DRAFT Preamble to the *IARC Monographs*

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The Preamble to the *IARC Monographs* describes the objective and scope of the programme, the principles and procedures used in developing *Monographs*, the types of evidence considered, and the scientific criteria that guide the evaluations. The Preamble should be consulted when reading a *Monograph* or list of evaluations.

1. Background

Soon after IARC was established in 1965, it received frequent requests for advice on the carcinogenic risk of various chemicals, including requests for lists of known and suspected human carcinogens. It was clear that it would not be a simple task to adequately summarize the complexity of the information that was available, and IARC began to consider means of obtaining international expert opinion on this subject. In 1970, the IARC Advisory Committee on Environmental Carcinogenesis recommended, ' . . . that a compendium on carcinogenic chemicals be prepared by experts. The biological activity and evaluation of practical importance to public health should be referenced and documented.' The IARC Governing Council adopted a resolution concerning the role of IARC in providing government authorities with expert, independent, scientific opinion on environmental carcinogenesis. As one means to that end, the Governing Council recommended that IARC should prepare monographs on the evaluation of carcinogenic risk of chemicals to man, which became the initial title of the series.

In the succeeding years, the scope of the programme broadened as *Monographs* were developed for groups of related chemicals, complex mixtures, occupational exposures, physical and biological agents, and lifestyle factors. In 1988 the phrase 'of chemicals' was dropped from the title, which assumed its present form, the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*.

The *Monographs* seek to identify the causes of human cancer. This is the first step in cancer prevention. The need for cancer prevention is as great today as it was when IARC was established, as the global burden of cancer is high and continues to increase. The annual number of new cancer cases was estimated at 10.1 million in 2000 and is expected to reach 15 million by 2020 (Stewart & Kleihues, 2003). With current trends in demographics and exposure, the cancer burden has been shifting from high-resource countries to low- and medium-resource countries. As a result of *Monograph* evaluations, national health agencies have been able to take, on scientific grounds, measures to reduce exposure to occupational carcinogens, tobacco smoke, ionizing and non-ionizing radiation, and other environmental sources of exposure to cancer-causing agents.

The criteria established in 1971 to evaluate carcinogenic risk to humans were adopted by the Working Groups whose deliberations resulted in the first 16 volumes of the *Monographs* series. Those criteria were subsequently updated by further ad-hoc Working Groups (IARC, 1977, 1978, 1979, 1982, 1983, 1987, 1988, 1991, 2005; Vainio et al., 1992).

2. Objective and scope

The objective of the programme is to prepare, with the help of international working groups of experts, and to publish in the form of *Monographs*, critical reviews and consensus evaluations of evidence on the carcinogenicity of a wide range of human exposures. The *Monographs* may also indicate where additional research efforts are needed, specifically when data immediately relevant to an evaluation are not available.

The term 'carcinogen' is used in these *Monographs* to denote an exposure that is capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing their severity or multiplicity. The induction of benign neoplasms may in some circumstances (see Section 9) contribute to the judgement that the exposure is carcinogenic. The terms 'neoplasm' and 'tumour' are used interchangeably.

Risk assessment is the use of scientific data to describe the adverse health effects of exposure to hazardous agents (National Research Council 1983, 1994). Risk assessment can be described as a series of distinct steps. *Hazard identification* assesses whether exposure to an agent is linked to specific adverse health effects (in this case, cancer). *Dose-response assessment* characterizes the relation between the dose of an agent and an adverse health effect. *Exposure assessment* determines the pathways and extent of human exposure to an agent. *Risk characterization* integrates the hazard, dose-response, and exposure assessments to describe the nature and magnitude of human risk, including inherent uncertainty. Thus a cancer *hazard* is an agent that is capable of causing cancer under some circumstances, while a cancer *risk* is an estimate of the carcinogenic effects expected from exposure to a cancer hazard.

Under this paradigm, the *Monographs* are an exercise in hazard identification, despite the historical presence of the word 'risk' in the title. The *Monographs* critically review and evaluate the published scientific evidence in order to assess whether an agent can alter the age-specific incidence of cancer in humans. The long-term objective is to publish up-to-date information on each carcinogenic hazard to which humans are exposed.

Some epidemiological and experimental studies indicate that different agents may act at different stages in the carcinogenic process, and several different mechanisms may be involved. The aim of the *Monographs* has been, from their inception, to evaluate evidence of carcinogenicity at any stage in the carcinogenesis process, independently of the underlying mechanisms. Information on mechanisms may, however, be used in making the overall evaluation (IARC, 1991, 2005; Vainio et al., 1992; see also Sections 10 and 12). As mechanisms of carcinogenesis are elucidated, IARC convenes international scientific conferences to determine whether a broad-based consensus has emerged on how specific mechanistic data can be used in an evaluation of human carcinogenicity. The results of such conferences are reported in IARC Scientific Publications, which, as long as they still reflect the current state of scientific knowledge, may guide subsequent *Monograph* Working Groups.

Although the *Monographs* have emphasized hazard identification, important issues may also involve dose-response assessment. In many cases, the same epidemiological and experimental studies used to evaluate a cancer hazard can also be used to estimate a dose-response relationship. A *Monograph* may undertake to estimate dose-response relationships within the range of the available epidemiological data, or it may compare the dose-response

information from experimental and epidemiological studies. In some cases, a subsequent publication may be prepared by a separate working group with expertise in quantitative exposure-dose and dose-response analysis.

The *Monographs* may assist national and international authorities in making risk assessments and in formulating decisions concerning any necessary preventive measures. The evaluations of IARC Working Groups are scientific, qualitative judgements about the evidence for or against carcinogenicity provided by the available data. These evaluations represent only one part of the body of information on which regulatory measures may be based. Other components of regulatory decisions may vary from one situation to another and from country to country, responding to different socioeconomic and national priorities. **Therefore, no recommendation is given with regard to regulation or legislation, which are the responsibility of individual governments and/or other international organizations.**

3. Selection of topics for the *Monographs*

Topics are selected on the basis of two main criteria: (a) there is evidence of human exposure, and (b) there is some evidence or suspicion of carcinogenicity. The term 'agent' is used to include individual chemical compounds, groups of related chemical compounds, physical agents (such as radiation) and biological factors (such as viruses). Exposures to mixtures of agents may occur in occupational and environmental settings and as a result of personal and cultural habits (such as smoking and dietary practices). Chemical analogues and compounds with biological or physical characteristics similar to those of suspected carcinogens may also be considered, even in the absence of data on a possible carcinogenic effect in humans or experimental animals.

The scientific literature is surveyed for published data relevant to an assessment of carcinogenicity. Ad-hoc Working Groups convened by IARC in 1984, 1989, 1991, 1993, 1998 and 2003 gave recommendations as to which agents should be evaluated in the *Monographs* series. Recent recommendations are available on the *Monographs* website (http://monographs.iarc.fr). IARC may schedule other topics as it becomes aware of new scientific information or as national health agencies identify an urgent public health need related to cancer.

As significant new data on subjects on which *Monographs* have already been prepared become available, re-evaluations are made at subsequent meetings, and revised *Monographs* are published. In some cases it may be appropriate to critically review only the new data published since a prior evaluation. This can be useful for updating a database, reviewing new data that resolve a previously open question, or identifying new tumour sites associated with a carcinogenic agent. Major changes in an evaluation (e.g. a new classification in Group 1 or a determination that a mechanism does not operate in humans, see Section 12) are more appropriately addressed by a full *Monograph* review.

4. Data for the *Monographs*

The *Monographs* intend to review all pertinent epidemiological studies and cancer bioassays in experimental animals. Other studies may be mentioned briefly, particularly when the information is considered to be a useful supplement to that in other reports or when they provide the only data available. Those that are judged to be inadequate or irrelevant to the

evaluation are generally omitted. If a group of similar studies is not reviewed, the reasons should be indicated.

Mechanistic and other relevant data are also reviewed, although the *Monographs* do not necessarily cite all the literature concerning the subject of an evaluation (see Section 10). Only those data considered by the Working Group to be relevant to making the evaluation are included.

With regard to biological and epidemiological data, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed by the Working Group. Government agency reports that have undergone peer review and are widely available are considered. Exceptions may be made on an ad-hoc basis to include reports, abstracts, and doctoral theses that are in their final form and publicly available, if their inclusion is considered pertinent to making a final evaluation (see Section 12). In the sections on chemical and physical properties, on analysis, on production and use and on occurrence, other sources of information may be used.

Inclusion of studies does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results, and limitations are clearly outlined in square brackets at the end of each study description (see Section 6). The reasons for not giving further consideration to an individual study also should be indicated in the square brackets.

5. Meeting participants

Five categories of participants can be present at *Monograph* meetings.

- The Working Group is responsible for the critical reviews and consensus evaluations that are developed during the meeting. The tasks 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; (v) to evaluate data relevant to the understanding of mechanism of action; and (vi) to make an overall evaluation of the carcinogenicity of the exposure to humans. Working Group Members generally have published significant research related to the carcinogenicity of the agents being reviewed, and IARC uses literature searches to identify most experts. Working Group Members are selected based on (a) knowledge and experience and (b) absence of real or apparent conflicts of interests. Consideration is also given to demographic diversity and balance of scientific findings and views.

- Invited Specialists are experts who also have critical knowledge and experience but have a real or apparent conflict of interests. These experts are invited when necessary to assist in the Working Group by contributing their unique knowledge and experience during subgroup and plenary discussions. They may also contribute text to the sections on exposure. Invited Specialists do not serve as meeting chair or subgroup chair, draft text that pertains to cancer data, or participate in the evaluations.

- Representatives of national and international health agencies often attend meetings because their agencies sponsor the programme or are interested in the subject of a *Monograph*. Representatives have no official responsibilities during the meeting.

- Observers with relevant scientific credentials may be admitted to a meeting in limited numbers. Priority will be given to achieving a balance of Observers from constituencies with differing perspectives. Observers should only observe the meeting and not attempt to influence it. They may not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in evaluation discussions. At the meeting, the meeting chair and subgroup chairs may grant Observers an opportunity to speak, generally after they have observed a discussion. Observers agree to respect the Guidelines for Observers at *IARC Monograph* meetings (available at http://monographs.iarc.fr).

- The IARC Secretariat consists of scientists affiliated with IARC. They serve as rapporteurs and participate in all discussions. When requested by the meeting chair or subgroup chair, they may also draft text or analyse data.

Before an invitation is extended, each potential participant 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. 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 *Monograph* (Cogliano et al 2004).

The names and affiliations of participants are available on the *Monographs* website (http://monographs.iarc.fr) approximately 2 months before each meeting. It is not acceptable for Observers or third parties to contact participants before a meeting or to lobby them at any time.

All participants are listed, with their addresses, at the beginning of each volume. Each participant who is a member of a Working Group serves as an individual scientist and not as a representative of any organization, government or industry.

6. Working procedures

A separate Working Group is responsible for developing each volume of *Monographs*. A volume contains one or more *Monographs*, which can cover either a single agent or a group of related agents. Approximately one year in advance of a meeting of a Working Group, the topics are announced on the *Monographs* website (http://monographs.iarc.fr) and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant biological and epidemiological data are collected by IARC from recognized sources of information on carcinogenesis, including data storage and retrieval systems such as MEDLINE. Meeting participants who are asked to prepare first drafts of specific sections generally supplement the IARC literature searches with their own searches.

For most chemicals and some complex mixtures, the major collection of data and the preparation of first drafts of the sections on chemical and physical properties, on analysis, on production and use and on occurrence are carried out under a separate contract funded by the

US National Cancer Institute. Industrial associations, labour unions, and other knowledgeable organizations may be asked to provide input to the sections on production and use, although this involvement is not required as a general rule. Information on production and trade is obtained from governmental, trade, and market research publications and, in some cases, by direct contact with industries. Separate production data on some agents may not be available for a variety of reasons (e.g. not collected or made public in all producing countries, production is small, publication could disclose confidential information). Information on uses may be obtained from published sources but is often complemented by direct contact with manufacturers. Efforts are made to supplement this information with data from other national and international sources.

Six months before the meeting, the material obtained is sent to meeting participants to prepare sections for the first drafts of *Monographs*. The first drafts are compiled by IARC staff and sent, prior to the meeting, to all participants of the Working Group for review.

The Working Group meets at IARC for seven to eight days to discuss and finalize the texts of the *Monographs* and to formulate the evaluations. The objectives of the meeting are peer review and consensus. During the first few days, four subgroups (covering exposure data, cancer in humans, cancer in experimental animals, and mechanistic and other relevant data) review the first drafts, develop a joint subgroup draft, and write summaries. Care is taken to ensure that each study summary is written or reviewed by someone not associated with the study being considered. During the last few days the Working Group meets in plenary session to review the subgroup drafts and develop the consensus evaluations.

After the meeting, the master copy of each *Monograph* is verified by consulting the original literature, edited and prepared for publication. The aim is to publish *Monographs* within six months of the Working Group meeting. Summaries are available on the *Monographs* website soon after the meeting.

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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, directly impinging on its interpretation, should be brought to the attention of the reader, a Working Group comment is given in square brackets.

7. Exposure data

Sections that indicate the extent of past and present human exposure, the sources of exposure, the people most likely to be exposed and the factors that contribute to the exposure are included at the beginning of each *Monograph*.

Most *Monographs* on chemical agents include sections on chemical and physical data, on analysis, on production and use, on occurrence, and on human occupational and environmental exposures. *Monographs* on biological agents have sections on taxonomy, structure and biology, methods of detection, human exposures, epidemiology of infection and

clinical disease other than cancer. Whenever appropriate, a *Monograph* may include other sections such as historical perspectives, description of an industry or habit, physical chemistry or taxonomy.

For chemical agents, the Chemical Abstracts Services Registry Number, the latest Chemical Abstracts Primary Name and the IUPAC Systematic Name are recorded; other synonyms are given, but the list is not necessarily comprehensive. For biological agents, taxonomy and structure are described, and the degree of variability is given, when applicable.

Information on chemical and physical properties that are relevant to identification, occurrence and biological activity are included. A description of technical products of chemicals includes trades names, relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in which the agent being evaluated is only one of the ingredients. For biological agents, mode of replication, life cycle, target cells, persistence and latency and host response are given.

The purpose of the section on analysis or detection is to give the reader an overview of current methods, with emphasis on those widely used for regulatory purposes. Methods for monitoring human exposure are also given, when available. No critical evaluation or recommendation of any of the methods is meant or implied. For biological agents, methods of detection and exposure assessment are described, including their sensitivity, specificity and reproducibility.

The dates of first synthesis and of first commercial production of a chemical or mixture are provided when available; for agents which do not occur naturally, this information may allow a reasonable estimate to be made of the date before which no human exposure to the agent could have occurred. The dates of first reported occurrence of an exposure are also provided when available. In addition, methods of synthesis used in past and present commercial production and different methods of production, which may give rise to different impurities, are described.

The countries where companies report production of the agent, and the number of companies in each country, are identified. Available data on production, international trade and uses are obtained for representative regions. It should not, however, be inferred that those areas or nations are necessarily the sole or major sources or users of the agent. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice nor does it imply judgement as to their therapeutic efficacy.

Information on the occurrence of an agent or mixture in the environment and information on human exposures is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are also included. Such data may be available from national databases (for example, those of the US Environmental Protection Agency). It is important that the Working Group attempt to obtain data on exposures in developing countries, and other UN agencies may be helpful in this regard. In the case of mixtures, industries, occupations or processes, information is given about all agents present. For processes, industries and occupations, a historical description is also given, noting variations in chemical composition, physical properties and levels of

occupational exposure with time and place. For biological agents, the epidemiology of infection is described.

Statements concerning regulations and guidelines (e.g. occupational exposure limits, maximal levels permitted in foods and water, pesticide registrations) are included as indications of potential exposures, but they may not reflect the most recent situation, since such limits are continuously reviewed and modified. The absence of information on regulatory status for a country should not be taken to imply that that country does not have regulations with regard to the exposure. For biological agents, legislation and control, including vaccines and therapy, are described.

8. Studies of cancer in humans

(a) Types of studies considered

Three types of epidemiological studies of cancer contribute to the assessment of carcinogenicity in humans–cohort studies, case-control studies and correlation (or ecological) studies. Rarely, results from randomized trials may be available. Case series and case reports of cancer in humans may also be reviewed.

Cohort and case-control studies relate individual exposures under study to the occurrence of cancer in individuals and provide an effect estimate as the main measure of association.

In correlation studies, the units of investigation are usually whole populations (e.g. in particular geographical areas or at particular times), and cancer frequency is related to a summary measure of the exposure of the population to the agent, mixture or exposure circumstance under study. Because individual exposure is not documented, however, a causal relationship is less easy to infer from correlation studies than from cohort and case-control studies. Case reports generally arise from a suspicion, based on clinical experience, that the concurrence of two events-that is, a particular exposure and occurrence of a cancer-has happened rather more frequently than would be expected by chance. Case reports usually lack complete ascertainment of cases in any population, definition or enumeration of the population at risk and estimation of the expected number of cases in the absence of exposure. The uncertainties surrounding interpretation of case reports and correlation studies make them inadequate, except in rare instances, to form the sole basis for inferring a causal relationship. When taken together with case-control and cohort studies, however, relevant case reports or correlation studies may add materially to the judgement that a causal relationship is present.

Epidemiological studies of benign neoplasms, presumed preneoplastic lesions and other end-points thought to be relevant to cancer are also reviewed by the Working Group. They may, in some instances, strengthen inferences drawn from studies of cancer itself.

(b) Quality of studies considered

It is necessary to take into account the possible roles of bias, confounding and chance in the interpretation of epidemiological studies. Bias is the operation of factors in study design or execution that lead erroneously to a stronger or weaker association than in fact exists between disease and an agent, mixture or exposure circumstance. Confounding is a form of bias that occurs when the relationship with disease is made to appear stronger or to appear

weaker than it truly is as a result of an association between the apparent causal factor and another factor that is associated with either an increase or decrease in the incidence of the disease. In evaluating the extent to which these factors have been minimized in an individual study, the Working Group considers a number of aspects of design and analysis as described in the report of the study. For example, when suspicion of carcinogenicity arises largely from a single small study, careful consideration should be given when interpreting subsequent studies that included these data in an enlarged population. Most of these considerations apply equally to case-control, cohort and correlation studies. Lack of clarity of any of these aspects in the reporting of a study can decrease its credibility and the weight given to it in the final evaluation of the exposure.

Firstly, the study population, disease (or diseases) and exposure should have been well defined by the authors. Cases of disease in the study population should have been identified in a way that was independent of the exposure of interest, and exposure should have been assessed in a way that was not related to disease status.

Secondly, the authors should have taken account – in the study design and analysis – of other variables that can 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, such as by matching, or in the analysis, by statistical adjustment. In cohort studies, comparisons with local rates of disease may or may not be more appropriate than those with national rates. Internal comparisons of disease frequency among individuals at different levels of exposure are also desirable in cohort studies, since they minimize the potential for confounding related to 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 time since exposure began 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) Meta-analysis and pooled analysis of population-based studies

Independent population-based studies of the same agent may lead to results that are ambiguous. Combined analyses of data from multiple studies are a means of resolving this ambiguity, and well-conducted analyses can be considered by the Working Group. There are two types of combined analysis. The first involves combining summary statistics such as relative risks from individual studies (meta-analysis), and the second involves a pooled analysis of the raw data from the individual studies (pooled analysis).

Advantages of combined analyses are increased precision due to increased sample size and the opportunity to explore potential confounders, interactions and modifying effects that may explain heterogeneity among studies in more detail. A disadvantage of combined analyses is the possible lack of compatibility of data from various studies due to differences in subject recruitment, data collection procedures, measurement methods and effects of unmeasured co-variates that may differ among studies. Despite these limitations, well conducted combined analyses may provide a firmer basis than individual studies for drawing conclusions about potentially carcinogenic agents.

Meta-analyses may be conducted by the Working Group during the course of preparing a *Monograph* and are identified as original calculations by placing the results within square brackets. These may be de-novo analyses or updates of previously conducted analyses that incorporate the results from new studies. Whenever possible, however, it is preferable that such analyses be conducted prior to the *Monograph* meeting. Publication of the results of such meta-analyses prior to or concurrently with the *Monograph* meeting is encouraged for purposes of peer review. It is important that the same criteria for data quality be applied to combined analyses as would be applied to individual studies and that such analyses take heterogeneity between studies into account.

(d) Inferences about mechanisms of carcinogenesis

Detailed analyses of both relative and absolute risks in relation to temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure, peak exposure (when appropriate) and time since exposure ceased, are reviewed and summarized when available. The analysis of temporal relationships can be useful in formulating models of carcinogenesis. In particular, such analyses may suggest whether a carcinogen acts early or late in the process of carcinogenesis, although at best they allow only indirect inferences about the mechanism of action.

Special attention is given to results that may allow inferences about putative mechanisms of action (IARC, 1991; Vainio et al., 1992; Toniolo et al., 1997; Vineis et al., 1999; Buffler et al., 2004). The presence or absence of mechanistic biomarkers should be considered in the evaluation of causality. Mechanistic biomarkers are molecular, cellular or other biological changes implicated in the sequence of events by which an exposure or agent contributes to cancer. They include:

- Biomarkers of internal dose, of damage to DNA, proteins, and other chemical or structural components of the cell;

- Biomarkers of early effects, also known as 'intermediate biomarkers', such as mutations, chromosomal aberrations, genetic and genomic instability, or epigenomic modifications;

- Biomarkers of cellular, tissue or organism responses, such as hormonal, inflammatory or immunological responses;

- Biomarkers of genetic susceptibility, such as genetic variations affecting gene-environment interactions, which can contribute to increase the plausibility of association by demonstrating a modulation of risk in relation with genetic variation affecting a suspected causal pathway.

When these biomarkers contribute to the evaluation of carcinogenicity in humans, they are included in the section on cancer in humans. When they are informative about the mechanisms of carcinogenesis, they are included in the section on mechanistic and other relevant data.

Molecular epidemiological data that identify associations between genetic polymorphisms and interindividual differences in susceptibility to the agent(s) being evaluated may contribute to the identification of carcinogenic hazards to humans. If the polymorphism has been experimentally demonstrated to modulate gene function in a way consistent with increased susceptibility, these data can serve as additional evidence for causality. Similarly, molecular epidemiological studies that measure cell functions, enzymes or metabolites thought to be the basis of susceptibility can be taken as evidence that reinforces biological plausibility. It should be noted, however, that when data on genetic susceptibility originate from multiple comparisons arising from subgroup analyses, this can generate false-positive results and inconsistencies across studies, and such data therefore require careful evaluation. If the known phenotype of a genetic polymorphism can explain the carcinogenic mechanism of the agent to be evaluated, these data can serve as additional evidence for causality.

(e) Criteria for causality

After the quality of individual epidemiological studies of cancer has been summarized and assessed, a judgement is made concerning the strength of evidence that the agent, mixture or exposure circumstance in question is carcinogenic for humans. In making their judgement, the Working Group considers several criteria for causality. A strong association (i.e. a large relative risk) is more likely to indicate causality than a weak association, although it is recognized that relative risks of small magnitude do not imply lack of causality and may be important if the disease or exposure is common. Associations that are replicated in several studies of the same design or using different epidemiological approaches or under different circumstances of exposure are more likely to represent a causal relationship than isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (such as differences in amount of exposure), and results of studies judged to be of high quality are given more weight than those of studies judged to be methodologically less sound.

If the risk of the disease in question increases with the amount of exposure, this is considered to be a strong indication of causality, although absence of a graded response is not necessarily evidence against a causal relationship. Demonstration of a decline in risk after cessation of or reduction in exposure in individuals or in whole populations also supports a causal interpretation of the findings.

Although a carcinogen may act upon more than one target, the specificity of an association (i.e. an increased occurrence of cancer at one anatomical site or of one morphological type) adds plausibility to a causal relationship, particularly when excess cancer occurrence is limited to one morphological type within the same organ. The biological plausibility and coherence of the overall database are also considered.

Although rarely available, results from randomized trials showing different rates among exposed and unexposed individuals provide particularly strong evidence for causality.

When several epidemiological studies show little or no indication of an association between an exposure and cancer, the judgement may be made that, in the aggregate, they show evidence of lack of carcinogenicity. Such a judgement requires first of all that the studies giving rise to it meet, to a sufficient degree, the standards of design and analysis described above. Specifically, the possibility that bias, confounding or misclassification of exposure or outcome could explain the observed results should be considered and excluded with reasonable certainty. In addition, all studies that are judged to be methodologically sound should be consistent with a relative risk of unity for any observed level of exposure and, when considered together, should provide a pooled estimate of relative risk that is at or near unity and has a narrow confidence interval, due to sufficient population size. Moreover, no individual study nor the pooled results of all the studies should show any consistent tendency for relative risk of cancer to increase with increasing level of exposure. It is important to note that evidence of lack of carcinogenicity obtained in this way from several epidemiological studies can apply only to the type(s) of cancer studied and to dose levels and intervals between first exposure and observation of disease that are the same as or less than those observed in all the studies. Experience with human cancer indicates that the period from first exposure to the development of clinical cancer is seldom less than 20 years; latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

9. Studies of cancer in experimental animals

All known human carcinogens that have been studied adequately in experimental animals have produced positive results in one or more animal species (Wilbourn et al., 1986; Tomatis et al., 1989). For several agents (aflatoxins, 4-aminobiphenyl, azathioprine, betel quid with tobacco, BCME and CMME (technical grade), chlorambucil, chlornaphazine, ciclosporin, coal-tar pitches, coal-tars, combined oral contraceptives, cyclophosphamide, diethylstilboestrol, melphalan, 8-methoxypsoralen plus UVA, mustard gas, myleran, 2-naphthylamine, nonsteroidal estrogens, estrogen therapy/steroidal estrogens, solar radiation, thiotepa and vinyl chloride), carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed the carcinogenicity in humans (Vainio et al., 1995). Although this association cannot establish that all agents and mixtures that cause cancer in experimental animals also cause cancer in humans, nevertheless, **in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents and mixtures for which there is *sufficient evidence of carcinogenicity* in experimental animals (see Section 12) as if they presented a carcinogenic risk to humans.** The possibility that a given agent may cause cancer through a species-specific mechanism that does not operate in humans (see Section 12) should also be taken into consideration.

The nature and extent of impurities or contaminants present in the chemical or mixture or agent being evaluated are given when available. Animal species, strain, sex, numbers per group, age at start of treatment, exposure route, dose levels, exposure duration, survival, and tumour information (incidence, latency, severity or multiplicity of neoplasms or preneoplastic lesions) are reported.

Other types of studies summarized may include: experiments in which the agent or mixture was administered in conjunction with known carcinogens or factors that modify carcinogenic effects; studies in which the end-point was not cancer but a defined precancerous lesion; experiments on the carcinogenicity of known metabolites and

derivatives; and studies of cancer in non-laboratory animals (e.g. livestock and companion animals) exposed to the agent.

For experimental studies of mixtures, 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 nonlinear dose-response relationships.

An assessment is made as to the relevance to human exposure of samples tested in experimental animals, which may involve consideration of: (i) physical and chemical characteristics, (ii) constituent substances that indicate the presence of a class of substances, (iii) the results of tests for genetic and related effects, including DNA adducts, proto-oncogene mutation and expression and suppressor gene inactivation. The relevance of results obtained, for example, with animal viruses analogous to the virus being evaluated in the *Monograph* must also be considered. They may provide biological and mechanistic information relevant to the understanding of the process of carcinogenesis in humans and may strengthen the plausibility of a conclusion that the biological agent that is being evaluated is carcinogenic in humans.

(a) Qualitative aspects

An assessment of carcinogenicity involves several considerations of qualitative importance, including (i) the experimental conditions under which the test was performed, including route and schedule of exposure, species, strain, sex, age, duration of follow-up; (ii) the consistency of the results, for example, across species and target organ(s); (iii) the spectrum of neoplastic response, from preneoplastic lesions and benign tumours to malignant neoplasms; and (iv) the possible role of modifying factors.

As mentioned earlier (see Section 4), the *Monographs* intend to summarize all pertinent published studies. Those studies in experimental animals that are inadequate (e.g. too short a duration, too few animals, poor survival; see below) or are judged irrelevant to the evaluation may be omitted. Guidelines for conducting adequate long-term carcinogenicity experiments have been outlined (e.g. Montesano et al., 1986).

Considerations of importance to the Working Group in the interpretation and evaluation of a particular study include: (i) how clearly the agent was defined and, in the case of mixtures, how adequately the sample characterization was reported; (ii) whether the dose was adequately monitored, particularly in inhalation experiments; (iii) whether the doses and duration of treatment were appropriate; (iv) whether the survival of treated animals was similar to that of controls; (v) whether there were adequate numbers of animals per group; (vi) whether animals of both sexes were used; (vii) whether animals were allocated randomly to groups; (viii) whether the duration of observation was adequate; and (ix) whether the data were adequately reported.

When benign tumours occur together with and (a) originate from the same cell type in an organ or tissue as malignant tumours in a particular study and (b) appear to represent a stage in the progression to malignancy, it may be valid to combine them in assessing tumour incidence (Huff et al., 1989). The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed. If an agent or mixture induces only benign neoplasms that appear to be end-points

that do not readily undergo transition to malignancy, it should nevertheless be suspected of being a carcinogen and requires further investigation.

(b) Quantitative aspects

The probability that tumours will occur may depend on the species, sex, strain and age of the animal, the dose of the carcinogen and the route and length of exposure. Evidence of an increased incidence of neoplasms with increased level of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms.

The form of the dose-response relationship can vary widely, depending on the particular agent under study and the target organ. Both DNA damage and increased cell division are important aspects of carcinogenesis, and cell proliferation is a strong determinant of dose-response relationships for some carcinogens (Cohen & Ellwein, 1990). Since many chemicals require metabolic activation before being converted into their reactive intermediates, both metabolic and pharmacokinetic aspects are important in determining the dose-response pattern. Saturation of steps such as absorption, activation, inactivation and elimination may produce nonlinearity in the dose-response relationship, as could saturation of processes such as DNA repair (Hoel et al., 1983; Gart et al., 1986).

(c) Statistical analysis of long-term experiments in animals

Factors considered by the Working Group include the adequacy of the information given for each treatment group: (i) the number of animals studied and the number examined histologically, (ii) the number of animals with a given tumour type and (iii) length of survival. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose (Peto et al., 1980; Gart et al., 1986). When there is no difference in survival between control and treatment groups, the Working Group usually compares the proportions of animals developing each tumour type in each of the groups. Otherwise, consideration is given as to whether or not appropriate adjustments have been made for differences in survival. These adjustments can include: comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour is discovered), in the case where most differences in survival occur before tumours appear; life-table methods, when tumours are visible or when they may be considered fatal because mortality rapidly follows tumour development; and the Mantel-Haenszel test or logistic regression, when occult tumours do not affect the animals' risk of dying but are incidental findings at autopsy.

In practice, classifying tumours as fatal or incidental may be difficult. Several survival-adjusted methods have been developed that do not require this distinction (Gart et al., 1986).

Formal statistical methods have been developed to incorporate historical control data into the analysis of data from an experiment. These methods assign an appropriate weight to historical and concurrent controls on the basis of the extent of between-study and within-study variability: little weight to historical controls when they show a high degree of variability, and greater weight when they show little variability. It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of the historical controls, particularly when historical controls show high between-study variability and are, thus, of little relevance to the current experiment. In analysing results for uncommon tumours, however, the analysis may

be improved by considering historical control data, particularly when between-study variability is low.

Meta-analyses or pooled analyses of animal experiments may be used as an aid in interpreting animal data.

10. Mechanistic and other relevant data

In coming to an overall evaluation of carcinogenicity in humans (see Section 12), the Working Group also considers mechanistic and other relevant data. The nature of the information selected for this section depends on the agent being considered. The Working Group should select representative studies to give a concise description of the data relevant to carcinogenesis, plus any additional data on toxic effects other than cancer that they consider to be important.

For chemicals or complex mixtures of chemicals such as those in some occupational situations and involving cultural habits (e.g. tobacco smoking), the data considered to be relevant are divided into those on absorption, distribution, metabolism and excretion; mechanisms of carcinogenesis, including genetic toxicity; susceptible individuals, populations, and life stages; toxic effects, including reproductive and developmental effects; and additional relevant data, including the biological effects related to physical and chemical properties and structure-activity relationships.

Concise information is given on absorption, distribution (including placental transfer), metabolism and excretion in both humans and experimental animals. Kinetic factors that may affect the dose-response relationship, such as saturation of uptake, protein binding, metabolic activation, detoxification and DNA repair processes, are mentioned. Studies that indicate the metabolic fate of the agent in humans and in experimental animals are summarized briefly, and comparisons of data from humans and animals are made when possible. Comparative information on the relationship between exposure and the dose that reaches the target site may be of particular importance for extrapolation between species.

Concise information is also given on the potential mechanisms by which an exposure or agent alters cell physiology in a way consistent with increased cancer risk. Over time, progress in the understanding of carcinogenesis have resulted in the use of different terms and concepts to describe the process by which a cell acquires and accumulates molecular and cellular modifications that contribute to cancer. The emergence of increasingly complex bioassays and bioinformatic methods provides new means to assess the overall nature and consequences of these modifications. Critical steps in this process include the acquisition by the cell of an enhanced proliferation capacity (through self-sufficiency in growth signal and insensitivity to growth-inhibitory signals), unlimited replicative capacity, evasion from apoptosis, relaxed control over genetic and genomic stability, sustained angiogenesis, and local and distant invasion capacity (Hanrahan & Weinberg, 2000). The acquisition of these characteristics may be driven by irreversible or reversible molecular and cellular modifications. Irreversible changes include genetic and genomic alterations, as well as permanent, epigenetic modifications. Reversible changes may include, among others, repairable damage to cellular components and untimely or excessive activation or inhibition of specific regulatory pathways. In many instances, an exposure or agent can affect several of the above critical steps through different mechanisms.

The Working Group should attempt to identify the possible mechanisms by which the agent may increase the risk of cancer. For each possible mechanism, a representative selection of key data from humans and experimental systems is summarized. Attention is given to data gaps and to data that may suggest the operation of other mechanisms. The section need not cite every study and should focus on giving a clear description of the mechanism and show whether it is or is not supported by the available literature. The relevance of the mechanism to humans is discussed, in particular, when mechanistic data are derived from experimental model systems.

For the agent, mixture or exposure circumstance being evaluated, the available data on end-points or other phenomena relevant to mechanisms of carcinogenesis from studies in humans, experimental animals and other experimental model systems are summarized within one or more of the following descriptive dimensions:

(i) Evidence for structural changes or structural damage at the molecular level, including, in particular, genotoxicity: for example, structure-activity considerations, formation of adducts to DNA and other biomolecules, formation of DNA strand breaks, mutagenicity (including effects on specific genes), chromosomal mutation/aneuploidy, or changes in DNA methylation.

(ii) Evidence for functional changes in cell signalling pathways involved in alterations of critical mechanisms of tumour occurrence, progression and development. These functional changes include, for example, activation or inactivation of enzymes involved in metabolic activation of xenobiotics, changes in DNA repair capacity, changes to the structure and amount of the products of cancer-related genes, changes to the pattern of post-translational modifications of proteins, consistent with activation or inhibition of signalling pathways (i.e. protein phosphorylation, acetylations and other covalent modifications), changes in chromatin structures, in DNA packing and in DNA metabolism, changes in the secretion of growth and survival regulatory factors or metabolites that may affect the behaviour of adjacent cells, and changes in the expression of cell differentiation markers and in intercellular communications.

(iii) Evidence for morphological, physiological or behavioural changes at the cell, tissue and organism level. These changes may include mitogenesis, compensatory cell proliferation, evasion from apoptosis, bypass of replicative senescence, hyperplasia, metaplasia or preneoplasia, angiogenesis, local or distant invasion, acute or chronic inflammation, and effects on the immune response.

These dimensions are not mutually exclusive, and an agent may fall within more than one of them. Thus, for example, the action of an agent on the expression of relevant genes could be summarized under all dimensions, for example, mechanisms relevant to mutagenesis in the first dimension, effects on the accumulation, localization and activity of the gene product and of its molecular targets in the second dimension, and effects on cell behaviour in the third dimension. In assessing these dimensions, evidence from dose and time relationships of carcinogenic effects, and of their contribution to the natural history of cancer, are considered. For example, consideration is given as to whether the mechanism may act early or late during tumour development.

Tests of genetic and related effects are described in view of the relevance of gene mutation and chromosomal damage to carcinogenesis (Vainio et al., 1992; McGregor et al.,

1999). The adequacy of the reporting of sample characterization is considered and, where necessary, commented upon; with regard to complex mixtures, such comments are similar to those described for animal carcinogenicity tests. The available data are interpreted critically by phylogenetic group according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations, aneuploidy and cell transformation. The concentrations employed are given, and mention is made of whether use of an exogenous metabolic system in vitro affected the test result. These data are listed in tabular form.

Positive results in tests using prokaryotes, lower eukaryotes, insects and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information about the types of genetic effect produced and about the involvement of metabolic activation. Some end-points described are clearly genetic in nature (e.g. gene mutations and chromosomal aberrations), while others are to a greater or lesser degree associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for tumour-promoting activity and for cell transformation may be sensitive to changes that are not necessarily the result of genetic alterations but that may have specific relevance to the process of carcinogenesis. Critical appraisals of these tests have been published (Montesano et al., 1986; McGregor et al., 1999).

Genetic or other activity manifest in humans and experimental mammals is regarded as being of greater relevance than that in other organisms. The demonstration that an agent or mixture can induce gene and chromosomal mutations in mammals in vivo indicates that it may have carcinogenic activity, although this activity may not be detectably expressed in all species. Relative potency in tests for mutagenicity and related effects is not a reliable indicator of carcinogenic potency. Negative results in tests for mutagenicity in selected tissues from animals treated in vivo provide less weight, partly because they do not exclude the possibility of an effect in tissues other than those examined. Moreover, negative results in short-term tests with genetic end-points cannot be considered to provide evidence to rule out carcinogenicity of agents or mixtures that act through other mechanisms (e.g. receptor-mediated effects, cellular toxicity with regenerative proliferation, peroxisome proliferation) (Vainio et al., 1992). Factors that may lead to misleading results in short-term tests have been discussed in detail elsewhere (Montesano et al., 1986; McGregor et al., 1999).

Information is given about which individuals, populations, and life-stages may have greater susceptibility to the agent, based on what is known about the agent's toxicokinetics and mechanisms of carcinogenesis. Relevant toxicokinetic data may include, for example, information about genetic polymorphisms of metabolism, differences in metabolic capacity due to life-stage or the presence of disease, differences in DNA repair capacity, and competition for or alteration of metabolic capacity by medications or other chemical exposures. Relevant data on mechanisms may include factors that make the mechanism more likely to occur in some groups, for example, a pre-existing hormonal imbalance, a suppressed immune system, or periods of higher-than-usual tissue growth or regeneration. Genetic polymorphisms that lead to differences in behaviour (e.g. addiction) are also included.

Data are given on acute and chronic toxic effects other than cancer. Toxic effects that confirm distribution and biological effects at the sites of tumour development, or toxicity that alters physiology in a way that could lead to tumour development, are emphasized. Effects on reproduction, teratogenicity, fetotoxicity and embryotoxicity are also summarized briefly. The adequacy of epidemiological studies of reproductive outcome and genetic and related

effects in humans is evaluated by the same criteria as are applied to epidemiological studies of cancer.

Structure-activity relationships that may be relevant to an evaluation of the carcinogenicity of an agent, the toxicological implications of the agent's physical and chemical properties, and any other data relevant to the evaluation that are not included elsewhere, are also described.

For biological agents–viruses, bacteria and parasites–other data relevant to carcinogenicity include descriptions of the pathology of infection, molecular biology (integration and expression of viruses, and any genetic alterations seen in human tumours) and other observations, which might include cellular and tissue responses to infection, immune response and the presence of tumour markers.

11. Summary and integration

This section is a summary of data that are presented in the preceding sections. Summaries will appear on the *Monographs* website (http://monographs.iarc.fr).

(a) Exposure data

Human exposure is summarized, as appropriate, on the basis of elements such as production, use, occurrence and exposure levels in the workplace and environment and measurements in human tissues and body fluids. Quantitative data and time trends are given when available, in comparing exposures in different occupations and environmental settings. Exposure to biological agents is described in terms of transmission, prevalence and persistence of infection.

(b) Cancer in humans

Results of epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized. The target organ(s) or tissue(s) where an increase in cancer was observed should be identified. Dose-response and other quantitative data may be given when available.

(c) Cancer in experimental animals

Data relevant to an evaluation of carcinogenicity in animals are summarized. For each animal species and route of administration, it is stated whether an increased incidence, reduced latency, or increased severity or multiplicity of neoplasms or preneoplastic lesions were observed, and the tumour sites are indicated. If the agent or mixture produced tumours after prenatal exposure or in single-dose experiments, this is also indicated. Negative findings and inverse relationships are also summarized. Dose-response and other quantitative data may be given when available.

(d) Mechanistic and other relevant data

Data relevant to the identification of the possible mechanism(s) of carcinogenicity are summarized. This includes information on toxicokinetics (absorption, distribution,

metabolism, excretion) and mechanistic data (e.g. on genetic toxicity, epigenetic effects). In addition, information on susceptible individuals, populations and life stages is summarized. This section also reports on other toxic effects, including reproductive and developmental effects, as well as additional relevant data, that are considered to be important.

(e) Integration

The reasoning that the Working Group used to reach its evaluation is presented and discussed. This section integrates the major findings from studies of cancer in humans, cancer in experimental animals, and mechanistic and other relevant data. It includes general statements of the principal line(s) of argument that emerged, the Working Group's conclusions on the strength of the evidence for each group of studies, citations to indicate which studies were pivotal to the Working Group's conclusions, and an explanation of the Working Group's reasoning in weighing data and making evaluations (see Section 12).

The discussion should not necessarily be limited to one specific line of argument favouring the Working Group's evaluation. It should, when the Working Group could not reach consensus, indicate the differences of scientific opinion that were evident during the meeting. There should be some indication of the relative degree of support for each alternative position.

12. Evaluation

Evaluations of the strength of the evidence for carcinogenicity arising from human and experimental animal data are made, using standard terms. There is also a characterization of the strength of the mechanistic evidence.

It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of carcinogenicity. In considering all of the relevant scientific data, the Working Group may assign the agent, mixture or exposure circumstance to a higher or lower category than a strict interpretation of these criteria would indicate.

These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency). A classification may change as new information becomes available.

An evaluation of degree of evidence, whether for a single agent or a mixture, is limited to the materials tested, as defined physically, chemically or biologically. When the agents evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single evaluation of degree of evidence.

(a) Carcinogenicity in humans

The applicability of an evaluation of the carcinogenicity of a mixture, process, occupation or industry on the basis of evidence from epidemiological studies depends on the variability over time and place of the mixtures, processes, occupations and industries. The Working Group seeks to identify the specific exposure, process or activity which is considered most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data on exposure and other aspects permit.

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

***Sufficient evidence of carcinogenicity*:** The Working Group considers that a causal relationship has been established between exposure to the agent, mixture or exposure circumstance and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence. A statement that there is *sufficient evidence* should be followed by a separate sentence that identifies the target organ(s) or tissue(s) where an increased risk of cancer was observed in humans.

***Limited evidence of carcinogenicity*:** A positive association has been observed between exposure to the agent, mixture or exposure circumstance and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

***Inadequate evidence of carcinogenicity*:** The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association between exposure and cancer, or no data on cancer in humans are available.

***Evidence suggesting lack of carcinogenicity*:** There are several adequate studies covering the full range of levels of exposure that human beings are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent, mixture or exposure circumstance and any studied cancer at any observed level of exposure. The results from these studies alone or combined should have tight confidence intervals with an upper limit close to the null value (i.e. a relative risk of 1.0). Bias and confounding should be ruled out with reasonable confidence, and the studies should have an adequate length of followup. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the cancer sites, conditions and levels of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

In some instances, the above categories may be used to classify the degree of evidence related to carcinogenicity in specific organs or tissues.

(b) Carcinogenicity in experimental animals

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

***Sufficient evidence of carcinogenicity*:** The Working Group considers that a causal relationship has been established between the agent or mixture and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals, (b) both sexes of a single species in a study conducted under Good Laboratory Practices (e.g. a US National Toxicology Program study) or (c) in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

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

***Limited evidence of carcinogenicity*:** The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; or (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; or (c) the agent or mixture increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential, or of certain neoplasms which may occur spontaneously in high incidences in certain strains.

***Inadequate evidence of carcinogenicity*:** The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations, or no data on cancer in experimental animals are available.

***Evidence suggesting lack of carcinogenicity*:** Adequate studies involving at least two species are available which show that, within the limits of the tests used, the agent or mixture is not carcinogenic. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the species, tumour sites, age at exposure, and conditions and levels of exposure studied.

(c) Mechanistic and other relevant data

Mechanistic and other evidence judged to be relevant to an evaluation of carcinogenicity and of sufficient importance to affect the overall evaluation is highlighted. This may include data on preneoplastic lesions, tumour pathology, genetic and related effects, structure-activity relationships, metabolism and pharmacokinetics, physicochemical parameters and analogous biological agents.

The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is assessed, using terms such as weak, moderate or strong. Then the Working Group assesses if that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans come from data on humans or biological specimens obtained from exposed humans. The data may be considered to be especially relevant if they show that the agent in question has caused changes in exposed humans that are on the causal pathway to carcinogenesis. Such data may, however, never become available, because it is at least conceivable that certain compounds may be kept from human use solely on the basis of evidence of their toxicity and/or carcinogenicity in experimental systems.

The conclusion that a mechanism operates in experimental animals is strengthened by findings of consistent results in different experimental systems, by demonstrating biological plausibility, and by coherence of the overall database. Strong support can be obtained from studies that experimentally challenge the hypothesized mechanism, by demonstrating that suppression of key mechanistic processes leads to suppression of tumour development. The Working Group should consider whether multiple mechanisms might contribute to tumour development, whether different mechanisms might operate in different dose ranges, whether separate mechanisms might operate in humans and experimental animals, and whether a

unique mechanism might operate in a susceptible group. The possible contribution of alternative mechanisms must be considered before concluding that tumours observed in experimental animals are not relevant to humans. An uneven level of experimental support for different mechanisms may reflect that disproportionate resources have been focused on investigating a favoured mechanism.

Current or anticipated levels of human exposure are not used to determine whether a mechanism operates in humans. In terms of the risk assessment paradigm (see Section 2), a conclusion that a mechanism does not operate in humans is a matter of hazard, not exposure or risk. Such a conclusion should be valid in the case of accidental and unanticipated human exposures that are difficult to foresee at present.

For complex exposures, including occupational and industrial exposures, the chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

(d) Overall evaluation

Finally, the body of evidence is considered as a whole, in order to reach an overall evaluation of the carcinogenicity to humans of an agent, mixture or circumstance of exposure.

An evaluation may be made for a group of agents that have been evaluated by the Working Group. In addition, when supporting data indicate that other, related agents for which there is no direct evidence of capacity to induce cancer in humans or in animals may also be carcinogenic, a statement describing the rationale for this conclusion is added to the evaluation narrative; an additional evaluation may be made for this broader group of agents if the strength of the evidence warrants it.

The agent, mixture or exposure circumstance is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent, mixture or exposure circumstance is a matter of scientific judgement, reflecting the strength of the evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data.

Group 1: The agent (mixture) is *carcinogenic to humans.*
The exposure circumstance entails exposures that are *carcinogenic to humans.*

This category is used when there is *sufficient evidence of carcinogenicity* in humans. Exceptionally, an agent (mixture) may be placed in this category when evidence of carcinogenicity in humans is less than *sufficient* but there is *sufficient evidence of carcinogenicity* in experimental animals and strong evidence in exposed humans that the agent (mixture) acts through a relevant mechanism of carcinogenicity.

Group 2

This category includes agents, mixtures and exposure circumstances for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost *sufficient*, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents, mixtures and exposure circumstances are assigned to either Group 2A (*probably carcinogenic to humans*) or Group 2B (*possibly carcinogenic to humans*) on the basis of epidemiological and experimental evidence of carcinogenicity and mechanistic and other relevant data. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used simply as descriptors of different levels of evidence of human carcinogenicity, with *probably carcinogenic* signifying a higher level of evidence than *possibly carcinogenic*.

Group 2A: The agent (mixture) is *probably carcinogenic to humans.*
The exposure circumstance entails exposures that are *probably carcinogenic to humans.*

This category is used when there is *limited evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals. In some cases, an agent (mixture) may be classified in this category when there *is inadequate evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent, mixture or exposure circumstance may be classified in this category solely on the basis of *limited evidence of carcinogenicity* in humans.

Group 2B: The agent (mixture) is *possibly carcinogenic to humans.*
The exposure circumstance entails exposures that are *possibly carcinogenic to humans.*

This category is used for agents, mixtures and exposure circumstances for which there is *limited evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals. It may also be used when there is *inadequate evidence of carcinogenicity* in humans but there is *sufficient evidence of carcinogenicity* in experimental animals. In some instances, an agent, mixture or exposure circumstance for which there is *inadequate evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals together with supporting evidence from mechanistic and other relevant data may be placed in this group. Possible carcinogenicity can be assessed solely on the basis of strong evidence from mechanistic and other relevant data.

Group 3: The agent (mixture or exposure circumstance) *is not classifiable as to its carcinogenicity to humans.*

This category is used most commonly for agents, mixtures and exposure circumstances for which the evidence of carcinogenicity is *inadequate* in humans and *inadequate* or *limited* in experimental animals.

Exceptionally, agents (mixtures) for which the evidence of carcinogenicity is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents, mixtures and exposure circumstances that do not fall into any other group are also placed in this category.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed, especially when exposures are widespread or the cancer data are consistent with differing interpretations.

Group 4: The agent (mixture) is *probably not carcinogenic to humans.*

This category is used for agents or mixtures for which there is *evidence suggesting lack of carcinogenicity* in humans and in experimental animals. In some instances, agents or mixtures for which there is *inadequate evidence of carcinogenicity* in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of mechanistic and other relevant data, may be classified in this group.

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