The use of structured frameworks can be invaluable in promoting harmonization in the assessment of chemical risk. IPCS has therefore updated and extended its mode of action (MOA) framework for cancer to address the issue of human relevance of a carcinogenic response observed in an experimental study. The first stage is to determine whether it is possible to establish an MOA. This comprises a series of key events along the causal pathway to cancer, identified using a weight-of-evidence approach based on the Bradford Hill criteria. The key events are then compared first qualitatively and then quantitatively between the experimental animals and humans. Finally, a clear statement of confidence, analysis, and implications is produced. The IPCS human relevance framework for cancer provides an analytical tool to enable the transparent evaluation of the data, identification of key data gaps, and structured presentation of information that would be of value in the further risk assessment of the compound, even if relevancy cannot be excluded. This might include data on the shape of the dose-response curve, identification of any thresholds and recognition of potentially susceptible subgroups, for example, the basis of genetic or life-stage differences.

**Keywords** Animal–Human Concordance, DNA-Reactive, Carcinogens, Human Relevance Framework for Cancer, Key Events, Mode of Action, Risk Assessment

Fundamental to the evolution of cancer risk assessment over the last three decades has been our increasing understanding of the biology of cancer and the identification of key events in carcinogenesis. Through the mid-1980s, national and international assessments of human cancer hazard and risk depended primarily on lifetime assays in rodents of potentially carcinogenic agents. For few agents was there sufficient human evidence on which to base retrospective cancer assessments, and...
fewer still would be expected to be detected prospectively, given modern controls on general exposures in the workplace and in the environment generally. Inherent in rodent-based assessments was the assumption that the observation of tumors in laboratory animals could be meaningfully extrapolated to identify potential human carcinogens, and, by the use of mathematical models, to provide upper bound estimates of risk at human doses of regulatory significance. During the same period, the potential significance of mutagenesis in carcinogenesis was becoming accepted by the scientific community. Subsequently, it has become increasingly apparent that an appreciable number of chemicals cause cancer in laboratory animals by processes that do not involve direct interaction with DNA. These developments in our understanding of the biological basis of carcinogenesis in both laboratory animals and humans have benefited risk assessment processes by providing more data on the pharmacokinetics and pharmacodynamics of suspect carcinogenic agents. Consideration of the biological processes involved in the carcinogenesis of specific compounds has led to the concept of mode of action (MOA).

A postulated MOA for carcinogenesis is a biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. It describes key cytological and biochemical events—that is, those that are both measurable and necessary to the observed carcinogenicity—in a logical framework. MOA contrasts with mechanism of action, which generally involves a sufficient understanding of the molecular basis for an effect and its detailed description, so causation can be established in molecular terms.

In 2001, as part of its efforts to harmonize risk assessment practices, the International Programme on Chemical Safety (IPCS) (WHO/LO/UNEP) published a framework for assessment of MOA for carcinogenesis in laboratory animals (animal MOA), based on Bradford Hill criteria for causality. The IPCS Human Relevance Framework (HRF) presented in this document updates this MOA framework and extends it to consider human relevance. It is an analytical tool to provide a means of evaluating systematically the data available on a specific carcinogenic response to a chemical in a transparent manner. While it is envisioned that the framework will be of value to risk assessors both within and outside of regulatory agencies, it will also be a valuable tool to the research community. Amongst reasons for using the framework are:

- To provide a generic approach to the analysis of data to contribute to harmonization.
- To encourage transparency of the consideration and use of available data and reasons for the conclusions drawn.
- To provide guidance in the presentation of data.
- To identify critical data deficiencies and needs.
- To inform the quantitative assessment of carcinogenic risk to humans.

These and other topics are discussed in more detail next.

THE ROLE OF THE INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY IN DEVELOPING THE FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS

The IPCS has been leading an effort to harmonize approaches to cancer risk assessment as part of its larger project on the Harmonization of Approaches to the Assessment of Risk From Exposure to Chemicals. The first phase of this work resulted in the publication of the IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis in experimental animals (Sonich-Mullin et al., 2001). As described in that publication, a major impediment to harmonization identified in the consideration of weight-of-evidence was the evaluation of MOA in animals. Sonich-Mullin et al. (2001) provided a framework for evaluating MOA of chemical carcinogenesis in animals and recognized the importance of moving on to the next step in the overall characterization of cancer hazard and risk in humans: the assessment of relevance of the MOA of animal carcinogenesis to humans. Adoption of the MOA framework concept is proceeding through its incorporation in the revised U.S. Environmental Protection Agency (EPA) Risk Assessment Guidelines for Carcinogens (U.S. EPA, 1999, 2005), and is now commonly used by other regulatory agencies and international organizations. In the United Kingdom (UK), the framework is being used for the assessment of pesticides and industrial chemicals. The UK Committee on Carcinogenicity (COC) has noted the framework’s value with regard to both harmonization between agencies and internal consistency in its latest guidelines (COC, 2004). It has also been adopted and is being used by agencies in Australia and in Canada, in the evaluation of Existing Chemicals under the Canadian Environmental Protection Act. The European Union has incorporated the framework into its technical guidance documents on evaluating new and existing industrial chemicals and biocides, including carcinogenicity. With regard to international organizations, of particular note is the use of the framework by the WHO/FAO Joint Meeting on Pesticide Residues (JMPR), for example, in its evaluation of pyrethrin extract and its incorporation into the resulting monograph.

The step to extend the MOA Framework to include consideration of human relevance has been undertaken by IPCS in cooperation with international partners. It was the subject of an IPCS international workshop convened in Bradford, United Kingdom, from 21 to 23 April 2005. This workshop prepared draft text for an IPCS Human Relevance Framework, including updating the 2001 Mode of Action Framework. The draft was published on the internet for public comment and sent to a number of WHO Collaborating Centres and IPCS Participating Institutions for peer review. An expert meeting convened in
London (December 2005) considered the comments received and finalized the framework. The framework text and the steps leading to its development are discussed in detail in the following sections.

THE 2001 IPCS CONCEPTUAL MOA FRAMEWORK FOR EVALUATING ANIMAL CARCINOGENESIS

Purpose of the Framework

The IPCS Mode of Action Framework for evaluating carcinogenesis in animals (Sonich-Mullin et al., 2001) remains a fundamental basis for the IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans. The animal MOA framework provides a generic approach to the principles commonly used when evaluating a postulated MOA for tumor induction in animals by a chemical carcinogen. Thus, the framework is a tool that provides a structured approach to the assessment of the overall weight of the evidence for the postulated MOA. In this context, a supported MOA would have evidence provided by robust experimental observations and mechanistic data to establish a biologically plausible explanation.

The framework is designed to bring transparency to the analysis of a postulated MOA and thereby to promote confidence in the conclusions reached through the use of a defined procedure that encourages clear and consistent documentation supporting the analysis and reasoning and that highlights inconsistencies and uncertainties in the available data. The purpose of the framework is to provide a systematic means of considering the weight of the evidence for a MOA in a given situation; it is not designed to give an absolute answer on sufficiency of the information, as this will vary depending on the circumstance. It is not a checklist of criteria, but rather an analytical approach. However, the process can be greatly aided by the presentation of tabular summaries of comparative data on incidence of key events and tumors.

The animal MOA framework analysis is an important step in the hazard characterization. It is envisioned that the animal MOA framework will contribute to risk assessments of chemical carcinogens across all sectors (drugs, industrial chemicals, pesticides, food additives, etc.). In the resulting risk assessment documentation, the framework analysis would be appropriately positioned within the hazard characterization section. In the absence of adequate epidemiological data, it may be regarded as an essential component in any discussion of human relevance, dose-response relationships, and risk characterization. It is also envisioned that the framework will be useful to both regulators and researchers in identifying research needs based on clear delineation of data gaps and inconsistencies.

MOA analysis can be used to establish whether a compound has an MOA that has been described previously or that it has a novel MOA. Thus, the output of an MOA analysis may serve to support the evaluation of a specific compound or contribute to the generation of a novel MOA. In the former, chemical-specific data play a vital role in the concordance analysis for human relevance. In the latter, it will be important to identify which events are key to the biological processes that represent the MOA.

Thus, an MOA, comprising the same set of key events, may apply to many different compounds. The evidence necessary to establish that a specific MOA is responsible for a given carcinogenic response will be substantial the first time such an MOA is proposed. As subsequent compounds are found to share this MOA, the “barrier” to acceptance will be lower, though it will always be necessary to establish rigorously that the key events comprising the MOA occur, and that they fulfill the criteria described later in this article. It will also be important to exclude other possible MOAs.

Scientific peer participation is a prerequisite for the development and acceptance of a novel postulated MOA. Peer participation includes both peer involvement in the development of an MOA and peer review by scientists who are independent of the process of development of the MOA. Publication in the scientific literature and presentation and discussion at scientific meetings and workshops constitute peer involvement that contribute to acceptance of an MOA by the scientific community.

While acceptance does not necessarily mean unanimity, most of the scientists reviewing an MOA analysis should agree that the relevant scientific information has been identified and appropriately analyzed, that “key events” have been identified and are supported by the information presented, that their relationship to carcinogenesis has been clearly established in the hypothesized MOA, and that alternate MOAs have been considered and rejected.

As knowledge advances, the characterization of a MOA will change. Additional key events may be identified, and others may be refined or even dropped. Nevertheless, significant changes to the key events also need some general acceptance, through peer review, such as described earlier.

Update of Framework Guidelines

In development of the IPCS Human Relevance Framework, the 2001 animal MOA Framework text has been updated and this revised version is presented here.

Introduction to Framework Analysis

This section describes the cancer endpoint or endpoints that have been observed and identifies which of these are addressed in the analysis. Prior to embarking on a framework analysis, there needs to be careful evaluation of the weight of evidence for a carcinogenic response in experimental animals. The nature of the framework is such that only one MOA is analyzed at a time; hence, for example, different tumor types associated with chemical treatment, even if recorded in the same animals, will require separate framework analyses to discern each tumor’s MOA. However, in considering the pathogenesis of a single
type of tumor, it should be recognized that it is possible that a chemical could induce that tumor type by more than one MOA. Hence, it might be necessary to undertake an analysis of more than one MOA for the same tumor type for a single chemical. Consistent with species- and tissue-specific variation in metabolic activation and detoxication, there is often only poor site concordance for genotoxic carcinogens. This will need to be kept in mind when comparing animal and human data. In contrast, consistent with the observation that most carcinogens acting by a nongenotoxic MOA perturb physiological processes that tend to be site specific, site concordance is reasonably assumed, at least as an initial premise in the human relevance framework.

1. Postulated Mode of Action (Theory of the Case)

This section comprises a brief description of the sequence of events on the path to cancer for the postulated MOA of the test substance. This explanation of the sequence of events leads into the next section, which identifies the events considered “key” (i.e., necessary and measurable), given the database available for the analysis.

2. Key Events

This section briefly identifies and describes the “key events”—measurable events that are critical to the induction of tumors as hypothesized in the postulated MOA. To support an association, a body of experiments needs to define and measure an event consistently. Pertinent observations include, for example, tumor response and key events in the same cell type, sites ofaction logically related to event(s), increased cell growth, specific biochemical events, changes in organ weight and/or histology, proliferation, perturbations in hormones or other signaling systems, receptor–ligand interactions, effects on DNA or chromosomes, and impact on cell cycle. For example, key events for tumors hypothesized to be associated with prolonged regenerative proliferation might be cytotoxicity as measured histopathologically and an increase in labeling index. As another example, key events for induction of urinary bladder tumors hypothesized to be due to formation of urinary solids composed primarily of calcium phosphate might include elevated urinary free calcium, phosphate, and pH, and formation of urinary solids, followed by irritation and regenerative hyperplasia of the urothelium.

3. Concordance of Dose-Response Relationships

This section should characterize the dose-effect/response relationships for each of the key events and for the tumor response, and discuss their interrelationships, in the context of the Bradford Hill criteria. Ideally, one should be able to correlate the dose dependency of the increases in incidence of a key event with increases in incidence or severity (e.g., lesion progression) of other key events occurring later in the process, and with the ultimate tumor incidence. Comparative tabular presentation of incidence of key events and tumors is often helpful in examining dose response. In the case of complex data sets, this is almost essential.

It is important to consider whether there are fundamental differences in the biological response (i.e., dose transitions) at different parts of the dose-response curve for tumor formation (Slikker et al., 2004). If so, key events relevant to the different parts of the dose-response curve will need to be defined and used in the framework analysis.

4. Temporal Association

This section should characterize the temporal relationships for each of the key events and for the tumor response. The temporal sequence of key events leading to the tumor response should be determined. Key events should be apparent before tumor appearance and should be consistent temporally with each other; this is essential in deciding whether the data support the postulated MOA. Observations of key events at the same time as the tumors (e.g., at the end of a bioassay) do not contribute to considerations of temporal association, but can contribute to analysis in the next section. Most often, complete data sets to address the criterion of temporality are not available.

5. Strength, Consistency, and Specificity of Association of Tumor Response with Key Events

This section should discuss the weight of evidence linking the key events, precursor lesions, and the tumor response. Stop/recovery studies showing absence or reduction of subsequent events or tumor when a key event is blocked or diminished are particularly important tests of the association. Consistent observations in a number of such studies with differing experimental designs increase that support, since different designs may reduce unknown biases or confounding. Consistency, which addresses repeatability of key events in the postulated MOA for cancer in different studies, is distinguished from coherence, however, which addresses the relationship of the postulated MOA with observations in the broader database (see point 7). Pertinent observations include tumor response and key events in the same cell type, sites of action logically related to event(s), and results from multistage studies and from stop/recovery studies.

6. Biological Plausibility and Coherence

One should consider whether the MOA is consistent with what is known about carcinogenesis in general (biological plausibility) and also in relation to what is known for the substance specifically (coherence). For the postulated MOA and the events that are part of it to be biologically plausible, they need to be consistent with current understanding of the biology of cancer. However, the extent to which biological plausibility can be used as a criterion against which weight of evidence is assessed...
may be limited due to gaps in our knowledge. Coherence, which addresses the relationship of the postulated MOA with observations in the broader database—for example, association of MOA for tumors with that for other endpoints—needs to be distinguished from consistency (addressed in point 6), which addresses repeatability of key events in the postulated MOA for cancer in different studies. For coherence, likeness of the case to that for structural analogues may be informative (i.e., structure–activity analysis). Information from other compounds that share the postulated MOA may be of value, such as sex, species, and strain differences in sensitivity and their relationship to key events. Additionally, this section should consider whether the database on the agent is internally consistent in supporting the purported MOA, including that for relevant noncancer toxicities. Some modes of action can be anticipated to evoke effects other than cancer, such as reproductive effects of certain hormonal disturbances that are carcinogenic.

7. Other Modes of Action

This section discusses alternative modes of action that logically present themselves in the case. If alternative modes of action are supported, they need their own framework analysis. These should be distinguished from additional components of a single MOA that likely contribute to the observed effect, since these would be addressed in the analysis of the principal MOA.

8. Uncertainties, Inconsistencies, and Data Gaps

Uncertainties should include those related to both the biology of tumor development and those for the database on the compound of interest. Inconsistencies should be flagged and data gaps identified. For the identified data gaps, there should be some indication of whether they are critical as support for the postulated MOA.

9. Assessment of Postulated Mode of Action

This section should include a clear statement of the outcome with an indication of the level of confidence in the postulated MOA—for example, high, moderate, or low. If a novel MOA is being proposed, this should be clearly indicated. However, if the MOA is the same as that proposed for other compounds, the extent to which the key events fit this MOA needs to be stated explicitly. Any major difference should be noted and their implications for the MOA should be discussed.

ADDRESSING THE ISSUE OF HUMAN RELEVANCE

In 2000, an IPCS Harmonization Project Cancer Planning Work Group convened in Carshalton, United Kingdom (IPCS, 2000). (This initial IPCS Working Group differed in membership from the subsequent IPCS working group convened to work on the human relevance project.) Among the recommendations of that meeting was the suggestion that IPCS and ILSI move forward together and in parallel on the development of the extension of the IPCS MOA Framework toward addressing human relevance. It was recognized that ILSI could provide much help in technical workshops. In June 2001, the International Life Sciences Institute Risk Science Institute (ILSI/RSI) with support from the US EPA and Health Canada formed a working group to examine key issues in the use of MOA information to determine the relevance of animal tumors. These efforts have resulted in several published reports that are described next. An IPCS Cancer Working Group, convened on 3–5 March 2004 in Arlington, VA, agreed that that these reports should form the starting point for further exploration of the issue of human relevance of animal tumors by IPCS with the goal of developing a unified IPCS Human Relevance Framework for use of MOA information in risk assessment for regulatory and other purposes (IPCS, 2004).

To address the issue of the human relevance of the MOA(s) determined in animals, ILSI/RSI charged its working group with expanding the IPCS MOA Framework to include evaluation of the human relevance of a cancer MOA determined in animals. The details of the process, the case studies, and the framework were published as a series of papers in the November 2003 issue of Critical Reviews in Toxicology (CRTJ) (Cohen et al., 2003; Meek et al., 2003). These articles describe the ILSI/RSI Human Relevance Framework (HRF) and provide guidance for its application. In addition, references to specific examples on which the framework is based are included. Several iterations of case studies of chemicals with generally well-known modes of action were used to develop the integrated framework. The intent was to provide guidance for a disciplined, transparent process evaluating the MOA in animals and each key event with respect to human relevance.

The ILSI/RSI HRF is based on three fundamental questions:

1. Is the weight of evidence sufficient to establish the mode of action (MOA) in animals?
2. Are key events in the animal MOA plausible in humans?
3. Taking into account kinetic and dynamic factors, are key events in the animal MOA plausible in humans?

Questions 2 and 3 involve qualitative and quantitative considerations, respectively, in a concordance analysis of human information in relation to the animal MOA and its key events. These are followed by an explicit description of confidence in the evaluation, identification of specific data gaps, and the implications for risk assessment. It was emphasized by ILSI/RSI that use of this framework would form part of the hazard characterization step of the overall risk assessment process.

DEVELOPMENT OF AN IPCS HUMAN RELEVANCE FRAMEWORK GUIDANCE DOCUMENT BASED ON THE IPCS MOA FRAMEWORK AND THE ILSI/RSI HRF

The 2004 IPCS Cancer Working Group discussed the type of document that would be produced as a result of its task to
extend the IPCS MOA Framework to address human relevance. It was recognized that one integrated guidance document that worked as a whole would be needed to facilitate uptake and use by regulatory and other risk assessment bodies. The guidance could be supplemented by publication of the other materials generated through the process (e.g., issues papers and case studies).

There was general agreement among Working Group members that the questions identified as the critical components of the ILSI/RSI HRF were important and in general appropriate for addressing the human relevance of an MOA determined in animals. However, several issues were identified that could benefit from additional clarification, development or expansion.

These refinements of the ILSI/RSI HRF were developed through discussions of the IPCS Cancer Working Group and at a Workshop convened for this purpose in Bradford, UK, on 21–23 April 2005 (IPCS, 2005). The resulting IPCS Human Relevance Framework (IPCS HRF) is presented as an approach to answering a series of three questions, leading to a documented, logical conclusion regarding the human relevance of the MOA underlying animal tumors. The application of the guidance results in a narrative with four sections that may be incorporated into the hazard characterization of a risk assessment. The sections are as follows (see Figure 1):

1. Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?
2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

![Diagram](image)

FIG. 1. IPCS general scheme illustrating the main steps in evaluating the human relevance of an animal MOA for tumor formation. The questions have been designed to enable an unequivocal answer yes or no, but recognizing the need for judgment regarding sufficiency of weight of evidence. Answers leading to the left side of the diagram indicate that the weight of evidence is such that the MOA is not considered relevant to humans. Answers leading to the right side of the diagram indicate either that the weight of evidence is such that the MOA is likely to be relevant to humans or that it is not possible to reach a conclusion regarding likely relevance to humans, due to uncertainties in the available information. In these cases, the assessment would proceed to risk characterization. It should be noted that only at this stage would human exposure be included in the evaluation.
3. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?


In applying this framework for a given chemical, tumors of each animal target organ observed are evaluated independently, with the assumption that different modes of action are possible in different organs, though based on this analysis, modes of action in different tissues may be similar. Similarly, an evaluation of the likelihood of congruence between target organ(s) in different species and in humans needs to be made, based on the MOA analysis.

**Is the Weight of Evidence Sufficient to Establish a Mode of Action in Animals?**

Answering this first question in the IPCS Human Relevance Framework requires application of the (updated) IPCS MOA Framework described earlier in this document. The steps in the MOA Framework, which are based on the Bradford Hill criteria for causality, are:

1. Postulated MOA.
2. Key events; associated critical parameters.
3. Dose-response relationships.
4. Temporal association.
5. Strength, consistency, and specificity of association of key events and tumor response.
6. Biological plausibility and coherence.
7. Possible alternative MOAs.
8. Uncertainties, Inconsistencies, and data gaps.
9. Conclusion about the MOA.

This process incorporates an evaluation of the weight of evidence for possible alternative MOAs at a given site, and an evaluation of the overall strength of evidence supporting the MOA under consideration. Ultimately, a decision concerning the weight of evidence supporting the MOA and the level of confidence in that decision must be made. It also identifies critically important data gaps that, when filled, would increase confidence in the proposed mode of action. It is also necessary to establish whether the postulated MOA has already been described for other chemicals, in which case human relevance will already have been evaluated, or whether the proposed MOA is novel, in which case human relevance needs to be assessed de novo.

For a given chemical, the primary sources of information for evaluating a MOA are likely to be data generated for that specific chemical in the animal model in which tumors were produced. Obviously, data from other sources can and should also be used, as appropriate, along with data on chemicals with similar chemical structures, the same or similar MOAs, or both. If the MOA for a chemical is novel, considerably more data will be required to support the conclusion that it is related to the carcinogenic process of the tumors induced by that chemical than subsequent examples of chemicals acting by the same MOA. The ILSI/RSI working group and the IPCS Bradford Workshop did not address the issue of how much data is sufficient to support a specific MOA for a given chemical per se, except by way of example within the case studies and recognition that acceptance of a novel MOA requires scientific consensus (described earlier). Consideration at this stage of the MOA analysis of potential variations between animals and humans also facilitates addressing subsequent steps in the framework.

**Can Human Relevance of the MOA Be Reasonably Excluded on the Basis of Fundamental, Qualitative Differences in Key Events Between Experimental Animals and Humans?**

The wording of this question was changed from that in the ILSI/RSI HRF, following discussion at the IPCS Workshop on the implications of a yes or a no answer to the original question. In answering the original question, only an unequivocal no would be sufficient to permit the conclusion that the animal MOA was not relevant to humans. Also, it was recognized that translation of the word “plausible” into other languages could be problematic. The question was therefore reworded to enable a yes/no answer, but qualified by the descriptor “reasonably,” based on recognition that decisions about the adequacy of weight of evidence are not absolute but involve scientific judgment based on transparent analysis of the available data.

This step represents a qualitative assessment of the relevance of the MOA to human cancer potential. Listing the critical specific key events that occur in the animal MOA and directly evaluating whether each of the key events might or might not occur in humans facilitates consideration and transparent presentation of the relevant information. Presentation in tabular form, referred to as a concordance table, can be helpful in delineating the relevant information (for an example see Meek et al., 2003, case study 6: kidney and liver tumors associated with chloroform exposure, Table 7; McGregor et al., this issue, case study on formaldehyde, Table 3). The key events (and possibly some of the critical associated processes) are listed with the information regarding these events for the animals in which the tumor was observed. It is intended that the information in these tables be brief, since a narrative explanation is expected to accompany the table. In the right-hand column, the effect on humans for each of the key events is evaluated. An additional column for the results in a different strain, species, sex, or route of administration that does not result in tumors can be useful if information is available for comparison to the model that leads to tumors. In addition, factors may be identified that, while not key themselves, can modulate key events and so contribute to differences between species or individuals. Such factors include genetic differences in pathways of metabolism, competing pathways of metabolism, and cell proliferation induced by concurrent pathology. Any such factors identified should be noted in a footnote to the concordance table.
The evaluation of the concordance of the key events for the MOA for a given chemical in humans is an evaluation of the MOA in humans, rather than an evaluation of the specific chemical. In general, details of the initial key events are likely to be more chemical-specific, for example, the enzyme induction response by phenobarbital in rodent liver, or the formation of a cytotoxic metabolite from chloroform by specific cytochrome P-450 enzyme(s). Later events are more generic to the MOA, for example pleiotropic stimulation of hepatic proliferation or regenerative hyperplasia. Information that can be utilized to evaluate the key events in humans can come from in vitro and in vivo studies on the substance itself, but also can involve basic information regarding anatomy, physiology, endocrinology, genetic disorders, epidemiology, and any other information that is known regarding the key events in humans. Information concerning an evaluation of the key event in humans exposed directly to the specific chemical is frequently unavailable.

As knowledge concerning the development of cancer evolves, it may become possible to combine some MOAs on the basis of the basic biology of the processes involved, thus relying less on chemical specific information to reach a conclusion on the human relevance of a given MOA.

In evaluating the concordance of the information in humans to that in animals, a narrative describing the weight of evidence and an evaluation of the level of confidence for the human information need to be provided. Some specific types of information that are useful include the following:

1. Cancer incidences at the anatomical site and cell type of interest, including age, sex, ethnic differences, and risk factors, including chemicals and other environmental agents.
2. Knowledge of the nature and function of the target site, including development, structure (gross and microscopic), and control mechanisms at the physiological, cellular, and biochemical levels.
3. Human and animal disease states that provide insight concerning target organ regulation and responsiveness.
4. Human and animal responses to the chemical under review or analogs following short-, intermediate-, or long-term exposure, including target organs and effects.

Obviously, a substantial amount of information is required to conclude that the given MOA is not relevant to humans. If such a conclusion is strongly supported by the data, then chemicals producing animal tumors only by that MOA would not pose a cancer hazard to humans and no additional risk characterization for this endpoint is required. Since there is no cancer hazard, there is no cancer risk for the tumor under consideration.

The question of relevance considers all groups and life stages. It is possible that the conditions under which an MOA operates occur primarily in a susceptible subpopulation or life stage, for example, in those with a preexisting viral infection, hormonal imbalance, or disease state. Special attention is paid to whether tumors could arise from early-life exposure, considering various kinetic and dynamic aspects of development during these life stages. Any information suggesting quantitative differences in susceptibility is identified for use in risk characterization.

Can Human Relevance of the MOA Be Reasonably Excluded on the Basis of Quantitative Differences in Either Kinetic or Dynamic Factors Between Experimental Animals and Humans?

The wording of this question was changed from that in the ILSI/RSI HRF, following discussion at the IPCS Workshop on the implications of a yes or a no answer to the original question. In answering the original question, only an unequivocal no would be sufficient to permit the conclusion that the animal MOA was not relevant to humans. The question was therefore reworded to enable a yes/no answer, but qualified by the descriptor “reasonably,” based on recognition that decisions about the adequacy of weight of evidence are not absolute but involve judgment based on transparent analysis of the available data.

For purposes of human relevance analysis, if the experimental animal MOA is judged to be qualitatively relevant to humans, a more quantitative assessment is required that takes into account any kinetic and dynamic information that is available from both the experimental animals and humans. Such data will of necessity be both chemical and MOA specific and will include the biologically effective doses required to produce the relevant dynamic responses from which neoplasia can arise. Kinetic considerations include the nature and time course of chemical uptake, distribution, metabolism, and excretion, while dynamic considerations include the consequences of the interaction of the chemical with cells, tissues, and organs. On occasion, the biologically effective dose that would be required to create these conditions would not be possible in humans. It may also be that quantitative differences in a biological process involved in a key event, for example, the clearance of a hormone, are so great that the animal MOA is not relevant to humans. However, the IPCS Workshop recognized that only infrequently is it likely that it will be possible to dismiss human relevance on the basis of quantitative differences. As with the qualitative assessment, a tabular comparison of quantitative data from the experimental animals and humans can facilitate the evaluation (for example, see Meek et al., 2003 case study 5; thyroid tumors associated with exposure to phenobarbital. Table 6; Dellarco et al., this issue, case study on thiazopyr, Table 4). Useful comparisons can also be made with key events identified from studies of other compounds believed to induce effects by a similar MOA. For example, in the case of thiazopyr, information on the effects of phenobarbital in humans was particularly informative in evaluating the relevance of the MOA. As molecular and kinetic approaches continue to evolve, understanding of the similarities and differences of responses in animals and humans will be improved. It may become apparent that qualitative differences in a key event between an animal model and humans will be
identified as being due to a specific quantitative difference, thus changing the answer to the second question (described earlier) to no.

As with question 2, if the conclusion to this question is yes, then chemicals producing animal tumors only by that MOA would not pose a cancer hazard to humans and no additional risk characterization for this endpoint is required.

**Statement of Confidence, Analysis, and Implications**

Following the overall assessment of each of the three questions, a statement of confidence is necessary that addresses the quality and quantity of data underlying the analysis, consistency of the analysis within the framework, consistency of the database, and the nature and extent of the concordance analysis. An evaluation of alternative modes of action, using comparable analyses and rigor, is also essential. A critically important outcome of adequate consideration of the weight of the evidence for an overall MOA and the qualitative and quantitative concordance is the identification of specific data gaps that can be addressed experimentally in future investigations to increase confidence.

Infrequently, there may be conclusive epidemiological data on the cancer risk from a chemical that shares the MOA of the compound under consideration—that is the compound does or does not cause cancer in humans. Obviously, such data would lend considerable weight to the conclusion of the human relevance evaluation. However, there may be occasions when, despite it being possible to establish an MOA in animals, there is insufficient information on the key events in humans to reach a clear conclusion on human relevance. In such circumstances it might be possible to bridge this data gap by using epidemiological data. For example, the database on key events in humans for compounds that act like phenobarbital via activation of the CAR receptor to induce hepatic tumors is incomplete. However, there are robust epidemiological data showing that exposure to phenobarbital for prolonged periods at relatively high doses does not cause cancer in humans. One possibility therefore, is to “read across” from these findings with phenobarbital to any other compound that shares its MOA in animals in inducing rodent liver tumors and to conclude that the tumors caused by such a compound are not relevant to the risk assessment of the compound in humans (Holsapple et al., 2006). Such a conclusion would be critically dependent on the reliability of the epidemiological data and the similarity between the MOA for the chemical under test to that of the compound for which there are epidemiological data available.

In applying the framework to case studies, it is apparent that much current research does not address key questions that would facilitate an analysis of an animal MOA or its relevance to humans. Often this has been because of lack of transparent delineation of key data gaps based on consideration of the data in analytical frameworks such as that presented here. Thus, use of the human relevance framework can be very informative to researchers from the outset in the design of their studies.

The output of formal human relevance analysis provides information that is useful for more than just determining whether or not an endpoint in animals is relevant to humans. Rather, consideration of the relevant information in a transparent, analytical framework provides much additional information that is critically important in subsequent steps in the risk characterization for relevant effects. Based on a human relevance analysis for a proposed MOA for relevant effects, it may be possible to predict, for example, site concordance or not of observed tumors in animals to humans. Application of the human relevancy framework also often provides information on relevant modulating factors that are likely to affect risk, such as hepatitis B and aflatoxin B1 (see Cohen et al., this issue, case study on 4-aminobiphenyl). Analysis often also provides an indication of those components of a proposed MOA which may only operate over a certain dose range. If a high experimental dose of a given compound is needed to result in an obligatory step in a MOA, then the relevance to human risk becomes a matter of exposure. Thus, the exposure assessment step of the subsequent risk characterization is critical to the proper evaluation of human cancer potential. In addition, information identified during the framework analysis can prove invaluable in hazard quantification based on the key events for the MOA.

Importantly, the human relevance analysis also contributes to identification of any special subpopulations (e.g., those with genetic predisposition) who are at increased risk and often provides information relevant to consideration of relative risk at various life stages. In some cases, this may not be based on chemical-specific information but rather inference, based on knowledge of the MOA, as to whether or not specific age groups may be at increased or decreased risk.

The data and their analysis using the framework should be reported in a transparent manner, enabling others to determine the basis of the conclusions reached with respect to the key events, the exclusion of other MOAs, and the analysis of human relevance. As the specific form of presentation will vary with the type of data available, it is not helpful to be prescriptive on how the information should be reported. However, presentation should include sufficient details on the context and thought processes to ensure transparency of the conclusions reached. The use of appropriate tables can be helpful in presenting certain data such as comparative analysis of key events in experimental animals and humans.

**Dissemination of the Framework**

To assist in the dissemination and application of the IPCS Human Relevance Framework, a database of generally accepted modes of action and informative cases should be constructed and maintained. This would comprise a series of MOAs and their associated key events, for reference by those developing framework analyses for compounds which may act by similar modes of action. The case studies would comprise worked examples that have been analyzed using the framework, to provide an indication of the relevant level of detail of the
Induces tumors only in rodents and humans and the comparable initial interactions with DNA by DNA-reactive carcinogens, it would be expected that, in general, DNA-reactive carcinogens would be assessed as progressing to the step of “yes, the key events in the animal MOA could occur in humans” in the ILSI/RSI HRF, as was the case for ethylene oxide (Meek et al., 2003), and “no” to the equivalent step in the IPCS HRF that asks the question, “Can human relevance of the MOA be reasonably excluded on the basis of fundamental qualitative differences in key events between animals and humans?”, as was the case for 4-aminobiphenyl (Cohen et al., this issue). In a recent paper, Preston and Williams (2005) presented a set of key events for tumor development that provided a guide for the use of the ILSI/RSI HRF with DNA-reactive carcinogens. This guide supported the view that for most DNA-reactive chemicals, the animal MOA would be predicted to be relevant to humans. However, it was also argued that there could be exceptions and that the ILSI/RSI HRF would be a valuable tool for identifying these. Use of the ILSI/RSI HRF and the IPCS HRF can also assist in quantifying differences in key events between rodents and humans that may be of value in extrapolating risk to humans. Not all rodent DNA-reactive carcinogens have been established to be human carcinogens as judged by the IARC review process. For some of these exceptions, this human–rodent difference in tumor response is attributable to lower exposure of humans to the agent or to the relative insensitivity of epidemiological studies to detect tumor responses at low exposure levels. However, there are other reasons for such differences that are based on biological considerations. For example, if a DNA-reactive carcinogen induces tumors only in a species-specific organ, it is possible that the animal MOA based on key events might not be relevant to humans, though available data on MOA would need to be considered to permit such a conclusion. Similarly, the generally more proficient DNA repair processes that occur in humans than in rodents (Hanawalt, 2001; Cortopassi and Wang, 1996) or a unique pathway of bioactivation in rodents could result in there being yes answers to the steps in the IPCS HRF that address the queries “Can human relevance of the MOA be reasonably excluded on the basis of fundamental qualitative differences in key events between animals and humans?” and/or “Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between animals and humans?” Alternatively, the IPCS HRF could provide quantitative information on these processes for use later in the risk characterization step.

The need in applying the IPCS HRF for DNA-reactive carcinogens is to develop a set of key events that would clearly describe the cancer process and use these as the guide for establishing the human relevance of a rodent tumor MOA for any particular DNA-reactive carcinogen under consideration.

**Application of the IPCS HRF to DNA- Reactive Carcinogens**

Because of similarities in the carcinogenic process between rodents and humans and the comparable initial interactions with DNA by DNA-reactive carcinogens, it would be expected that, in general, DNA-reactive carcinogens would be assessed as progressing to the step of “yes, the key events in the animal MOA could occur in humans” in the ILSI/RSI HRF, as was the case for ethylene oxide (Meek et al., 2003), and “no” to the equivalent step in the IPCS HRF that asks the question, “Can human relevance of the MOA be reasonably excluded on the basis of fundamental qualitative differences in key events between animals and humans?” as was the case for 4-aminobiphenyl (Cohen et al., this issue). In a recent paper, Preston and Williams (2005) presented a set of key events for tumor development that provided a guide for the use of the ILSI/RSI HRF with DNA-reactive carcinogens. This guide supported the view that for most DNA-reactive chemicals, the animal MOA would be predicted to be relevant to humans. However, it was also argued that there could be exceptions and that the ILSI/RSI HRF would be a valuable tool for identifying these. Use of the ILSI/RSI HRF and the IPCS HRF can also assist in quantifying differences in key events between rodents and humans that may be of value in extrapolating risk to humans. Not all rodent DNA-reactive carcinogens have been established to be human carcinogens as judged by the IARC review process. For some of these exceptions, this human–rodent difference in tumor response is attributable to lower exposure of humans to the agent or to the relative insensitivity of epidemiological studies to detect tumor responses at low exposure levels. However, there are other reasons for such differences that are based on biological considerations. For example, if a DNA-reactive carcinogen induces tumors only in a species-specific organ, it is possible that the animal MOA based on key events might not be relevant to humans, though available data on MOA would need to be considered to permit such a conclusion. Similarly, the generally more proficient DNA repair processes that occur in humans than in rodents (Hanawalt, 2001; Cortopassi and Wang, 1996) or a unique pathway of bioactivation in rodents could result in there being yes answers to the steps in the IPCS HRF that address the queries “Can human relevance of the MOA be reasonably excluded on the basis of fundamental qualitative differences in key events between animals and humans?” and/or “Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between animals and humans?” Alternatively, the IPCS HRF could provide quantitative information on these processes for use later in the risk characterization step.

The IPCS Human Relevance Framework and Risk Assessment

Among the strengths of the framework are its flexibility, general applicability to carcinogens acting by any MOA, and ability to explore the impact of each key event on the carcinogenic response. This includes determination of the nature of the dose-response curve, the identification and location of thresholds for individual key events, and their consequences for the overall tumor response curve. In addition, by considering the kinetic and dynamic factors involved in each key event, it may be possible to reach conclusions regarding the relevance or not of the carcinogenic response to specific subpopulations, for example, in early life, in those with particular diseases or in those with specific polymorphisms. Alternatively, application of the framework can provide quantitative information on the differences between such groups. Application of the framework can also more generally inform the risk characterization of the chemical, even when it is concluded that the carcinogenic response per se is not relevant to humans.

As stated at the outset, MOA analysis and its human relevance counterpart are aspects of the hazard identification and characterization phases of risk assessment (National Research Council, 1983; Meek et al., 2003). Consistent with this paradigm, the human relevance case studies referred to in the present report contribute to, but do not complete, a risk assessment for the chemicals under study. This is because a complete risk characterization requires not only evaluation of doses in the range of observations from experimental or occupational hygiene studies but also extrapolation to human exposure levels of interest in daily and lifetime activities. Hazard characterization—and related MOA analysis—deals with the potential for harm in general terms, while the complete risk assessment puts this potential hazard into context with respect to exposure for decision makers. Risk characterization seeks to describe the relationship between these effects and the dose(s) to which humans are exposed in order to understand and estimate the nature and likelihood of effects in humans who are generally exposed at lower dose levels.

Understanding dose response can have a profound effect on hazard characterization and therefore is an important component of the MOA analysis, particularly when nonlinear processes or dose transitions are inherent in the relevant biology. Similarly, quantifying hazard in the context of dose informs the process of risk assessment by suggesting extrapolation models that are consistent with our understanding of the biology.

Estimating these generally lower human exposure levels is the task of the exposure analysis component of the risk
assessments. This usually involves extensive analysis of data collected from environmental media, plant and animal tissues, as well as that derived from pharmacokinetic models. This process also depends on analyses of human activity patterns, and life-stage and lifestyle factors that may bring about exposure. Ideally, based on this information, a range of exposure scenarios is developed for different groups (men, women, children, infants, special groups, based for example on ethnicity or occupation) for use in identifying populations of concern. While hazard characterization, which is largely included in the framework analysis, involves quantification (dose-response analysis), estimating external exposures and contextualizing the hazard with respect to these estimates comprise subsequent steps in the risk assessment process. For example, in the case of melamine (Meek et al., 2003, case study 7) it was concluded that the animal MOA was potentially relevant to humans. However, recognition that bladder carcinoma formation occurred only at very high doses carried forward to the subsequent stages of the risk assessment, exposure assessment, and risk characterization. The full risk assessment established that human exposures would not achieve levels necessary to produce bladder carcinomas, by a substantial margin.

CONCLUSIONS

This IPCS Human Relevance Framework has been developed based on experience gained from the original 2001 IPCS MOA framework and consideration of the 2003 ILSI/RSI human cancer relevance framework. Many aspects of these frameworks have been adopted but a number of changes have been made to improve clarity and to introduce some elements not previously considered (e.g., sensitive subpopulations). The utility and role of the framework as an analytical tool within hazard characterization and within the overall risk assessment/characterization paradigm, that is, informing human relevance and dose-response extrapolation, have been emphasized. A number of general points and conclusions follow from the development of this framework:

1. Prior to embarking on a framework analysis, there needs to be careful evaluation of the weight of evidence for a carcinogenic response in experimental animals.
2. Peer involvement and independent review are essential prerequisites for the general acceptance and scientific defensibility of a new MOA.
3. The framework is applicable to all MOAs for carcinogens, including DNA reactivity.
4. Although human relevance is likely to be assumed for most DNA-reactive carcinogens, the human relevance analysis is a valuable approach to enhance understanding, improve characterization of the hazard and risk, and identify exceptions.
5. When dealing with a chemical that may operate through a novel MOA, the analysis is focused on the chemical and entails a detailed evaluation via the HRF. However, when a specific chemical produces a tumor response consistent with an already established and peer reviewed MOA through which other chemicals have been shown to operate, the analysis is then focused on the established MOA and a determination of whether the chemical produces its carcinogenic effect via the same key events established for the pathway.
6. When evaluating the human relevance of a tumor response found in experimental animals, the concordance analysis of key events is for the MOA and is not necessarily a chemical-specific evaluation. Chemical-specific and generic information relevant to the carcinogenic process can be valuable in the analysis. As knowledge advances, MOAs will become less chemical specific and will be based even more on the key biological processes involved, allowing greater generalization of human relevance from one compound to another.
7. The biological understanding and significance of the key events can inform the approach to dose-response extrapolation for cancer risk, and thus understanding of the MOA can have a profound effect on the hazard and risk characterization, particularly when nonlinear processes or dose transitions are inherent in the relevant biology.
8. It is recommended that a database of generally accepted MOAs and informative case studies be established and maintained. This should provide examples that add to the existing case studies developed by ILSI/RSI and IPCS, and that are instructive in the application of the framework analysis. This database is particularly important as experience continues to evolve in the development of modes of action of carcinogens.
9. It is important to consider potentially susceptible subgroups and different life stages in the analysis.

In conclusion, the IPCS Human Relevance Framework provides a rigorous and transparent approach for judging whether data support a postulated mode of carcinogenic action for a chemical and for evaluating its relevance for humans. The scientific community is encouraged to use this approach as a means to increase the use of mechanistic information in cancer risk assessment and are encouraged to provide feedback which may lead to additional refinements in the future. The framework is of value both to the risk assessment and research communities in furthering our understanding of carcinogenic processes, in identifying critical data gaps and informing the design of studies related to MOAs. When a carcinogenic response is considered potentially relevant to humans, information obtained on the key events during the analysis can prove invaluable in subsequent hazard quantification of the compound. It should be possible to extend the framework to noncancer endpoints, and further work on this is recommended. Thus, application of the IPCS Human Relevance Framework would be an invaluable tool for harmonization across endpoints.
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REFERENCES


