, 40 mg/kg bw per day in females; Friedman and Beliles, 2002); inhalation at ≤ 90 ppm (195 mg/m^3; Nemec et al., 2008); or inhalation ≤ 100 ppm (217 mg/m^3; Saillenfait et al., 1993). [The Working Group noted the decreased body-weight gain in dams and associated decreases in fetal body weights reported by Saillenfait et al. (1993).] In contrast, ovarian atrophy and cysts were reported in female mice after chronic exposures to acrylonitrile by oral administration (gavage) at ≤ 20 mg/kg bw per day (5 days per week for 2 years), whereas no effect on sperm count and motility was observed in male mice after subchronic oral administration (gavage) at 20 mg/kg bw per day for 14 weeks (NTP, 2001; Ghanayem et al., 2002), and no dose-related effect on seminiferous tubules was noted in male F344 rats after 2 years of oral (drinking-water) exposure at ≤ 8.4 mg/kg day (Johannsen and Levinskas, 2002a) (see Section 3.1). [The Working Group noted that the anogenital distance was increased in F_1 males exposed by inhalation to acrylonitrile at 45 ppm and 90 ppm, but not in F_2 males exposed to 45 ppm (90 ppm was not evaluated in F_2 males); owing to the lack of effect in the F_2 generation, and inability to determine any molecular mechanism, Nemec et al. (2008) concluded that this was not an effect of exposure to acrylonitrile.] Although effects on haematological markers were not consistently reported in experimental animals, small reductions, particularly in haemoglobin and haematocrit, were observed after ≤ 24 months of exposure in female F344 rats after oral (drinking-water) exposure (see Section 3.2), as well as in male Sprague-Dawley rats after oral (gavage or drinking-water) exposure to similar daily doses (i.e. approximately 10 mg/kg bw per day; Johannsen and Levinskas, 2002a, b).

Among 144 cases of acrylonitrile poisoning that occurred in industrial settings in China between 1977 and 1994, the majority (95%)

were associated with convulsion, as well as less frequent reports of variation in heart rate, dizziness, headache, feebleness, and chest tightness, after acute exposures to high concentrations of 40–560 mg/m^3 in approximately half of the cases for which reliable exposure estimates were available (Chen et al., 1999). [The Working Group noted that acute exposures at high concentrations may not have any clear relationship to carcinogenicity.]

Synopsis

[Overall, the Working Group noted that the urinary metabolite specific to acrylonitrile (CEMA) was associated with serum end-points for liver oxidative stress or injury in a few cross-sectional studies. Increases in self-reported symptoms were reported in several studies of workers exposed to acrylonitrile, and decrements in neurobehavioural outcomes were reported in one study. Acrylonitrile appears to be a skin sensitizer. Adverse effects on reproduction were noted in many studies among male acrylonitrile-exposed workers, but particularly in female workers, as well as female spouses of male workers, across a wide range of exposure conditions (i.e. 0.9–153 mg/m^3); similar effects were not observed in many studies in rodents.]

5. Summary of Data Reported

5.1 Exposure characterization

Acrylonitrile is a chemical with a high production volume. It is a liquid under standard conditions but evaporates easily into air. Acrylonitrile is produced mainly by petrochemical processes. Almost all acrylonitrile is used as a monomer to prepare polyacrylonitrile and copolymers such as acrylonitrile–butadiene–styrene or styrene–acrylonitrile. One major application of these polymers is for fibres for textiles, clothing, and carpets. Other major uses are for resins in the construction, automobile, marine, and aerospace industries, as well as for rubber and plastics in consumer products and food-contact materials.

The presence of acrylonitrile in ambient air and indoor environments is mainly attributed to anthropogenic sources such as vehicle exhaust emissions, industrial emissions, solid waste facilities, and tobacco smoke. There are few data on acrylonitrile in water, soil, food, and consumer products.

Occupational exposure to acrylonitrile mainly occurs in industries involved in the production of monomers, fibres, resins, and polymers. Workers can also be exposed to acrylonitrile in chemical production industries, downstream industries such as tyre production and plastic moulding, as well as in hospitals during electrosurgical procedures. The main pathways of exposure in occupational settings are inhalation and skin contact. Workers exposed to acrylonitrile, depending on the industry, might have co-exposure to aromatic hydrocarbons, alkenes, metals, and dyes.

The primary route of exposure for the general population is tobacco smoking, including exposure to secondhand smoke. Other potential sources of exposure include air pollution and emissions from three-dimensional (3D) printers. Both *S*-(2-cyanoethyl) mercapturic acid (CEMA, also referred to as CYMA) and 2-[2-(cyanoethyl) sulfanyl]acetic acid (CESA) are validated biomarkers to measure mid-term and recent acrylonitrile exposure.

5.2 Cancer in humans

Data on the association between acrylonitrile exposure and cancer come largely from cohorts of occupationally exposed workers. Exposure assessments for acrylonitrile varied markedly among studies, ranging from qualitative and semiquantitative to quantitative exposure characterization. This was a major consideration in

the evaluation of the evidence. The Working Group considered epidemiological studies on the association between acrylonitrile exposure and cancers of the respiratory system, digestive tract, genitourinary system, other solid cancers, and haematological malignancies.

The data of highest quality were available for lung cancer, with 14 studies in total. Studies with cumulative exposure characterization and with consideration for confounding by smoking behaviours and co-exposures were given more weight. The most informative results came from a large, high-quality retrospective cohort of eight facilities in the USA representing many of the major sectors of acrylonitrile-production operations (monomer, fibre, and resin). The results showed consistent evidence of a quantitative exposure–response relation for lung cancer mortality after accounting for chance and major sources of confounding and healthy-worker survivor bias. In a large, pooled case–control study conducted using occupational histories in seven European countries, an exposure–response relation was also observed between acrylonitrile exposure and lung cancer incidence after adjusting for confounding by tobacco and co-exposures.

Reports from several studies that included a comparison of incidence or mortality with that in external reference populations showed a few excesses among workers exposed to acrylonitrile. These results were likely to be subject to healthy-worker biases, which probably diminished the effect estimates for the relation between acrylonitrile and lung cancer. Further limitations in the assessment of exposure to acrylonitrile would be expected to lead to nondifferential exposure misclassification, which would also bias effect estimates towards the null. Despite these downward biases, excesses have been reported in four additional retrospective cohorts. Although these studies contributed to the literature reporting an association between acrylonitrile and lung cancer, the poor-quality exposure assessment and imprecise effect estimates further limited the conclusions that could be drawn from these studies.

Overall, the Working Group considered that the body of evidence supported a causal relation between acrylonitrile exposure and lung cancer because the results of high-quality studies supported a positive association that was deemed unlikely to be explained by chance, confounding, or bias.

Some additional credible associations were also observed between acrylonitrile exposure and bladder cancer. Six cohort studies were available on the mortality or incidence experience of workers with potential exposure to acrylonitrile. The estimates were imprecise but frequently elevated, although the level of detail on exposure was variable. For bladder cancer, the most compelling findings came from mortality data that included a positive exposure–response relation based on quantitative data; however, because of high survival rates for this cancer type, the evaluation of mortality provides incomplete information on the occurrence of disease. Furthermore, the available studies were imprecise, and bias, confounding, and chance could not be ruled out with reasonable confidence.

For other cancer types that have been evaluated, including mesothelioma, melanoma, lymphohaematopoietic malignancies, and cancers of the stomach, oesophagus, colon and rectum, pancreas, kidney and renal system, prostate, brain and central nervous system, and breast, the available studies lacked consistency, were of insufficient quality, or lacked statistical precision and thus did not permit a conclusion to be drawn about an association with acrylonitrile.

5.3 Cancer in experimental animals

Treatment with acrylonitrile caused an increase in the incidence of malignant neoplasms in both sexes of two species (mouse and rat) in multiple studies.

In a well-conducted study that complied with Good Laboratory Practice in male and female $B6C3F_1$ mice treated by oral administration (gavage), acrylonitrile caused forestomach squamous cell carcinoma in males and females.

In an oral administration (drinking-water) study in male and female Sprague-Dawley rats (Spartan substrain), acrylonitrile caused focal or multifocal glial cell tumour (astrocytoma) of the nervous system, carcinoma of the Zymbal gland, and squamous cell carcinoma of the forestomach in males and females; and carcinoma of the mammary gland and mucous cystadenocarcinoma without metastasis of the small intestine in females.

In another study in male and female Sprague-Dawley rats (Spartan substrain) treated by oral administration (drinking-water), acrylonitrile caused carcinoma of the Zymbal gland in males and females, and brain and spinal cord astrocytoma in females.

In a study in male and female Fischer 344 rats treated by oral administration (drinking-water), acrylonitrile caused brain astrocytoma in males and females, and spinal cord astrocytoma in males.

In a study in male and female Sprague-Dawley rats (Spartan substrain) treated by oral administration (gavage), acrylonitrile caused brain astrocytoma and carcinoma of the Zymbal gland in males and females, squamous cell carcinoma of the forestomach and intestinal adenocarcinoma in males, and carcinoma of the mammary gland in females.

In a study in male and female Sprague-Dawley rats (Spartan substrain) treated by inhalation (whole-body exposure), acrylonitrile caused focal only or multifocal only glial cell tumour (astrocytoma) of the nervous system (brain and spinal cord, combined) and carcinoma of the Zymbal gland in males and females, mucinous cystadenocarcinoma without metastasis of the small intestine in males, and carcinoma (all types) and adenocarcinoma of the mammary gland in females.

In a two-generation study in Sprague-Dawley rats treated by inhalation (whole-body exposure), acrylonitrile caused encephalic glioma (oligodendroglioma) and carcinoma of the Zymbal gland in a first experiment in the male offspring, and encephalic glioma (oligodendroglioma), extrahepatic angiosarcoma, malignant tumours and, more specifically, adenocarcinoma of the mammary gland in the female offspring. In a second (concurrent) experiment in the same study, acrylonitrile caused leukaemia (a variety of tumours of the haematopoietic and lymphoid tissues at different sites) in the female offspring.

In a three-generation study of reproductive toxicity (not specifically designed to evaluate the carcinogenicity of acrylonitrile) in male and female Charles River [CRL:CDBS CD (SD) BR] rats treated by oral administration (drinking-water), acrylonitrile caused astrocytoma of the brain or spinal cord in the female offspring.

5.4 Mechanistic evidence

Regarding the absorption, distribution, metabolism, and excretion of acrylonitrile, data were available from studies in exposed humans and experimental systems. Acrylonitrile is readily absorbed after inhalation and ingestion and through the skin to the bloodstream and is distributed to all major organs. The main portion of absorbed acrylonitrile is excreted in the form of metabolites in urine; however, excretion is not complete within 24 hours. Metabolism in humans is very similar to that observed in rats, the main pathways being metabolic activation to cyanoethyl oxide (CEO) predominantly catalysed by cytochrome P450 (CYP) 2E1 and conjugation with glutathione, eventually yielding CEMA, which is excreted in the urine. CEO, the key electrophilic metabolite of acrylonitrile, releases cyanide in the course of biotransformation and is detoxified via two metabolic pathways: (i) epoxide

hydrolase-catalysed hydrolysis; and (ii) glutathione conjugation, which leads to two major mercapturic acids, *S*-(1-cyano-2-hydroxyethyl)-mercapturic acid (CHEMA) and 2-hydroxyethylmercapturic acid (HEMA).

There is consistent and coherent evidence that acrylonitrile exhibits key characteristics of carcinogens.

Acrylonitrile is an electrophile or can be metabolically activated to an electrophile. Suggestive evidence comes from studies in exposed humans. Acrylonitrile forms adducts with haemoglobin and serum albumin that are consistently observed even at low exposure levels; however, no acrylonitrile–DNA adducts have been reported in exposed humans. Consistent and coherent evidence comes from studies in experimental systems. In multiple studies in rats exposed to radiolabelled acrylonitrile, binding to nucleic acids, haemoglobin, and multiple tissue proteins was reported. A single DNA adduct was detected, namely, *N*7-(2-oxoethyl)guanine (*N*7-OXE-Gua), at very low levels. In vitro, both acrylonitrile and CEO bind to proteins. Six deoxynucleoside adducts, including *N*7-OXE-Gua, were identified when DNA was treated with high concentrations of CEO in vitro; acrylonitrile adducts with nucleobases have also been identified only after exposure to acrylonitrile at extremely high concentrations for long reaction times. There is a paucity of data in human primary cells.

Acrylonitrile is genotoxic. There is suggestive evidence that acrylonitrile causes genotoxicity in exposed humans. In five studies assessing chromosome-level mutations in exposed humans across various occupational sectors, positive results were reported in the overall or subcomponent analysis of three of the four worker cohorts. Aneuploidy and DNA-strand breaks were also reported in a separate cross-sectional study of chemical plant workers. However, these studies were of limited informativeness. There is a paucity of data in human primary cells. There is consistent and coherent evidence that acrylonitrile induces genetic alterations including mutations in experimental systems ranging from bacteria to rodents. Furthermore, in three studies in human lymphoblastoid cell lines, positive results were reported for the induction of gene mutations. Acrylonitrile can cause DNA damage (observed in the comet assay) in rodents, both in vitro and in vivo. Typically, genotoxicity caused by acrylonitrile requires metabolic activation.

Acrylonitrile induces oxidative stress. There is a paucity of data in exposed humans and no data in human primary cells. Consistent and coherent evidence comes from studies in experimental systems. On the basis of these data, there is clear evidence from numerous studies in vivo that acrylonitrile induces oxidative damage to DNA, generation of ROS, increases in levels of antioxidant enzymes, and decreases in levels of glutathione. Similar effects occur in studies in non-human mammalian systems in vitro.

Acrylonitrile causes immortalization. There is a paucity of data in exposed humans and in human primary cells. Consistent and coherent evidence comes from studies in experimental systems. Evidence from human cells in vitro is limited to one study in which acrylonitrile-associated induction of anchorage-independent growth was reported in neonatal foreskin fibroblasts, and three studies reporting induction of p53 and $p21^{WAF1}$, which are biomarkers of senescence in other cell types. Positive evidence comes from non-human cells in vitro: morphological transformation was reported in Syrian hamster embryo cells, and morphological transformation and anchorage-independent growth in murine fibroblasts.

Acrylonitrile alters cell proliferation, cell death, or nutrient supply. There is a paucity of data in exposed humans and no data in human primary cells. Consistent and coherent evidence comes from studies in experimental systems. In studies in rodents, hyperplasia has been shown to be induced in multiple tissues with acrylonitrile at several doses. Hyperplasia and/or hyperkeratosis, mostly in the forestomach of mice and rats, were reported in several studies of exposure of up to 2 years.

There is suggestive evidence that acrylonitrile induces chronic inflammation in experimental systems. There is a paucity of data in exposed humans and no data in human primary cells. Active chronic inflammation in the forestomach was observed in female mice after 14 weeks of exposure. In other studies, increases in inflammatory markers were reported after 28 days of exposure.

There is suggestive evidence that acrylonitrile is immunosuppressive in exposed humans and in experimental systems. In three occupational studies in exposed humans, decreases in blood lymphocytes were observed in workers assumed to be exposed to acrylonitrile via inhalation; however, these studies were of limited informativeness because of co-exposures. There were no data in human primary cells. In experimental systems, animals were exposed subcutaneously, by gavage, or via drinking-water. Acrylonitrile had effects on total lymphocyte counts, immunoglobulins, phagocytic activity, and measures of both the humoral immune response and delayed-type hypersensitivity. A possible effect of acrylonitrile on neutrophils and their function has also been identified; however, in these studies a limited number of end-points was measured, and acrylonitrile was tested at high doses.

There is suggestive evidence that acrylonitrile modulates receptor-mediated effects in experimental systems. There is a paucity of data in exposed humans and no data in human primary cells. In two independent studies, reduction of corticosterone plasma levels was reported in rats.

There was a paucity of data or no data available for the key characteristics "alters DNA repair or genomic instability" and "induces epigenetic alterations".

6. Evaluation and Rationale

6.1 Cancer in humans

There is *sufficient* evidence in humans for the carcinogenicity of acrylonitrile. Acrylonitrile causes cancer of the lung. Positive associations have been observed between acrylonitrile and cancer of the bladder.

6.2 Cancer in experimental animals

There is *sufficient* evidence in experimental animals for the carcinogenicity of acrylonitrile.

6.3 Mechanistic evidence

There is *strong* evidence that acrylonitrile exhibits key characteristics of carcinogens in experimental systems.

6.4 Overall evaluation

Acrylonitrile is *carcinogenic to humans* (Group 1).

6.5 Rationale

The evaluation of acrylonitrile as Group 1 is based on *sufficient* evidence for cancer in humans. There was also *sufficient* evidence for cancer in experimental animals, and *strong* evidence of key characteristics of carcinogens in experimental systems.

There is *sufficient* evidence that exposure to acrylonitrile causes lung cancer in humans. This *sufficient* evidence was primarily based on results from a well-conducted pooled cohort study showing quantitative exposure–response associations after accounting for major sources of confounding and bias, including smoking, co-exposures, and the healthy-worker survivor bias. As the study represented many of the major sectors of acrylonitrile-production operations (monomer, fibre, and resin) and was robust to sensitivity analyses, the results were particularly informative. Other smaller studies showing an excess mortality in individuals with higher exposure to acrylonitrile offered additional support for this conclusion, and their results were deemed to have potential bias towards the null. Although in some smaller studies no excess risks were observed in occupationally exposed cohorts compared with the general population, those studies were considered likely to be biased downwards because of healthy-worker biases and towards the null because of nondifferential exposure misclassification.

There was *limited* evidence that exposure to acrylonitrile causes bladder cancer in humans, as positive associations were observed, but there was remaining doubt over the consistency of evidence and the informativeness of mortality outcomes for a cancer with relatively high survival rates. Therefore, bias and confounding could not be ruled out.

For all other cancer types in humans, the evidence was considered *inadequate*, as associations were not seen consistently across the available studies, associations were imprecise, or studies were few in number.

The *sufficient* evidence in experimental animals is based on an increase in the incidence of malignant neoplasms in both sexes of two species (mouse and rat) in multiple studies, including one well-conducted study that complied with Good Laboratory Practice.

There is *strong* evidence that acrylonitrile exhibits key characteristics of carcinogens. This finding is based on consistent and coherent evidence that acrylonitrile is an electrophile or can be metabolically activated to an electrophile, is genotoxic, induces oxidative stress, causes immortalization, and alters cell proliferation, cell death, or nutrient supply in experimental systems.

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