WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

INTERNAL REPORT 06/001

Report of the Advisory Group to Review the Amended Preamble to the *IARC Monographs*

6–8 December 2005

LYON, FRANCE
2006

FOREWORD

During 2005, IARC amended the Preamble to the *IARC Monographs*. The Preamble describes the principles and procedures used in developing *IARC Monographs*, including the scientific criteria that guide the evaluations. The objective was to reflect scientific developments and procedural changes that have occurred since the Preamble was last amended in 1991.

The process began in March 2005, when IARC asked meeting chairs from the previous 10 years and subgroup chairs from the previous 5 years for suggestions on which parts of the Preamble should be revised, based on their experience. Their suggestions were considered by an international Advisory Group that met in May 2005 to recommend updates to the Preamble. The report of the May Advisory Group discussed a series of issues and made several recommendations (IARC Internal Report No. 05/001).

The recommendations of the May Advisory Group and the earlier suggestions formed the basis of a draft Preamble prepared by IARC staff. In August 2005, IARC made available the draft Preamble and other materials on the *IARC Monographs* programme website (http://monographs.iarc.fr) and invited the general public, the scientific community, national health agencies and other organizations to comment. Comments received after a two-month period were considered by a larger Advisory Group that met in December 2005 to review the amended Preamble.

Herein is the report the December 2005 Advisory Group. Its recommendations have been incorporated into the amended Preamble, which was given a final review by that Advisory Group. The amended Preamble will be used from the February 2006 *Monographs* meeting onwards.

IARC thanks the German Federal Ministry of Health and Social Security for financial support for the May and December Advisory Group meetings. IARC also thanks the Members of the May and December Advisory Groups, the meeting chairs and subgroup chairs who made useful suggestions, and the individual and institutional commentors who submitted valuable suggestions and perspectives. These contributions have all helped to enhance and renovate the *IARC Monographs* programme.

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LIST OF PARTICIPANTS

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[1] Advisory Group members serve in their individual capacities as scientists and not as representatives of their government or any organization with which they are affiliated. Affiliations are provided for identification purposes only.

[2] Also served on the May 2005 Advisory Group to recommend updates to the Preamble.

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Acknowledgement

IARC thanks the German Federal Ministry of Health and Social Security for financial support for this Advisory Group meeting.

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European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Belgium
International Institute of Synthetic Rubber Producers (IISRP), USA
International Union, United Automobile, Aerospace and Agricultural Implement Workers of America (UAW), USA
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[7] Serves on review panels for the US Environmental Protection Agency and the Minnesota Department of Health, for which compensation will be received through Versar and Eastern Research Group, respectively. (These agencies sometimes use private contractors to convene review meetings).

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General comments

The Advisory Group (AG) was generally impressed with the new version of the Preamble and commended the Secretariat on a document that addressed the deficiencies seen in the previous version while maintaining the integrity of the *Monographs* Programme. The Secretariat had done an excellent job of considering and including the comments suggested by previous Working Group Chairs, the May Advisory Group (MAG) and others who offered advice in advance of the development of a draft Preamble. In addition, the solicitation of outside comments prior to the meeting of the AG provided a broad perspective of the issues that are of concern to interested parties in the draft Preamble and will definitely lead to an improved Preamble and improved *Monographs* Programme.

The majority of the AG comments were focused on clarification of the intent of the wording in the draft Preamble rather than substantive changes in the outlined process. However, there were a few issues that the AG wished to highlight as important modifications suggested for the final Preamble. These include:

1. Restructure to two basic sections – General Principles and Procedures, Scientific Review;
2. Changes to the tone and tenor of the levels of evidence used to evaluate carcinogenicity data from laboratory experiments;
3. The use and utility of mechanistic data in modifying both degrees of evidence and the final classification in Working Group deliberations;
4. Clarification of the role of invited experts and representatives in the Working Group evaluations; and
5. Balance and conflict of interest.

Each of these issues were discussed within the context of the recommendations of the AG that are given below and are broken down into sections that follow those of the draft Preamble.

Structure of the Preamble

In essence, the first six sections of the draft Preamble refer to procedural issues related to the formation, composition and management of a *Monographs* Working Group and could be captured as subheadings under the title 'Part A: General Principles and Procedure'. The core of the scientific review conducted by the Working Group and guidance for the final evaluations are given in Sections 7–12. These could also be grouped under a single title of 'Part B: Scientific Review and Evaluation'. Subsequently, by numbering the sections of Part B, a structure is created in which the Sections of the *Monographs* relate to the numbering in the Preamble. Thus, the new Preamble would have the following structure:

1. Background and brief introduction

This text is fairly short and enhances the historical perspective through which one can view the development of the *Monographs* Programme. In the one-paragraph introduction, the word 'scientific' should be inserted before 'principles' to emphasize that the Preamble defines both the processes used and the scientific principles that support these processes in making decisions for any one agent, mixture or exposure circumstance. In addition, it was suggested that the following text be added to the end of the introductory paragraph:

> The Preamble is primarily a statement of scientific principles, rather than a specification of working procedures. The working procedures through which any IARC Working Group implements these principles are not specified in detail, remain predominantly the prerogative of any individual Working Group and usually involve operations that have been established as being effective during previous *Monographs* meetings.

The AG also recommends that the Secretariat develop a more detailed informational document describing the Preamble and its overall objectives and how it is employed in making Working Group decisions. This document does not need to be part of the formal Preamble but could exist on the IARC web server or as a short document for distribution to interested parties.

2. Objective and Scope

In Section 2, the term 'consensus' is used to describe the final evaluations of the Working Group. In common with three of the public comments (Huff, ECETOC, IISRP), the AG felt this term could lead to confusion. The AG discussed the terminology that might best be used to describe the decision-making process. While the word 'consensus' was considered to be a useful term, it was evident that no single word would adequately cover all options that a

Working Group might legitimately use in arriving at an evaluation. In the light of these considerations, the following was suggested:

> IARC Working Groups strive to achieve a consensus evaluation. Consensus reflects broad agreement among Working Group Members, but not necessarily unanimity. The Working Group Chair may elect to call a vote on issues when consensus is not readily achieved to determine the diversity of opinion among Working Group Members.

Also in Section 2, the public comments (NRDC, Melnick, Huff, ECETOC, IISRP) highlighted concerns regarding the definition of a carcinogen. The AG felt the definition was adequate with a minor exception noted below:

> In these *Monographs*, an agent, mixture or exposure circumstance is termed 'carcinogenic' when it 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.

By placing the *IARC Monographs* into their proper context in the overall process of risk assessment, others may understand clearly what part of the process is being addressed. However, members of the AG noted that the process of risk assessment is described differently from country to country. To avoid confusion, the AG suggested that the third and fourth paragraphs of Section 2 be replaced with the following text:

> For the *Monographs*, 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. The *Monographs* are an exercise in evaluating a hazard, 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.

While the last paragraph of Section 2 covered the use of the *IARC Monographs* in risk assessment and regulatory decisions, the AG felt there was a broader use and this should be noted. The following modifications to the last paragraph suggest the changes that are needed:

> The *Monographs* are used by national and international authorities to make risk assessments, formulate decisions concerning any necessary preventive measures, provide effective cancer control programmes and decide among the myriad of options that govern a public health decision. The evaluations of IARC Working Groups are scientific, qualitative judgements about the evidence for or against carcinogenicity based on the available data. These evaluations represent only one part of the body of information on which public health decisions may be based. Public health options vary from one situation to another and from country to country, and relate to many factors including different socioeconomic and national priorities. Therefore, no recommendation is given with regard to regulation or legislation, which are the responsibility of the individual governments and/or other international organizations.

Additional public comments pertained to various parts of Section 2. The AG felt that the remaining text used by the Secretariat in the draft Preamble was clear and concise and did not need additional modification.

3. Selection of Topics for the *Monographs*

Only a few public comments related to this section and the AG felt that none of them warranted a change in the draft Preamble. The AG accepted the text as written.

4. Data for the *Monographs*

In the third paragraph of Section 4, the Draft Preamble discusses the inclusion of government reports and limits them to those that have undergone peer-review. The AG felt this wording was too restrictive and suggested that it should be removed from the Preamble. Instead, the AG suggested this wording:

> Government agency reports that are publicly available may be considered.

One public comment (Grilli) noted the existence, in some cases, of data on agents being reviewed in the *Monographs* that could not be included due to the requirement that they be publicly available. Most notably, this could pertain to toxicological information on pharmaceuticals and/or pesticides which has historically been labelled as proprietary and not subject to public disclosure. While it was recognized that the restriction of data to be considered to published scientific research has the potential to preclude consideration of information that is confidential or of otherwise restricted availability but which might impact the evaluation, the AG felt that the strength of the *Monographs* series would be reduced if evaluations were made using data that may not be shared with other scientists and the public at large. Thus, while the AG was concerned that such data are not in the open scientific literature, it fully supported the Secretariat in their use of only 'publicly available' data in the evaluations. That said, prior to each *Monographs* meeting, the AG encourages IARC to seek actively data from different sources (published and unpublished) using multiple mechanisms such as a call for data through the IARC website, to request submission of publications from developing countries, to prepare review articles from publications in local journals and to request data from government agencies. If necessary, during a Working Group meeting, unpublished data could be reviewed and/or analysed by the Working Group Members.

The third sentence of paragraph 3 in Section 4 has too much detail and inappropriately elevates the utility of abstracts (public comment by ECETOC, IISRP). While the AG supported the use of any information, including abstracts, by a Working Group if it is critical to their evaluations, a better wording of this sentence was considered to be:

> Exceptions may be made on an ad-hoc basis to include doctoral theses and other material that are in their final form and publicly available, if their inclusion is considered pertinent to making a final evaluation (see Section 12).

Several other public comments were provided on Section 4, but the AG felt that these were not appropriate for the Preamble.

5. Meeting Participants

There was general support by the AG on the clarification in the Preamble of the roles of the meeting participants. With only minor suggestions (see below), the AG endorsed the description and restrictions given in this section.

Two public comments noted a lack of clarity in the roles of and restrictions placed on Invited Specialists. The AG recognized the importance of using Invited Specialists as a resource for technical information that may assist a Working Group in its deliberations. However, because of the potential for conflict of interest, the AG recommends that Invited Specialists continue to be used by IARC in a limited capacity, and that their involvement be structured in such a way so as not to influence the evaluations. In this context, the AG felt that the role of Invited Specialists in drafting text for the Working Group should be restricted to non-influential issues in exposure such as a general description of data on production and use.

Three public comments suggested (ACC, ECETOC, IISRP) that meeting participants with conflicts of interest simply be required to state these conflicts and not be limited in their role in the Working Group. The AG disagreed with this position and fully supported the limits outlined in the draft Preamble.

Four public comments (Greenberg, Melnick, ACC, UAW) mentioned balance as a key issue in developing a Working Group. The AG agreed that balance of perspectives is an important consideration, but noted that conflict of interest does not necessarily imply prejudice. The restriction of the role of Observers to that of participants who only observe and do not attempt to influence the meeting reduces significantly the concern about balancing conflicts of interest among this category of participant. In contrast, Invited Specialists play an important and critical role by bringing their knowledge and experience to the subgroup and plenary sessions. The data that they emphasize, the particular interpretations they present and the lines of research that they may have explored naturally reflect the particular experience and employment of the Invited Specialists and may also reflect the interests and perspectives of their employers. For these reasons, IARC should consider the evenness of Invited Specialists in certain situations, for example, when the volume and nature of the information that they contribute could appear to influence the evaluation. IARC should re-evaluate the issue of whether or not to balance Invited Specialists or Observers after gaining experience with the new procedures that are currently in place.

For clarity, the AG suggested that the wording regarding Observers be changed to note that they are "… admitted by IARC to a meeting…".

One public comment (Huff) suggested that the role of Representatives be restricted with regard to both numbers and manner of participation similarly to that of Observers. The AG partially agreed and suggested that the Preamble include the sentence:

> Representatives may not serve as Meeting Chair or Subgroup Chair, draft any part of a monograph or participate in the discussions on the evaluations.

The number of Representatives should be decided by the IARC and the AG had no opinion on this issue.

The definition used for the IARC Secretariat appeared to be too restrictive and could prevent temporary visitors to IARC from being included in the list of Working Group Members. The AG suggested the following wording for the first sentence:

> The IARC Secretariat consists of scientists who are designated by IARC and who have relevant expertise.

The possibility that members of the IARC Secretariat be obliged to make a Declaration of Interest was discussed by the AG, and it was concluded that IARC should consider this possibility.

The AG felt that all other public comments were either dealt with appropriately in the draft Preamble or were too detailed to be included.

6. Working Procedures

Two public comments (ECETOC, IISRP) requested that the first drafts of the *IARC Monographs* be made available for public comment [repetition]. The AG noted that, although the draft Preamble refers to the initial write-ups as first drafts, this is a mischaracterization. The initial write-ups of the scientific reviews are in the form of draft working papers, which contain initial compilations and reviews of data that are designed to initiate the discussions and deliberations of a Working Group at the start of a *Monographs* meeting. The working papers typically undergo several cycles of deliberation, review and revision before they achieve a form that could be considered as draft sections of a monograph. Public release of working papers ahead of the meeting would therefore be inappropriate as it could frequently lead to misconceptions regarding the ultimate review and characterization of the evidence by a Working Group and politicize the development process of the *Monographs*.

One reason to release material early is the possibility that data that were not being considered by the Working Group may be identified. The AG felt that a better approach to addressing gaps in data would be a call for relevant data prior to the development of the working papers, coupled with careful selection of experts for the Working Group. In a related comment (Huff), it was noted that, if draft working papers are provided to observers prior to the meeting, they should be made available to others who cannot afford to attend the meeting but who are interested in the issue. The AG noted this as a concern, and recommends that working papers not be sent to Observers ahead of the meeting. Should this occur, public release of working papers or other more restricted releases should be considered. Nevertheless, the AG did not believe release of pre-deliberational drafts to be in the best interest of the *Monographs* programme.

A number of other comments were provided to IARC regarding Section 6. Some related to mixing disciplines in the various breakout groups during a Working Group meeting (UAW, ECETOC). The AG felt that these issues should not be included in the Preamble but recommends that IARC consider them when forming Working Groups. The remaining public comments pertaining to Section 6 were felt to be inappropriate for the Preamble.

Sections 7–10

The AG felt that the core of the scientific review conducted by a Working Group will receive major guidance from Sections 7, 8, 9 and 10. In view of the many public comments on these sections and the subtle changes in language that the Group wanted to incorporate, the AG decided to provide IARC with a modified draft of these sections rather than comments on what should be changed. In many cases, the changes the AG made to these sections address public comments, but not all public comments were deemed appropriate and many were therefore not included in the changes. Where appropriate, the AG inserted commentary enclosed in square brackets ([]) into the draft text to explain some changes or support individual passages. The AG did not provide further commentary on these sections and felt that the new drafts provide an ample description of their intent. The suggested wording for Sections 7–10 is given in the Appendix.

11. Summary and Integration

There was broad support within the AG and from the public comments for an integration section that explains the basis for the conclusion. It was felt that this section will improve the transparency of the evaluations and increase public confidence and understanding. In general, the AG felt that the language used in the draft Preamble was clear and concise. One comment (Tomatis) suggested a change in the title was needed to replace 'Integration' with 'Rationale'. The AG agreed that this would be an improvement.

Finally, this section should not provide new data and the last sentence of section (c) should therefore be altered to read:

> Dose–response and other quantitative data may be summarized when available.

12. Evaluation

Several comments (IISRP, Grilli, Huff) suggested that the actual names and/or numbers of categories be altered to provide greater flexibility in and/or clarity of interpretation. The AG felt that the current categories used by the IARC were adequate, had stood the test of time and should remain effectively the same.

In the evaluation process, consideration of mechanistic data in their entirety occurs at the final stage of the evaluation. However, specific mechanistic findings may be taken into account in determining the confidence that should be vested in particular epidemiological or experimental studies. Hence, although mechanistic information is not excluded from the determination of *sufficient* or *limited evidence*, these determinations are primarily expressions of the outcome from epidemiological and experimental studies, respectively.

One public comment (Tomatis) suggested that the identification of target organ(s) in the description of the levels of evidence of carcinogenicity in humans could mislead readers into believing that other organs have been deemed to be free of agent-induced cancers. The AG recognized this possibility and suggested the following sentence be added to the end of the paragraph on "***Sufficient evidence of carcinogenicity***":

> Identification of a specific target organ or tissue does not preclude the possibility that the agent may cause cancer at other sites.

There was considerable debate in both the AG and the public comments (Huff, NRDC, UAW, ACC, CONCAWE, ECETOC, IISRP] regarding the proposed change to include positive findings in both sexes in a single species from a Good Laboratory Practice study as providing '*sufficient evidence* of carcinogenicity'. The AG supported the recommendation of the MAG and suggested that IARC keep this designation in the Preamble. The debate centred around the issue of the quality of studies versus the independence of laboratories. The AG felt that, if a study of males and females in a single experiment was very well conducted and provided significant detail on the characterization of the animal exposures, care and feeding in the laboratory and the evaluation of pathogens together with a high quality of pathology with external review, then positive results in both males and females could satisfy the criterion of a causal inference in two experiments. The Working Group would still be expected to use their best scientific judgement in making a decision on whether there was sufficient evidence, but the AG felt that the clarification of this issue in the Preamble was warranted.

Given the historical relevance of the two examples listed as (a) and (c) in the draft Preamble, the AG felt that (b) should be included as a separate sentence and that the reference to the NTP be removed. The following language was suggested:

> ***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 or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide *sufficient evidence*.
>
> 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 or age at onset, or at an increased incidence at multiple sites.

There was a suggestion to delete "certain neoplasms which may occur spontaneously in high incidences in certain strains," from the category for '*limited evidence* of carcinogenicity' in animals (Huff) because statistical significance would be achieved only with an incidence that was considerably increased. The AG agreed and suggested that this text be removed, noting that the Working Group may still reduce the degree of evidence if, for a specific agent, the results warrant such a reduction.

The AG also spent a considerable amount of time discussing the use of specialized toxicological studies and the potential categories under which they may be included. Of particular interest were initiation–promotion studies and studies in genetically modified animals. This discussion was initiated due to difficulties associated with the classification of these types of data that had been encountered in recent *Monographs* meetings. The AG felt that the descriptions given for *limited* and *sufficient evidence* do not provide adequate guidance to ensure some degree of consistency in the evaluations made by Working Groups from one *Monographs* meeting to another. However, the AG did not wish to add multiple new examples to the degrees of evidence used for animal experiments. It was felt that the best solution would be to include a single additional description of the weakest level of evidence one might accept as providing *limited evidence* of carcinogenicity from the special studies into this category and expect that reasonable scientists who evaluated other special studies would act accordingly. Agents that only show promoting activity in one or more well-conducted initiation–promotion study, while showing a causal inference for increased carcinogenic activity, would need additional mechanistic data or data from other sources to conclude that this causal inference was *sufficient evidence* of carcinogenicity. Examples of other types of data that may raise this degree of evidence could include multiple initiation–promotion studies in several species and several different organ systems that consistently demonstrate promotional activity, an initiation–promotion study that shows a causal increase in the initiating capacity of the agent or a single two-year carcinogenicity study in a single sex of a single species that demonstrates a causal association. A more detailed discussion of these issues is provided in *IARC Scientific Publications No. 146* and Working Groups may wish to consult this volume when faced with special studies. Hence, it was proposed to add this case to the list of circumstances enumerated under *limited evidence* of carcinogenicity.

In addition, the AG felt that it would be useful to include explicitly these types of study in the list of those to be considered when evaluating the evidence in experimental animals. It

was suggested that the following wording be added to the beginning of Section 12(b) together with a reference to the discussion of the use of these data in *IARC Scientific Publications No. 146*:

> Carcinogenicity 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.

In the light of these recommendations, the AG drafted new text to describe the evaluation of evidence in experimental animals as follows:

> ***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; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent or mixture increases the incidence of benign neoplasms or lesions of uncertain neoplastic potential only; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

Several comments (Huff, Tomatis, ECETOC, IISRP, UAW) noted that the second to last paragraph under 12(c) beginning with "Current or anticipated…" needed to be clarified and/or revised. The AG agreed in principle that this paragraph could be expanded, but did not provide any specific language.

Several public comments related to the proposed change to allow a classification of *possibly carcinogenic to humans* (Group 2B) solely on the basis of strong evidence from mechanistic and other relevant data. The AG supported this clarification by IARC and noted that there is increasing confidence in our understanding of mechanisms which is supported by the science. Other public comments suggested this should be based on the full statement regarding use of mechanistic data given in *IARC Scientific Publications No. 146* (IISRP, ECETOC). The AG encouraged the IARC to consider this possibility.

One public comment (Melnick) supported extending this concept to allow mechanistic data to place a compound into Group 2A. The AG felt that this was possible, but only when the compound is clearly a member of a mechanistic class for which one or more members of the class have *sufficient evidence* of carcinogenicity that places some members in Group 1 or Group 2A.

The IARC Secretariat was encouraged to define a strategy to address situations in which unanimity on an overall evaluation cannot be reached. The AG recommends that the portion of Section 11 that refers to integration be moved to the end of Section 12 as a new topic (e). In cases of differing scientific interpretation, the AG felt that the overall evaluation should reflect the majority view but that the minority view be provided an opportunity to present a brief summary of the alternative position and the scientific rationale for this position. In establishing the majority view, the Working Group Chair may elect initially to take a non-binding poll of the Working Group to establish the extent of agreement and/or disagreement among the Members. The AG discussed the actual wording of this paragraph and proposed an alternative wording which is given below:

> The reasoning that the Working Group used to reach its [consensus] evaluation is presented and discussed. This section integrates the major findings from studies of cancer in humans, studies of cancer in experimental animals and mechanistic and other relevant data. It includes general 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, citations to indicate which studies were pivotal to these conclusions and an explanation of the reasoning of the Working Group in weighing data and making evaluations (see Section 12). When there are significant differences of scientific interpretation among Working Group Members, a brief summary of the alternative interpretations is provided, together with their scientific rationale.

APPENDIX

7. Exposure data

The scope of the *IARC Monographs* has expanded beyond chemicals to include complex mixtures, occupational exposures, lifestyle factors, physical and biological agents and other potentially carcinogenic exposures. In respect of the various classes of agent, the specification and use of appropriate indicators of exposure are undertaken by the Working Group and may be outlined in the General Remarks of the relevant *Monographs* volume.

Data 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, analysis, production and use, occurrence and 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. Those on physical agents that are forms of radiation include sections on energy, range of the radiation and on source and routes of exposure. Those on foreign bodies, fibres and respirable particles include sections on sources and routes of exposure and size range and relative dimension of the particles. Whenever appropriate, a monograph may include other sections such as historical perspectives or the description of an industry or habit.

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 provide 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, plants, 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 (ref. NHANES). In order to understand more fully the carcinogenic risk of an agent, it is important to obtain a full range of data on human exposure. Information on exposure should include relevant findings from both developed and developing countries. Some of these data are not distributed widely and may be available from government reports and other sources. In the case of mixtures, industries, occupations or processes, information is given about all agents known to be 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 date 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, 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

This section includes all epidemiological studies. Studies of biomarkers included when they are relevant to an evaluation of carcinogenicity to humans.

(a) Types of studies considered

Several types of epidemiological study of cancer contribute to the assessment of carcinogenicity in humans—cohort studies, case–control studies, correlation (or ecological) studies and intervention studies. Rarely, results from randomized trials may be available. Case reports and case series 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 estimate of effect (such as relative risk) as the main measure of association. Intervention studies may provide strong evidence for making causal inferences, as exemplified by cessation of smoking and the decrease in risk for lung cancer.

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. In correlation studies, individual exposure is not documented, which renders this kind of study more prone to confounding. In some circumstances, however, correlation studies may be more informative than analytical study designs, as exemplified by exposure to arsenic in drinking-water (IARC, Vol. 84).

In some instances, case reports and case series have provided important information about the carcinogenicity of agents [response to one of the public comments]. These types of study 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 and case series 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 that surround the interpretation of case reports, case series 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, these types of study 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 effect 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. 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, 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 into account — in the study design and analysis —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-analyses and pooled analyses

Independent epidemiological studies of the same agent may lead to results that are difficult to interpret. 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 analyses. 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) (ref).

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 the potential carcinogenicity of agents.

Meta-analyses relevant to a particular monograph may be available as published studies and hence be available for consideration by the Working Group. Alternatively, meta-analyses may be undertaken prior to a *Monographs* meeting, and may occur as a consequence of the topic of the *Monographs* volume being publicized on the IARC website. Publication of the results of such meta-analyses prior to a *Monographs* meeting is a requirement for their consideration. IARC may commission a meta-analysis or pooled analysis that is pertinent to a particular *Monographs* meeting. Finally, as a means of gaining insight from the results of multiple individual studies, ad-hoc calculations that combine data from different studies may be conducted by the Working Group in the course of a *Monographs* meeting. The results of such original calculations, which would be specified in the monograph by presentation in square brackets, might involve updates of previously conducted analyses that incorporate the results of more recent studies or de-novo analyses. Irrespective of the source of data for the meta-analyses and pooled analyses, it is important the same criteria for data quality be applied as those that would be applied to individual studies and to ensure also that sources of heterogeneity between studies be taken into account.

(d) Temporal effects

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 cessation of exposure, are reviewed and summarized when available. Analyses of temporal relationships may be useful

in making causal inferences. In addition, 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.

(e) Use of biomarkers in epidemiological studies

Biomarkers indicate molecular, cellular or other biological changes and are increasingly used in epidemiological studies for various purposes (IARC, 1991; Vainio *et al*., 1992; Toniolo *et al*., 1997; Vineis *et al*., 1999; Buffler *et al*., 2004; Bonassi *et al*., 2005). These may include evidence of exposure, of early effects, of cellular, tissue or organism responses of individual susceptibility and/or host responses and inference of a mechanism. This is a rapidly evolving field that encompasses developments in genomics, epigenomics and other emerging technologies (see Section 10).

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 demonstrated experimentally to modify the functional activity of the gene product in a manner that is consistent with increased susceptibility, these data may be useful in making causal inferences. 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, data on this phenotype may be useful in making causal inferences.

(f) 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 (Hill, 1965). A strong association (e.g. a large relative risk) is more likely to indicate causality than a weak association, although it is recognized that estimates of effect 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.

A number of scenarios may increase confidence in a causal relationship. On the one hand, an agent may be specific in causing tumours at one site or of one morphological type. On the other, carcinogenicity may be evident through causation of multiple tumour types. Temporality, precision of estimates of effect, biological plausibility and coherence of the

overall database are also considered. Data on biomarkers may be employed in an assessment of the biological plausibility of epidemiological observations.

Although rarely available, results from randomized trials that show different rates of cancer 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, a 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 (*a*) be consistent with an estimate of effect of unity for any observed level of exposure and, when considered together, (*b*) provide a pooled estimate of relative risk that is at or near unity and (*c*) have 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 sometimes longer 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 for carcinogenicity in experimental animals have produced positive results in one or more animal species (Wilbourn *et al*., 1986; Tomatis *et al*., 1989). For several agents (e.g. aflatoxins, diethylstilboestrol, solar radiation, vinyl chloride), carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed their 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 that agents and mixtures for which there is *sufficient evidence* of carcinogenicity in experimental animals (see Section 12) present a carcinogenic hazard to humans. In the absence of additional scientific information, these agents or mixtures are considered to pose a carcinogenic hazard to humans. An example of additional scientific information would be data that demonstrate that a given agent causes cancer in animals through a species-specific mechanism that does not operate in humans or data that demonstrate that the mechanism in experimental animals also operates in humans (see Section 12).

The Working Group considers all available long-term studies on cancer in experimental animals with the agent under review. In all experimental settings, the nature and extent of impurities or contaminants present in the mixture or agent being evaluated are given when available. Animal species, strain (including genetic background where applicable), sex, numbers per group, age at start of treatment, exposure route, dose levels, duration of exposure, survival and information on tumours (incidence, latency, severity or multiplicity of neoplasms or preneoplastic lesions) are reported.

Other studies summarized may include: experiments in which the agent or mixture was administered in conjunction with known carcinogens or factors that modify carcinogenic effects (initiation–promotion studies, co-carcinogenicity studies and studies in genetically modified animals); 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 studies of mixtures, consideration is given to the possibility of changes in the physicochemical properties of the individual substances during collection, storage, extraction, concentration and delivery. Another consideration is that chemical and toxicological interactions of components in a mixture may alter dose response relationships. The relevance to human exposure of the test mixture administered in the animal experiment is also assessed. This may involve consideration of the following aspects of the mixture tested: (i) physical and chemical characteristics, (ii) identified constituents that may indicate the presence of a class of substances and (iii) the results of genetic toxicity and related tests.

The relevance of results obtained with an agent that is analogous (e.g. similar structures or similar viruses) to the one being evaluated in the monograph is also considered. Such results 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 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 (including genetic background where applicable), 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 judged irrelevant to the evaluation or judged to be inadequate (e.g. too short a duration, too few animals, poor survival; see below) may be omitted. Guidelines for conducting long-term carcinogenicity experiments have recently been published (e.g. OECD reference).

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 monitored adequately, particularly in inhalation experiments; (iii) whether the doses, duration of treatment and route of exposure 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 both male and female animals were used; (vii) whether animals were allocated randomly to groups; (viii) whether the duration of observation was adequate; and (ix) whether the data were reported adequately.

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, they are usually combined in the assessment of 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, genetic background and age of the animal, the dose of the carcinogen and the route, timing and duration 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. Mechanisms such as induction of DNA damage or repair, altered cell division and cell death rates and changes in intercellular communication are important determinants of dose–response relationships for some carcinogens. 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 non-linearity in the dose–response relationship (Hoel *et al*., 1983; Gart *et al*., 1986), as could saturation of processes such as DNA repair. The dose–response relationship can also be affected by differences in survival among the treatment groups.

(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; Portier & Bailar, 1989; Beiler & Williams, 1993). The choice of the most appropriate statistical method requires consideration of whether or not there are differences in survival among the treatment groups; for example, reduced survival because of non-tumour-related mortality can preclude the occurrence of tumours later in life. When detailed information on survival is not available, comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour is discovered) can be useful when significant differences in survival occur before tumours appear. The lethality of the tumour also requires consideration: the time of death provides an indication of the time of tumour onset for rapidly fatal tumours, and can be evaluated using life-table methods; non-fatal or incidental tumours that do not affect survival can be evaluated using methods such as the Mantel-Haenzel test for changes in tumour prevalence. Methods, such as the Poly-K test, that do not require information on tumour lethality, which is often difficult to determine, can also be used. When data are available on the number and/or size of tumours seen in experimental animals (e.g. papillomas on mouse skin, liver tumours observed through NMR [?nuclear magnetic resonance]), other more complicated statistical procedures may be needed (Kopp-Schneider & Portier; Dunson *et al*.).

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 less 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. Historical controls should be selected to resemble the concurrent controls as closely as possible with respect to species, gender, and strain, as well as other factors such as the basal diet and general laboratory environment that may affect tumour–response rates in control animals (Haseman *et al*., 1984; Greim; Fung; …).

Although meta-analyses and combined analyses of animal experiments are conducted less often than are similar analyses of epidemiological studies due to differences in experimental protocols, both meta-analyses and combined analyses of animal experiments can be useful aids in interpreting animal data when the experimental protocols are sufficiently similar.

10. Mechanistic and other relevant data

Mechanistic and other relevant data provide evidence of carcinogenicity and also help in assessing the relevance and importance of findings of cancer in animals and humans. The nature of the assessment of mechanistic and other relevant data to be evaluated depends on the agent being considered. The Working Group considers representative studies to give a concise description of the relevant data and issues that they consider to be important. Thus, in Section 4 of a monograph, not every available study is typically cited. Relevant topics to be addressed may include toxicokinetics, mechanisms of carcinogenesis, susceptible individuals, populations, life stages, other relevant data and other adverse effects. When data on biomarkers are informative about the mechanisms of carcinogenesis, they are included in this section.

These topics are not mutually exclusive, thus the same studies may be discussed in multiple subsections. For example, a mutation in a gene coding for an enzyme that metabolizes the agent under study could be discussed in the subsections on toxicokinetics, mechanistic data and individual susceptibility if it also exists as an inherited polymorphism. To assess these topics, data on dose, duration and life-stage relationships of carcinogenic effects and on 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.

(a) Toxicokinetics

Toxicokinetics refers to the absorption, distribution, metabolism and elimination of agents in humans, experimental animals and, where relevant, cellular systems. Examples of kinetic factors that may affect the dose–response relationships include tissue half-life, uptake, protein binding, metabolic activation and detoxification. 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 important for extrapolation of hazards between species and in clarifying the role of in-vitro findings.

(b) Data on mechanisms of cancer development

To narrow the focus, the Working Group attempts 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 relevance of the mechanism to humans is discussed, in particular, when

mechanistic data are derived from experimental model systems. Changes in the micro-environment of the affected cells, tissues or organs can be divided into three, non-exclusive levels as described below.

(i) Changes in physiology

Physiological changes refer to exposure-related modifications to the physiology and/or response of cells, tissues and organs. Examples of physiological changes include mitogenesis, compensatory cell division, evasion of apoptosis and/or senescence, presence of inflammation, hyperplasia, metaplasia and/or preneoplasia, angiogenesis, alterations in cellular adhesion, changes in steroidal estrogens and/or androgens and changes in immune surveillance.

(ii) Functional changes at the cellular level

Functional changes refer to exposure-related alterations in the signalling pathways used by cells to manage critical processes that are related to increased risk for cancer. Examples of functional changes include modified activities for enzymes involved in the metabolism of xenobiotics, alterations in the expression of key genes that regulate DNA repair, alterations in the cytokines that govern movement of cells through the cell cycle, changes in the patterns of post-translational modifications of proteins, changes in regulatory factors that alter apoptotic rates, changes in secretion of factors related to the stimulation of DNA replication and transcription and changes in gap–junction-mediated intercellular communication.

(iii) Changes at the molecular level

Molecular changes refer to exposure-related changes in key cellular structures at the molecular level, including, in particular, genotoxicity. Examples of molecular changes include formation of DNA adducts and DNA strand breaks, mutations in genes, chromosomal aberrations, aneuploidy and changes in DNA methylation patterns. Greater emphasis should be given to irreversible effects.

The use of mechanistic data in the identification of a carcinogenic hazard is specific to the mechanism being addressed and is not readily described for every possible level and mechanism discussed above.

Genotoxicity data are discussed here to illustrate the key issues involved in the evaluation of mechanistic data.

Tests for genetic and related effects are described in view of the relevance of gene mutation and chromosomal mutation/aneuploidy to carcinogenesis (Vainio *et al*., 1992; McGregor *et al*., 1999; refs). The adequacy of the reporting of sample characterization is considered and, when 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 and aneuploidy. 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, cell transformation and gap–junction intercellular communication 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 to be 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. 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 that rules out the carcinogenicity of agents or mixtures that act through other mechanisms (e.g. receptor-mediated effects, cellular toxicity with regenerative cell division, peroxisome proliferation) (Vainio *et al*., 1992). Factors that may give misleading results in short-term tests have been discussed in detail elsewhere (Montesano *et al*., 1986; McGregor *et al*., 1999).

When there is evidence that an agent acts by a specific mechanism that does not involve genotoxicity (e.g. hormonal dysregulation, immune suppression and calculi and other deposits that cause chronic irritation), that evidence is presented critically and reviewed in the context of rigorous criteria for the operation of that mechanism in carcinogenesis (e.g. IARC Scientific Publication 147).

(c) Other data relevant to mechanisms

For biological agents such as viruses, bacteria and parasites, other data relevant to carcinogenicity may 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 that might include cellular and tissue responses to infection, immune response and the presence of tumour markers.

For physical agents that are forms of radiation, other data relevant to carcinogenicity may include descriptions of damaging effects at the physiological, cellular and molecular level, as for chemical agents, and descriptions of how these effects occur. ‘Physical agents’ may also be considered to include foreign bodies, such as surgical implants of various kinds, and poorly soluble fibres, dusts and particles of various sizes, the pathogenic effects of which are a result of their physical presence in tissues or body cavities rather than from degradation products. Other relevant data for such materials may include characterization of cellular, tissue and physiological reactions to these materials and descriptions of pathological conditions other than neoplasia with which they may be associated.

(d) Activity classes

A description should be provided of any 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.

High-output data, such as those derived from gene expression microarrays, and high-throughput data, such as those that result from the evaluation of hundreds of agents for a single end-point, pose a unique problem for the use of mechanistic data in the evaluation of a carcinogenic hazard. In the case of high-output data, there is the possibility to over-interpret changes in individual end-points (e.g. changes in expression in one gene) without evaluating the consistency of that finding in the broader context of the other end-points evaluated (e.g. other genes with linked transcriptional control). High-output data can be used in evaluating mechanisms, but all end-points measured in a single experiment need to be considered in the proper context. For high-throughput data where the number of observations far exceeds the number of end-points measured, the utility for identifying common mechanisms across multiple agents is enhanced. These data can be used to identify mechanisms that not only seem plausible, but have a consistent pattern of carcinogenic response across entire classes of related compounds.

(e) Individual susceptibility

Individuals, populations and life-stages may have greater or lesser susceptibility to an agent, based on knowledge of the toxicokinetics and mechanisms of carcinogenesis of that agent and other factors. Examples of host and genetic factors that affect individual susceptibility include sex, genetic polymorphisms of metabolic genes of the agent under evaluation, differences in metabolic capacity due to life-stage or the presence of disease, differences in DNA repair capacity, competition for or alteration of metabolic capacity by medications or other chemical exposures, 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). Such data can substantially increase the strength of the evidence from epidemiological data and focus the linkage of in-vivo and in-vitro laboratory studies to humans.

(f) Other adverse effects

Finally, data on acute, subchronic and chronic adverse effects other than cancer are summarized. Adverse effects that confirm distribution and biological effects at the sites of tumour development, or alterations in physiology that could lead to tumour development, are emphasized. Effects on reproduction, embryonic and fetal survival and development are 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, but giving fewer details.

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