IARC HANDBOOKS

ABSENCE OF EXCESS BODY FATNESS

VOLUME 16

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IARC HANDBOOKS OF CANCER PREVENTION

International Agency for Research on Cancer

World Health Organization

WORKING PROCEDURES

The Working Procedures of the *IARC Handbooks of Cancer Prevention* describe the objective and scope of the programme, the scientific principles and procedures used in developing a *Handbook*, the types of evidence considered, and the scientific criteria that guide the evaluations. The Working Procedures should be consulted when reading a *Handbook* or a summary of evaluations made by the *IARC Handbooks*. These Working Procedures apply to the review and evaluation of primary prevention.

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

Prevention of cancer is one of the key objectives of the International Agency for Research on Cancer (IARC). The aim of the *IARC Handbooks of Cancer Prevention* series is to review and evaluate scientific information on interventions that may reduce the incidence of or mortality from cancer. As a result of the *IARC Handbooks* evaluations, national and international health agencies have been able, on scientific grounds, to take measures to develop interventions or recommendations that will reduce the risk of developing cancer.

The criteria guiding the evaluations were first established in 1995 at the inception of the *IARC Handbooks* series, and were revised in subsequent volumes.

2. Objective and scope

The objective of the *IARC Handbooks* programme is the preparation of critical reviews and evaluations of the evidence that a particular intervention can prevent cancer. The evaluations, which are prepared by a Working Group of international experts, are scientific judgements about the available evidence on efficacy, effectiveness, and safety of a wide range of cancer-preventive interventions. No recommendation is given with regard to national or international regulations or legislation, which are the responsibility of individual governments and/or other international authorities. The *IARC Handbooks* may assist national and international authorities in devising programmes of health promotion and cancer prevention, and in making benefit–risk assessments.

In this document, the term "intervention" refers to any chemical, activity, or strategy that is subject to evaluation in a *Handbook*. Cancer-preventive interventions encompass pharmacological, immunological, dietary, and behavioural interventions that may delay, block, or reverse carcinogenic processes, or reduce underlying risk factors.

Preventive interventions can be applied across a continuum of: (1) the general population; (2) subgroups with particular predisposing host or environmental risk factors, including genetic susceptibility to cancer; (3) persons with precancerous lesions; and (4) cancer patients at risk of

developing second primary tumours. Use of the same interventions in the treatment of cancer patients to control the growth, metastasis, and recurrence of tumours is considered to be patient management and not prevention, although data from clinical trials of such interventions may be pertinent when reaching an evaluation.

3. Selection of interventions for review

Interventions to be evaluated in the *IARC Handbooks* series are selected on the basis of one or more of the following criteria:

- The available evidence suggests potential for significantly reducing the incidence of cancer.
- There is a substantial body of human, experimental, clinical and/or mechanistic data suitable for evaluation.
- The intervention is in widespread use and of putative protective value, but of uncertain efficacy and safety.

If significant new data become available on an intervention for which a *Handbook* exists, a re-evaluation may be made at a subsequent meeting of the Working Group.

4. Data for the *IARC Handbooks*

Each *Handbook* considers all pertinent intervention trials and observational epidemiological studies, and all relevant cancer bioassays in experimental animals. Those studies that are judged by the Working Group to be uninformative for the evaluation (e.g. because of methodological limitations or small numbers) may be cited but not summarized. When such studies are not reviewed, the reasons are indicated.

Mechanistic and other relevant data are also reviewed. A *Handbook* does not necessarily cite all the mechanistic literature concerning the intervention being evaluated (see Part B, Section 4). Only those data considered by the Working Group to be relevant to making an evaluation are included.

With regard to intervention trials, epidemiological studies, cancer bioassays, and mechanistic and other relevant data, in the interests of transparency, only reports that have been published or accepted for publication in the openly available peer-reviewed scientific literature are reviewed. The same publication requirement applies to studies originating from IARC, including meta-analyses or pooled analyses commissioned by IARC in advance of a meeting (see Part B, Section 2c). Data from government-agency reports that are publicly available in final form are also considered. Exceptionally, doctoral theses and other material that are in their final form and publicly available may be reviewed.

Data on exposure and other information on an intervention under consideration are also reviewed. In the sections on chemical and physical properties, on analysis, on production and use, and on occurrence and exposure, the Working Group may consider published and unpublished sources of information.

In some cases it may be appropriate to review only the data published subsequent to a previous evaluation; this can be useful for updating a database, to resolve a previously open question, or to identify new organ sites associated with a protective effect of the intervention. Major changes (e.g. a large body of additional data that may lead to a new classification; see Part B, Section 6) are more appropriately addressed by a full review and re-evaluation of the entire body of data.

Inclusion of a study does not imply acceptance of the adequacy of the study design or of the authors' analysis and interpretation of the results; any limitations noted by the Working Group are clearly outlined in square brackets at the end of each study description (see Part B). The reasons for not giving further consideration to an individual study also are indicated in the square brackets.

5. Meeting participants

Five categories of participant can be present at meetings of the *IARC Handbooks*:

(a) Member of the Working Group

The Working Group is responsible for the critical reviews and evaluations that are developed during the meeting. The tasks of members of the Working Group are: (i) to ascertain that all appropriate data have been collected; (ii) to select the data relevant for the evaluation on the basis of scientific merit; (iii) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (iv) to evaluate the results of epidemiological and experimental studies on cancer-preventive effects; (v) to evaluate data relevant to the understanding of mechanisms of cancer prevention; and (vi) to make an overall evaluation of the cancer-preventive effect of the intervention in humans. Members of the Working Group are selected on the basis of (a) knowledge and experience; and (b) absence of real or perceived conflicts of interests. Members of the Working Group generally have published significant research related to the cancer-preventive effects of the interventions being reviewed, and have in most cases been identified as experts by IARC on the basis of literature searches. Consideration is also given to demographic diversity and balance of scientific findings and views. Each member of the Working Group serves as an individual scientist and not as a representative of any organization, government, or industry.

(b) Invited Specialist

Invited Specialists are experts who have knowledge and experience that is critical to consideration of the intervention being evaluated, but who also have a real or perceived conflict of interests. These experts are invited when necessary to assist the Working Group by contributing their unique knowledge and experience during subgroup and plenary discussions. They may also contribute text on non-influential issues, for example for the general description of the intervention or for the exposure (see Part B, Section 1). Invited Specialists do not serve as meeting chair or subgroup chair, do not draft text that pertains to the description or interpretation of data directly relevant to the evaluations, and do not participate in the evaluations.

(c) Representative

Representatives of national and international health agencies may attend meetings because such agencies sponsor the *IARC Handbooks* programme or are interested in the subject of a meeting. Representatives do not serve as meeting chair or subgroup chair, do not draft any part of a *Handbook*, and do not participate in the evaluations.

(d) Observer

Observers with relevant scientific credentials are admitted to an *IARC Handbook* meeting in limited numbers. Attention will be given to achieving a balance of Observers from constituencies with differing perspectives. They are invited to observe the meeting and should not attempt to influence it. Observers do not serve as meeting chair or subgroup chair, do not draft any part of a *Handbook*, and do not participate in the evaluations. At the meeting, the meeting chair and subgroup chairs may grant Observers an opportunity to speak, generally after a discussion has been completed by the Working Group. Observers agree to respect the Guidelines for Observers at *IARC Handbooks* meetings (available from http://handbooks.iarc.fr).

(e) The IARC Secretariat

The Secretariat consists of IARC scientific staff who have relevant expertise. They serve as rapporteurs and participate in discussions. When requested by the meeting chair or subgroup chair, they may also draft text or prepare tables

and analyses. Members of the Secretariat do not participate in the evaluations.

(f) Declaration of Interests

Before an invitation is extended, each potential participant, including the IARC Secretariat, completes the WHO Declaration of Interests to report financial interests, employment and consulting, and individual and institutional research support related to the subject of the meeting or any tobacco-related interests. IARC assesses these interests to determine whether there is a conflict that warrants some limitation on participation. The declarations are updated and reviewed again at the opening of the meeting. Interests related to the subject of the meeting are disclosed to the meeting participants and in the published volume (Cogliano et al., 2004).

The names and principal affiliations of participants are published on the website of the *IARC Handbooks* programme (http://handbooks.iarc.fr) approximately two months before each meeting. It is not acceptable for Observers or third parties to contact other participants before a meeting or to lobby them at any time. Meeting participants are asked to report all such contacts to IARC (Cogliano et al., 2005). The names and principal affiliations of all meeting participants are also listed at the beginning of the corresponding volume of the *Handbooks*.

B. SCIENTIFIC REVIEW AND EVALUATION

A wide range of findings must be taken into account before a particular intervention can be recognized as preventing cancer, and a systematic approach to data presentation has been adopted for *Handbooks* evaluations.

The available studies are summarized by the Working Group, with particular regard to the qualitative aspects discussed below. In general, numerical findings are indicated as they appear in the original report; units are converted when necessary for easier comparison. The Working Group may conduct additional analyses of the published data and use them in their assessment of the evidence; the results of such supplementary analyses are given in square brackets. When an important aspect of a study that directly impinges on its interpretation should be brought to the attention of the reader, a Working Group comment is given in square brackets.

The *IARC Handbooks* evaluate a wide range of interventions for primary prevention, including those involving chemical or pharmacological agents (e.g. drugs, vitamins, minerals, other nutritional supplements), immunological agents (vaccination), foods, behaviour changes (e.g. weight control, physical activity), and public-health policies (e.g. smoking restrictions). The structure of a *Handbook* typically comprises the following sections:

1. Exposure data
2. Studies of cancer prevention in humans
3. Studies of cancer prevention in experimental animals
4. Mechanistic and other relevant data
5. Summary
6. Evaluation and rationale

In addition, a section entitled "General Remarks" at the front of the volume discusses the reasons why the interventions were scheduled for evaluation, and key issues the Working Group encountered during the meeting.

The following part of the Working Procedures discusses the types of evidence considered and summarized in each section of a *Handbook*, followed by the scientific criteria that guide the evaluations.

1. Characteristics and occurrence of the intervention

Each *Handbook* includes general information identifying and describing the intervention. As preventive interventions can range from community-based interventions to measures targeted to individuals (e.g. behavioural, dietary, pharmacological measures), this information may vary substantially between interventions. Depending on the intervention, this section may include information on production and use, occurrence and exposure, prevalence, risk factors, and regulations and guidelines.

Given the wide variety of preventive interventions, this section will have an outline specific to each *Handbook*.

2. Studies of cancer prevention in humans

This section includes all pertinent experimental and observational epidemiological studies of cancer prevention in humans, with cancer as an outcome (see Part A, Section 4). Studies of biomarkers as indicators of the intervention are included in Section 4 when they are relevant to an evaluation of the cancer-preventive effect in humans.

(a) Types of study considered

This section focuses on studies that assess the prevention of cancer as an outcome in humans. Relevant evidence is normally provided by experimental studies (for example, randomized clinical trials and community intervention trials), and analytical observational studies, primarily cohort studies and case–control studies. For certain interventions applied at the population level, well-designed ecological studies (studies measuring both outcome and exposure on the aggregate, or population, level) or interrupted time-series studies may also be informative. Cross-sectional studies, descriptive epidemiological studies, case-series, and case reports are usually not reviewed. The uncertainties that surround the interpretation of such studies make them inadequate, except in exceptional circumstances, to form the basis for inferring a preventive relationship. However, when considered together with experimental and analytical observational studies, these types of study can sometimes contribute to the decision of the Working Group as to whether or not a causal relationship exists.

Intervention studies are experimental in design – that is, the use of, or exposure to, the intervention is assigned by the investigator. Experimental studies can provide the strongest and most direct evidence of a protective or preventive effect; however, the use of such studies is limited for practical and ethical reasons and the subjects are often drawn from select groups that may not represent the population at large.

In exceptional cases, epidemiological studies on advanced pre-neoplastic lesions and other end-points thought to be relevant to cancer are also reviewed in this section. The results of such studies may strengthen inferences drawn from other studies.

(b) Quality of studies considered

In considering whether a particular study should contribute to the evaluation of an intervention, the Working Group considers the following aspects:

- The relevance of the study;
- The appropriateness of the design and analysis to the question being asked;
- The adequacy and completeness of the presentation of the data; and
- The degree to which chance, bias, and confounding may have affected the results; for drugs or other marketed products, this bias assessment should include review of the funding source.

Aspects that are particularly important in evaluating randomized controlled trials are: the selection of participants, the nature and adequacy of the randomization procedure, evidence that randomization achieved an adequate balance between the groups, exclusion criteria used before and after randomization, compliance with the intervention in the intervention group, and "contamination" of the control group with the intervention. Other considerations are the means by which the end-point was determined and validated (either by screening or by other means of detection of the disease), the length and completeness of follow-up of the groups, and the adequacy of the analysis.

It is necessary to take into account the possible roles of bias, confounding, and chance in the interpretation of cohort and case–control studies. Bias is the effect of factors in study design or execution that leads erroneously to a stronger or weaker association than in fact exists between an intervention and outcome. Confounding is a form of bias that occurs when the relationship with the outcome is made to appear stronger or weaker than it is in reality, due to an association between the apparent causal factor and another factor that is associated with either an increase or a decrease in the incidence of the disease. The role of chance is related to biological variability and the influence of sample size on the precision of estimates of effect.

In evaluating the extent to which these factors have been minimized in an individual study, consideration is given to a number of aspects of design and analysis as described in the report of the study. Most of these considerations apply equally to all types of study. Lack of clarity regarding any of these aspects in the reporting of a study can decrease its credibility and the weight given to it in the final evaluation.

Firstly, the study population, target organ, and exposure should have been well defined by the authors. Cancer occurrence in the study population should have been identified in a way that was independent of the intervention of interest, and exposure to the intervention should have been assessed in a way that was not related to disease status.

Secondly, the authors should have taken into account – in the study design and analysis – other variables that could influence the risk of disease and may have been related to the exposure of interest. Potential confounding by such variables should have been dealt with either in the design of the study (e.g. by matching) or in the analysis (by statistical adjustment). Internal comparisons of frequency of disease among individuals with different levels of exposure are desirable in cohort studies, since they minimize the potential for confounding related to the difference in risk factors between an external reference group and the study population.

Thirdly, the authors should have reported the basic data on which the conclusions are founded, even if sophisticated statistical analyses were employed. At the very least, they should have given the numbers of exposed and unexposed cases and controls in a case–control study, and the numbers of cases observed and expected in a cohort study. Further tabulations by duration of exposure and other temporal factors are also important. In a cohort study, data on all cancer sites and all causes of death should have been given, to reveal the possibility of reporting bias. In a case–control study, the effects of investigated factors other than the exposure of interest should have been reported.

Finally, the statistical methods used to obtain estimates of relative risk, absolute rates of cancer, confidence intervals, and significance tests, and to adjust for confounding should have been clearly stated by the authors. These methods have been reviewed for case–control studies (Breslow & Day, 1980) and for cohort studies (Breslow & Day, 1987).

(c) Quantitative aspects

The Working Group gives special attention to quantitative assessment of the preventive effect of the intervention under study, by assessing data from studies investigating different doses or levels of exposure. The Working Group also addresses issues of timing and duration of use or exposure. Such quantitative assessment is important to clarify the circumstances under which a preventive effect can be achieved, as well as the dose or level of exposure at which a toxic effect has been shown.

(d) Criteria for preventive effects

After summarizing and assessing the individual studies, the Working Group makes a judgement concerning the strength of the evidence that the intervention in question prevents cancer in humans. In making its judgement, the Working Group considers several criteria for each relevant cancer site.

Evidence is frequently available from different types of study and is evaluated as a whole. Findings that are replicated in several studies of the same design or in studies using different approaches are more likely to provide evidence of a true protective effect than are isolated observations from single studies.

Evidence of protection derived from intervention studies of good quality is particularly informative. Evidence of a substantial and significant reduction in risk, including a "dose"–response relationship, is more likely to indicate a true effect. Nevertheless, a small effect, or an effect without a dose–response relationship, does not imply lack of real benefit and may be important for public health if the cancer is common.

The Working Group evaluates possible explanations for inconsistencies across studies, including differences in use of, or exposure to, the intervention, differences in the underlying risk of cancer, and metabolism and genetic differences in the population, as well as differences in study methodology. The results of studies judged to be of high quality are given more weight. Note is taken both of the applicability of preventive action to several cancers and of possible differences in activity, including the possibility of different findings between cancer sites.

3. Studies of cancer prevention in experimental animals

(a) Types of study considered

Animal models are an important component of research into cancer prevention. Models that permit evaluation of the effects of cancer-preventive interventions on the occurrence of cancer in most major organ sites are available. Animal models for such studies include: those in which cancer is produced by the administration of a chemical or physical carcinogen; those involving genetically engineered animals; and those in which tumours develop spontaneously. Most cancer-preventive interventions investigated in such studies can be placed into one of three categories: interventions that prevent molecules from reaching or reacting with critical target sites (blocking agents); interventions that decrease the sensitivity of target tissues to carcinogenic stimuli; and interventions that prevent evolution of the neoplastic process (suppressing agents). There is increasing interest in the use of combinations of interventions as a means of improving efficacy and minimizing toxicity; animal models are useful in evaluating such combinations. The development of optimal strategies for intervention trials in humans can be facilitated by the use of animal models that mimic the neoplastic process in humans.

Specific factors to be considered in such experiments are: (1) the temporal requirements of administration of the cancer-preventive interventions; (2) dose–response effects; (3) the site specificity of cancer-preventive activity; and (4) the number and structural diversity of

carcinogens whose activity can be reduced by the intervention being evaluated.

An important variable in the evaluation of the cancer-preventive response is the time and duration of administration of the intervention in relation to any carcinogenic treatment, or in transgenic or other experimental models in which no carcinogen is administered. Furthermore, concurrent administration of an intervention may result in a decreased incidence of tumours in a given organ and an increase in incidence in another organ of the same animal. Thus, in these experiments it is important that multiple organs be examined.

For all these studies, the nature and extent of impurities or contaminants present in the cancer-preventive intervention or interventions being evaluated are given when available. Also, consideration is given to the possibility of changes in the physicochemical properties of the test substance during collection, storage, extraction, concentration, and delivery. Chemical and toxicological interactions of the components of mixtures may result in non-linear dose–response relationships.

As certain components of commonly used diets of experimental animals are themselves known to have cancer-preventive activity, particular consideration should be given to the interaction between the diet and the apparent effect of the intervention being studied. Likewise, restriction of diet may be important. The appropriateness of the diet given relative to the composition of human diets may be commented on by the Working Group.

(b) Quality of studies considered

An assessment of the experimental prevention of cancer involves several considerations of qualitative importance, including: (1) the experimental conditions under which the test was performed (route and schedule of exposure, species, strain, sex and age of the animals studied, duration of the exposure, and duration of the study); (2) the consistency of the results, for example across species and target organ(s); (3) the stage or stages of the neoplastic process studied, from pre-neoplastic lesions and benign tumours to malignant tumours; and (4) the possible role of modifying factors.

In the interpretation and evaluation of a particular study, the Working Group takes into consideration: (1) how clearly the intervention was defined and, in the case of mixtures, how adequately the sample composition was reported; (2) the composition of the diet and the stability of the intervention in the diet; (3) whether the source, strain, and quality of the animals was reported; (4) whether there were adequate numbers of animals, of appropriate age, per group; (5) whether males and females were used, if appropriate; (6) whether animals were allocated randomly to groups; (7) whether appropriate respective controls were used; (8) whether the dose and schedule of treatment with the known carcinogen were appropriate in assays of combined treatment; (9) whether the doses of the cancer-preventive intervention were adequately monitored; (10) whether the agent(s) was absorbed, as shown by blood concentrations; (11) whether the survival of treated animals was similar to that of controls; (12) whether the body and organ weights of treated animals were similar to those of controls; (13) whether the duration of the experiment was adequate; (14) whether there was adequate statistical analysis; and (15) whether the data were adequately reported.

(c) Quantitative aspects

The incidence of tumours may depend on the species, sex, strain, and age of the animals, the dose of carcinogen (if any), the dose of the agent, and the route and duration of exposure. A decreased incidence and/or decreased multiplicity of tumours in adequately designed studies provide evidence of a cancer-preventive effect. A dose-related decrease in incidence and/or multiplicity further strengthens this association.

The nature of the dose–response relationship can vary widely, depending on the agent and the target organ. Saturation of steps such as absorption, activation, inactivation, and elimination may produce non-linearity in the dose–response relationship (Hoel et al., 1983; Gart et al., 1986), as could saturation of the detoxication processes. The dose–response relationship can also be affected by differences in survival between the treatment groups.

(d) Statistical analyses

Factors considered in the statistical analysis by the Working Group include: (1) the adequacy of the data for each treatment group; (2) the initial and final effective numbers of animals studied and the survival rate; (3) body weights; and (4) tumour incidence and multiplicity.

The statistical methods used should be clearly stated and should be the generally accepted techniques defined for this purpose. In particular, the statistical methods should be appropriate for the characteristics of the expected data distribution and should account for interactions in multifactorial studies. Consideration is given as to whether the appropriate adjustment was made for differences in survival.

If available, recent data on the incidence of specific tumours in historical controls, as well as in concurrent controls, are taken into account in the evaluation of tumour response.

4. Mechanistic and other relevant data

In evaluating an intervention, effects other than cancer are described and weighed. Furthermore, information that facilitates an understanding of the applicability of findings to different species, or to different human populations is particularly important; this includes metabolic, kinetic, and genetic data. Whenever possible, quantitative data, including information on dose, duration, and potency, are considered.

The focus of this section is on studies in humans, including intervention trials and epidemiological studies with cancer-relevant molecular biomarkers or intermediate end-points as an outcome. Studies in experimental systems can strengthen the evidence for the potential cancer-preventive effect of an intervention observed in humans, and can elucidate the mechanism(s) of cancer prevention. A brief summary of important findings in experimental systems is therefore included.

Evaluation of the results of intervention studies in humans includes consideration of quality, as described above. Study quality factors generally consider the adequacy of the methods and the reporting of results, addressing: (1) the description of the methods; (2) the appropriateness of control populations; (3) whether toxic effects were considered in the outcome; (4) whether the data were appropriately compiled and analysed; (5) whether appropriate quality controls were used; (6) whether appropriate concentration ranges were used; (7) whether adequate numbers of independent measurements were made per group; and (8) the relevance of the end-points.

The observation of effects on the occurrence of lesions presumed to be pre-neoplastic, or the emergence of benign or malignant tumours, may aid in assessing the mode of action of the intervention being considered. Particular attention is given to assessing the reversibility of these lesions and their predictive value in relation to cancer development.

(a) Toxicokinetics

Information is given on absorption, distribution (including placental transfer), metabolism, and excretion in humans. If human data are sparse, evidence from experimental animals may be summarized. Studies in humans that indicate the metabolic pathways and fate of an intervention are summarized. Data indicating long-term accumulation in human tissues are included.

Observations are made on inter-individual variations and relevant metabolic polymorphisms. Physiologically based pharmacokinetic models and their parameter values are relevant and are included whenever they are available.

Information from experimental systems, including on the fate of the compound within tissues and cells (transport, role of cellular receptors, compartmentalization, binding to macromolecules) may be briefly summarized.

The metabolic consequences of interventions are described.

(b) Mechanisms of cancer prevention

For a rational implementation of cancer-preventive measures, it is essential not only to assess protective end-points but also to understand the mechanisms by which the intervention exerts its anticarcinogenic action. Data on mechanisms will be primarily from studies in humans. Data from relevant experimental models can also be summarized, including studies of the inhibition of tumorigenesis in vivo, studies of intermediate biomarkers in vivo, analyses of interactions between agents and specific molecular targets, and studies of specific end-points in vitro. Information on the mechanisms of cancer-preventive activity inferred from relationships between chemical structure and biological activity can also be included.

Cancer-preventive interventions may act at different levels: (1) extracellular, for example inhibiting the uptake or endogenous formation of carcinogens, or forming complexes with, diluting, and/or deactivating carcinogens; (2) intracellular, for example trapping carcinogens in nontarget cells, modifying transmembrane transport, modulating metabolism, blocking reactive molecules, inhibiting cell replication, or modulating gene expression or DNA metabolism; or (3) at the level of the cell, tissue, or organism, for example affecting cell differentiation, intercellular communication, proteases, signal transduction, growth factors, cell adhesion molecules, angiogenesis, interactions with the extracellular matrix, hormonal status, and the immune system.

Many cancer-preventive interventions are known or suspected to act by several mechanisms, which may operate in a coordinated manner and allow them a broader spectrum of anticarcinogenic activity. Therefore, a range of possible mechanisms of action are taken into account in the evaluation of cancer prevention. These can be conceptually organized to encompass impacts on one or more related key characteristics of carcinogens (Smith et al., 2016), particularly interference with: (1) metabolic activation of carcinogens; (2) mutagenesis; (3) DNA repair or genomic instability; (4) epigenetic effects; (5) oxidative stress; (6) inflammation; (7) immune function; (8) receptor-mediated effects; (9) immortalization; or (10) cell proliferation, cell death, or nutrient supply.

(c) Susceptible populations

This section summarizes studies of cancer in humans that have addressed differential susceptibility due to toxicokinetics, mechanisms of cancer prevention, and other factors. Such studies may identify individuals, populations, and life-stages with greater or lesser susceptibility. Examples of host and genetic factors that affect individual susceptibility include sex, genetic polymorphisms of genes involved in the metabolism of the intervention, differences in metabolic capacity due to life-stage or the presence of disease, differences in DNA repair capacity, competition for alteration of metabolic capacity by medications or other chemical exposures, a pre-existing hormonal imbalance that is exacerbated by a chemical exposure, a suppressed immune system, periods of higher-than-usual tissue growth or regeneration, and genetic polymorphisms that lead to differences in behaviour (e.g. addiction). Genotyping is being used increasingly, not only to identify subpopulations at increased or decreased risk for cancers but also

to characterize variation in the biotransformation of and response to cancer-preventive interventions. Such data can substantially increase the strength of the evidence from epidemiological data and enhance the linkage of in vivo and in vitro laboratory studies to humans.

(d) Adverse effects

Relevant clinical or other evidence that would impact any recommendations may be summarized as appropriate.

5. Summary of data

This section is a summary of data presented in the preceding sections.

(a) Exposure data

Data are summarized, as appropriate, on elements such as characteristics and production or implementation of the intervention, and patterns of use or exposure in human populations. Quantitative data and time trends are given to compare exposure, use, or implementation in different regions and settings.

(b) Cancer prevention in humans

Results of epidemiological studies pertinent to an assessment of the cancer-preventive effect in humans are summarized. The target organ(s) or tissue(s) in which a decrease in cancer occurrence was observed is identified. Dose–response and other quantitative data may be summarized when available.

(c) Cancer in experimental animals

Data relevant to an evaluation of a cancer-preventive effect in animals are summarized. For each animal species, study design, and route of administration, it is stated whether decreased incidence, increased latency, or decreased severity or multiplicity of tumours or preneoplastic lesions were observed, and the tumour sites are indicated. Negative findings, positive relationships, dose–response, and other quantitative data are also summarized.

(d) Mechanistic and other relevant data

Human data relevant to the toxicokinetics (absorption, distribution, metabolism, elimination) and the possible mechanism(s) of cancer prevention are summarized. In addition, human studies on cancer susceptibility including on genetic polymorphisms, susceptible populations and life-stages are summarized. This section also reports briefly on adverse effects as well as any additional relevant data from experimental systems that are considered to be influential for the evaluation of a cancer-preventive effect.

6. Evaluation and rationale

Evaluations of the strength of the evidence for cancer-preventive effects from studies in humans and experimental animals are made using standard terms. Similarly, an evaluation of the strength of the mechanistic evidence is given.

It is recognized that the criteria for these evaluation categories, described below, cannot encompass all factors that may be relevant to an evaluation of cancer-preventive effects. In considering all the relevant scientific data, the Working Group may assign the intervention to a higher or lower category than a strict interpretation of these criteria would indicate.

The evaluation categories refer only to the strength of the evidence that an intervention prevents cancer, and not to the extent of its cancer-preventive effects (potency). The evaluations may change as new information becomes available.

Evaluations are inevitably limited to the intervention as actually implemented and observed, for example to the cancer sites, conditions, and duration of observation covered by the available studies.

(a) Cancer-preventive effects in humans

The evidence relevant to cancer prevention in humans is classified into one of the following categories:

Sufficient evidence of cancer-preventive effects: The Working Group considers that a preventive relationship has been established between the intervention and the risk of cancer in humans. That is, a preventive association has been observed in studies in which chance, bias, and confounding could be ruled out with confidence. A statement that there is sufficient evidence is followed by a sentence identifying the organ(s) or tissue(s) for which a preventive effect has been observed in humans. Identification of preventive effects in a specific organ or tissue does not preclude the possibility that the intervention may prevent cancer at other sites.

Limited evidence of cancer-preventive effects: A reduced risk of cancer is associated with the intervention for which a preventive effect is considered credible by the Working Group, but chance, bias, or confounding could not be ruled out with confidence.

Inadequate evidence of cancer-preventive effects: The available studies are not of sufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of a cancer-preventive effect of the intervention, or no data on the prevention of cancer by this intervention in humans are available.

Evidence suggesting lack of cancer-preventive effects: When several epidemiological studies show little or no indication of an association between an intervention and a reduced risk of cancer, a judgement may be made that the studies, taken together, show evidence of lack of a preventive effect. Such a judgement requires that the studies meet the standards of design and analysis described above. Specifically, the possibility that bias, confounding, or misclassification of the intervention or the outcome could explain the observed results should be considered and excluded with confidence.

(b) Cancer-preventive effects in experimental animals

Cancer-preventive effects in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in vivo bioassays that focus on one or more of the critical stages of carcinogenesis.

Evidence for cancer prevention in experimental animals is classified into one of the following categories:

Sufficient evidence of cancer-preventive effects: The Working Group considers that a causal relationship has been established between the intervention and a decreased incidence and/or multiplicity of spontaneous or chemically induced malignant neoplasms, or of an appropriate combination of benign and malignant neoplasms in an adequate number (four or more) of independent studies carried out at different times, or in different laboratories, or under different protocols.

Limited evidence of cancer-preventive effects: The data indicate a cancer-preventive effect, but are limited for making a definitive evaluation because, for example: (a) the evidence of a cancer-preventive effect is restricted to a small number (fewer than four) of experiments; or (b) the intervention decreases the incidence and/or multiplicity of benign neoplasms only.

Inadequate evidence of cancer-preventive effects: The studies cannot be interpreted as showing either the presence or absence of a preventive effect because of major methodological or quantitative limitations: unresolved questions regarding the adequacy of the design, conduct, or interpretation of the study, or few or no data on cancer prevention in experimental animals are available.

Evidence suggesting lack of cancer-preventive activity: Adequate evidence from conclusive studies in several models shows that, within the limits of the tests used, the intervention has no cancer-preventive effects.

(c) Mechanistic data on cancer-preventive effects

Mechanistic and other evidence judged to be relevant to an evaluation of a cancer-preventive effect and of sufficient importance to affect the overall evaluation is brought forward to the evaluation.

The strength of mechanistic evidence supporting the cancer-preventive effect is evaluated, using terms such as 'weak', 'moderate', or 'strong'. Indications that a particular mechanism operates in humans are strongest. The data may be considered to be especially relevant if they show in humans that the intervention in question has caused suppression of effects that are on the pathway to cancer. The mechanistic evidence can be strengthened by findings of consistent results in different experimental designs, by the demonstration of biological plausibility, and by coherence of the overall database.

The Working Group considers whether multiple mechanisms might contribute to cancer prevention, whether different mechanisms might operate in different dose ranges or at different sites, or whether separate mechanisms might operate in a susceptible group.

For complex interventions, such as food categories, the chemical composition and the potential contribution of different nutrients known to be present may be considered by the Working Group in its overall evaluation of cancer prevention.

(d) Overall evaluation

Finally, the body of evidence is considered as a whole, and summary statements are made that encompass the effects of the intervention with regard to cancer-preventive effects in humans. The overall evaluation is described according to the wording of one of the following standard categories. The categorization of an intervention is a matter of scientific judgement that reflects the strength of the evidence derived from studies in humans and in experimental animals, and from mechanistic and other relevant data.

(i) The intervention prevents cancer (Group A)

This category is used for interventions for which there is *sufficient evidence* of a cancer-preventive effect in humans.

The sites on which the evidence in humans is based are given.

(ii) The intervention probably prevents cancer (Group B1)

This category is used for interventions for which there is *limited evidence* of a cancer-preventive effect in humans and *sufficient evidence* in animals. An intervention may also be classified in this category when there is *limited evidence* in humans, less than *sufficient evidence* in experimental animals, and strong supporting evidence from mechanistic and other relevant data that the mechanism(s) of prevention also operates in humans.

The sites on which the evidence in humans is based are given.

(iii) The intervention possibly prevents cancer (Group B2)

This category is used for interventions for which there is *inadequate evidence* in humans and *sufficient evidence* in experimental animals. An intervention may also be classified in this category when there is *inadequate evidence* in humans, *limited evidence* in experimental animals, and strong supporting evidence from mechanistic and other relevant data that the mechanism(s) of prevention also operates in humans.

(iv) The intervention is unclassifiable as to its cancer-preventive effects (Group C)

This category is used for interventions for which the evidence is *inadequate* in humans and less than *sufficient* in experimental animals. Interventions that do not fall into any other group are also placed in this category.

(v) The intervention probably does not prevent cancer (Group D)

This category is used for interventions for which there is *evidence suggesting lack of a cancer-preventive effect* both in humans and in experimental animals.

(e) Rationale

The reasoning that the Working Group used to reach its evaluation is presented and discussed. This section integrates the major findings from studies in humans, studies in experimental animals, and mechanistic and other relevant data. It includes concise statements of the principal line(s) of argument that emerged, the conclusions of the Working Group on the strength of the evidence for each group of studies, and an explanation of the reasoning of the Working Group in weighing data and making evaluations. The human populations that were the subject of study should be identified. Additionally, important health concerns identified – such as adverse effects, including cancer-causing properties – should be clearly addressed.

When there are significant differences in scientific interpretation among Working Group members, a brief summary of the alternative interpretations is provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

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