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Use of mode of action in risk assessment: Past, present, and future

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Abstract

The evolution of chemical risk assessment has been marked by a steadily increasing expectation for the use of chemical-specific dosimetric and mechanistic information to tailor the risk assessment approach. The information to be used can range from the broad physical properties of the chemical to detailed information on the mechanism by which it causes a particular toxic outcome, and the risk assessment decisions effected can in turn range from how to define equivalent exposures across species to whether a particular animal outcome is relevant to a human health assessment. A concept that has proven useful in support of these considerations is the "mode of action," a term coined by the USEPA in their new guidelines for carcinogen risk assessment. This paper describes the increasing use of mode-of-action considerations in risk assessment, beginning with early examples involving quantitative dosimetry on the one hand, and qualitative relevance on the other, which foreshadowed the current interest in mode of action. It then describes more recent developments regarding the use of the mode-of-action concept for the selection of a low-dose extrapolation approach, for harmonization of cancer and noncancer risk assessment approaches, and for cross-chemical evaluations. Finally, examples of recent controversies associated with the use of mode-of-action information in risk assessment are provided to demonstrate the challenges that must be overcome to assure the continued viability of the mode-of-action approach.

Keywords: Mode of action; Risk assessment; Methodology; Trichloroethylene; Arsenic

1. Role of mode-of-action information in risk assessment

The flow of the risk assessment process is depicted in Fig. 1. Risk assessment generally begins with the observation of toxicity associated with exposure to a chemical. In the qualitative evaluation, the nature of the observed toxicity, together with information regarding the nature of the chemical, provides insight into the mode of action; that is, the sequence of events by which the active form of the chemical or a product of its metabolism interacts with the organism, leading to the observed response.[1] Information derived from this mechanistic evaluation, in turn, identifies the elements of tissue dosimetry that will influence the dose–response relationship. The risk assessment can then attempt to make use of the insights derived during the qualitative evaluation by incorporating

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[1] In the USEPA's Draft Final Guidelines for Carcinogen Risk Assessment (USEPA, 2003a), the term 'mode of action' is defined as: "a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. A 'key event' is an empirically observable precursor step that is itself a necessary element of the mode of action or is a marker for such an element. Mode of action is contrasted with 'mechanism of action,' which implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action. The toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action as the term is used here. There are many examples of possible modes of carcinogenic action, such as mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression."

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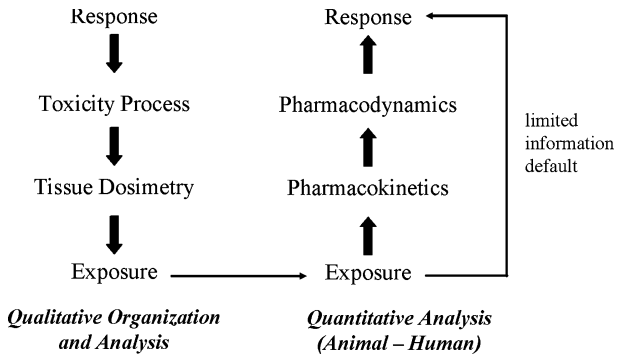

Fig. 1. The risk assessment process (from Clewell et al., 2002, with permission).

quantitative toxicokinetic and toxicodynamic information into the approach.

The simplest risk assessment approach (limited information default in Fig. 1) is to consider the chemical–biological system to be a black box; that is, given an input (the dose of chemical), an output is observed (the toxic response), with no consideration of the nature of the interaction. This approach implicitly assumes equivalence in both toxicokinetics and toxicodynamics (except as addressed by safety/uncertainty factors), and does not provide an opportunity for tailoring the risk assessment on the basis of chemical-specific information.

In general, both qualitative and quantitative studies provide important information for tailoring the risk assessment to a specific chemical. Qualitative mode-of-action information elucidates the manner in which the chemical alters the biological system, and qualitative dosimetry information identifies the form of the chemical/metabolite that is associated with the biological effect. This qualitative information in turn defines the requirements for the quantitative models of tissue dosimetry (toxicokinetics) and mode of action (toxicodynamics) that will be needed to support the dose–response assessment. The relationships between the qualitative concepts and the quantitative tools are illustrated in Fig. 2.

The new USEPA (2003a) cancer guidelines describe the review and interpretation of mode-of-action information to make qualitative decisions about the most appropriate risk assessment approach. As described in the guidelines, modeling both within the observable range and for extrapolation outside the range of observation would preferably be based on a biologically based dose–response (BBDR) model, if available.[2] This approach makes use of mode-of-action information to assess relevant measures of tissue dose along with the interactions of the active chemical moiety with cells and tissues to create a model that relates target tissue exposure to toxic response. To do this successfully, the BBDR model must be used in concert with a physiologically based pharmacokinetic (PBPK) model that specifies the toxicokinetic relationship between applied dose or environmental exposure and the selected metric of target tissue dose for different exposures and for different species.

In the case where a BBDR model is not available, a default dose–response method must be used, e.g., linear extrapolation. However, the PBPK model can still be used to support the dosimetry aspect of the risk assessment, in place of default dosimetry options (Clewell et al., 2002). In this case, the mode of action still plays a crucial role in assessing the relevant measure of tissue dose to be predicted with the PBPK model. For example, existing default dosimetry approaches, including body weight scaling (USEPA, 2003a) and the USEPA's inhalation dosimetry guidelines (USEPA, 1994) implicitly assume that the toxicity results from an effect of the parent chemical. As a result, these default dosimetry options may not provide appropriate dosimetry for modes of action not involving direct effects of the parent chemical, such as the case of a chemical whose toxicity results from the effects of a reactive metabolite (Clewell et al., 2002).

2. History of the application of mode-of-action information in risk assessment

In the earliest formal risk assessment methodology (Dourson and Stara, 1983; Lehman and Fitzhugh, 1954), a NOAEL[3] was divided by one or more "safety" or "uncertainty" factors (UFs) to obtain a human exposure guideline. For environmental risk assessments based on an oral animal study, the administered dose at the NOAEL, expressed in mg/kg/day, was divided by a factor of 10 to address the possibility that humans could be more sensitive than the experimental animal to the effects of the chemical, and a second factor of 10 was applied to consider uncertainty regarding variation in sensitivity across a human population. A similar approach was applied for inhalation risk assessments, except that the dosimetry was based either on air concentration or "inhaled dose." This generic approach was applied to all chemicals regardless of the nature of the toxicity or any chemical-specific information that might be available. The NOAEL/UF approach is still applied in noncancer risk assessment; however, it has become more sophisticated. In particular, chemical-specific information is routinely used in the derivation of reference concentrations

[2] The USEPA (IRIS) defines a BBDR model as a "predictive tool used to estimate potential human health risks by describing and quantifying the key steps in the cellular, tissue, and organismal responses as a result of chemical exposure."

[3] The USEPA (IRIS) defines a no-observed-adverse-effect-level as the highest exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered adverse, nor precursors to adverse effects.

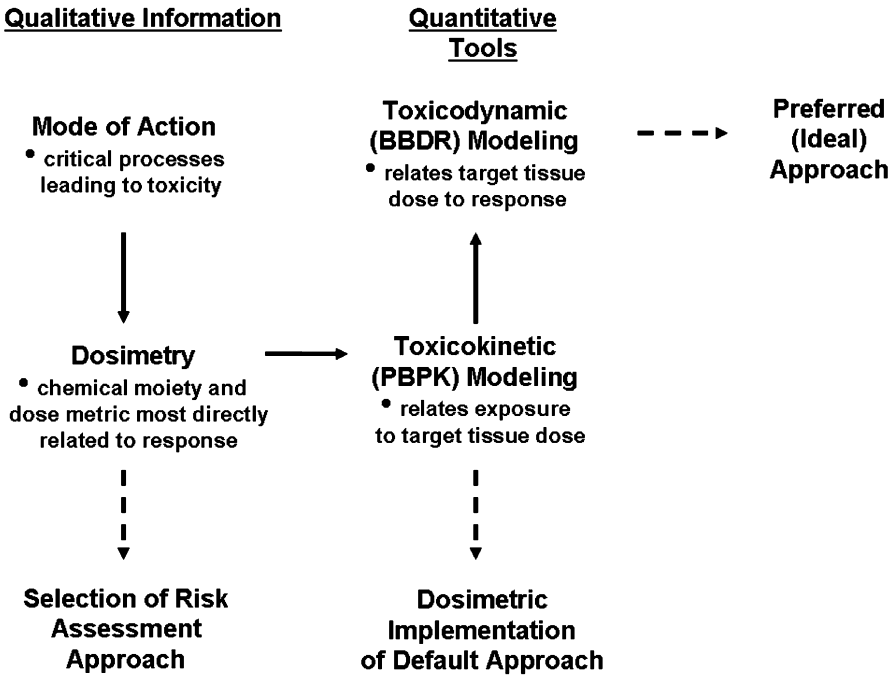

Fig. 2. Mode-of-action driven incorporation of chemical-specific information in risk assessment (from Clewell and Andersen, 2004, with permission).

(RfCs) and reference doses (RfDs), both to inform uncertainty factor selection (Dourson et al., 1996; Haber et al., 2001) and, in the case of RfCs, to select the appropriate dosimetry approach (USEPA, 1994).[4]

The USEPA (1994) inhalation dosimetry guidelines represent a major first step forward in the use of mode-of-action directed risk assessment approaches. Specifically, they provide different procedures for performing cross-species dosimetry depending on the physicochemical properties (solubility, reactivity) of the chemical and the location (portal of entry vs. remote) of the toxicity. The methodology described in these guidelines is also the default approach for inhalation dosimetry in the new cancer guidelines (USEPA, 2003a).

The development of a separate risk assessment methodology for carcinogens (USEPA, 1986) can in retrospect be viewed as resulting from mode-of-action considerations. The driver for the development of quantitative cancer risk assessment was a concern that, due to the nature of the cancer process, even doses of a carcinogen well below those observed to induce tumors in animals could entail an unacceptable risk of cancer to humans. This genotoxic mode-of-action concept was inconsistent with the existing NOAEL/UF approach.[5] As a result, low-dose linear extrapolation approaches were developed, culminating in the linearized multistage model (Crump et al., 1976), which was adopted by the USEPA for their cancer risk assessments based on animal studies.

The new cancer guidelines (USEPA, 2003a) effectively reverse this historical separation of cancer and noncancer risk assessment. They provide for multiple dose–response options for carcinogen risk assessment, driven by mode-of-action considerations. Specifically, in the absence of a BBDR, mode-of-action considerations dictate whether a linear dose–response assessment should be performed to derive a cancer potency estimate, or whether there is sufficient evidence of a highly nonlinear dose–response to justify the use of a harmonized RfC/RfD derivation. This harmonization of the cancer and noncancer risk assessment approaches has received widespread support (Bogdanffy et al., 2001a,b).

Other uses of mechanistic (mode-of-action) information in risk assessment can also be identified. For example, the determination of whether a specific animal outcome is relevant to human health requires detailed consideration of the potential for the mechanism of toxicity in the animal to occur in humans (USEPA, 1988, 1991). Another instance is the recent requirement that cumulative risk assessments be performed for exposure to multiple chemicals with a common mechanism of toxicity (USEPA, 2002a).

3. Examples of the use of mode-of-action information in risk assessment

Table 1 enumerates some of the potential uses of mode-of-action information in risk assessment, and lists

[4] Examples on IRIS of the consideration of mode-of-action data resulting in a reduction in uncertainty factors include acephate and nitrate.

[5] The USEPA (IRIS) states, for example, that an RfD "is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis." A similar statement is made for an RfC.

Table 1
Applications of mode of action in risk assessment

Application	Examples
Determination of human relevance	Atrazine, MTBE (USEPA)
Cross-chemical extrapolation	Vinyl fluoride (IARC)
Criterion for cumulative risk assessment	Organophosphates (USEPA)
Precursor/biomarker selection	Liver weight (USEPA)
Harmonization of cancer/noncancer approaches	Chloroform, perchlorate (USEPA)
Selection of dosimetric approach	Vinyl chloride, EGBE (USEPA)
Development of CSAFs	Boron and compounds (USEPA)
Development of BBDR model	Formaldehyde (USEPA)

chemicals for which risk assessments have been conducted that demonstrate the way in which the mode of action can be used for that purpose. The order of presentation in Table 1 is roughly aligned with the flow of Figs. 1 and 2; that is, from the qualitative aspects of the risk assessment (hazard identification) to the quantitative aspects (dose–response assessment). Each of these applications will be discussed in turn in the following sections.

3.1. Determination of human relevance

Mode-of-action information plays a key role in the determination of whether a particular animal toxicity is relevant to human health. The qualitative implications of mode-of-action information for the human relevance of an animal tumor have frequently been the subject of debate. Examples include rodent forestomach tumors (for which there is no corresponding organ in the human), in the case of butylated hydroxyanisole (Whysner and Williams, 1996a), and bladder tumors resulting from irritation by crystalline deposits, in the case of saccharin (Whysner and Williams, 1996b). An example of a mode-of-action evaluation leading to a conclusion that an animal tumor endpoint was not relevant to the human health assessment can be found in the inhalation cancer risk assessment for 1,1-dichloroethylene (USEPA, 2002b). The previous inhalation cancer assessment for 1,1-dichloroethylene had been based on an increased incidence of kidney adenocarcinomas in male rats. The reassessment concluded that new data suggesting that the kidney adenocarcinomas could be a sex- and species-specific response reduced the weight of evidence for carcinogenicity by the inhalation route of exposure. As a result, the reassessment did not derive an inhalation unit risk.

In some cases a particular toxic endpoint and associated mode of action have been evaluated for human relevance, independent of any particular chemical-specific risk assessment. The conclusions of these more global determinations can then be applied to any chemical meeting the criteria for the identified mode of action. For example, in 1991 the USEPA's Risk Assessment Forum recommended that male rat renal tubule tumors arising as a result of a process involving accumulation of α-2-u-globulin should not contribute to the qualitative weight of evidence that a chemical poses a human carcinogenic hazard, and should not be included in dose–response calculations for the estimation of human risk (USEPA, 1991). Risk assessments on IRIS which have used mode-of-action evaluation to justify the application of this recommendation to a specific chemical include methyl *tert*-butyl ether, *para*-dichlorobenzene, and d-limonene.

For thyroid follicular cell carcinogenesis, on the other hand, mode-of-action information was used by the USEPA's Risk Assessment Forum to modify the quantitative aspect of the cancer risk assessment; specifically, to justify the application of a threshold dose–response paradigm rather than the customary USEPA assumption of low-dose linearity (USEPA, 1988). Consistent with this recommendation, while an RfD has been developed for ethylenethiourea based on thyroid hyperplasia, no cancer risk assessment has been published using the thyroid tumors observed in the same study. The threshold dose–response approach recommended by the Risk Assessment Forum is currently being implemented in the USEPA's ongoing risk assessment for perchlorate (USEPA, 2002c).

3.2. Cross-chemical extrapolation

In developing their threshold limit values (TLV-TWA), it is common practice for the American Conference of Government Industrial Hygienists (ACGIH) to make use of toxicity information on toxicologically similar compounds to inform cases where data are lacking on the compound of interest. For example, the ACGIH (2002) documentation for propylene imine states: "on the basis of its lesser toxicity in comparison with ethylene imine (TLV-TWA of 0.5 ppm), a TLV-TWA of 2 ppm is recommended for propylene imine. Because propylene imine resembles ethylene imine in its physiologic action, a skin notation is also recommended." Similarly, the ACGIH (2002) documentation for propargyl alcohol states: "based on the structural and apparent toxicological similarity to allyl alcohol, a TLV-TWA of 1 ppm, on half that of allyl alcohol, is recommended for propargyl alcohol."

Another example of the use of mode-of-action considerations for cross-chemical extrapolation is the IARC (1995) categorization of vinyl fluoride as category 2a, probably carcinogenic to humans, despite inadequate human evidence, by analogy with vinyl chloride, for which there is adequate human evidence.

The USEPA has also considered mode-of-action as the basis for using data across chemicals. For example, the RfD for nitrite is based on a study conducted with nitrate, recognizing that the critical effect for nitrate

(methemoglobinemia) results from its reduction to nitrite in vivo (USEPA, 1985). Another example is provided by the IRIS entry for 4,6-dinitro-*o*-cyclohexylphenol, which was derived by analogy to 2,4-dinitrophenol, assuming a common mode of action.

An important use of cross-chemical extrapolation is the development of toxicity equivalence factors (TEFs), in which the toxicity of related compounds (congeners) is based on a measure of their relative potency compared to a well-studied reference compound. Examples of systems of TEFs that have been developed include those for polycyclic aromatic hydrocarbons, using benzo-*a*-pyrene as the reference compound (USEPA, 2001a), and those for "dioxin-like" compounds, using 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as the reference compound (USEPA, 2003b). A key assumption underlying the use of TEFs is that the various compounds act by a common mode of action (Safe, 1998).

3.3. Cumulative risk assessment

As with TEFs, one of the criteria for the performance of a cumulative risk assessment across multiple chemicals is the all of the chemicals considered demonstrate a common mechanism of toxicity (USEPA, 2002a). For example, cumulative risk assessments can be performed across multiple organophosphate and carbamate pesticides, which share a common mode of action involving inhibition of cholinesterase. In this case, it is possible to specify a single measure of response, inhibition of cholinesterase activity. The challenge lies in determining the contribution of a particular chemical under the conditions of a mixed exposure, where there is a potential for interactions in both toxicokinetics (metabolism) and toxicodynamics (reversible enzyme inhibition vs. irreversible phosphorylation).

3.4. Justification of the use of a precursor or biomarker for a critical effect

A precursor, by definition, must precede (temporally) the associated critical effect. It is often assumed, though not necessarily true, that the precursor will occur at lower doses than the critical effect. A biomarker, on the other hand, need only bear a demonstrable dose–response relationship to the effect, and may reflect an ongoing toxic process. Examples of useful biomarkers include hemoglobin adducts and liver enzyme assays. A classic example of the use of a biomarker as a surrogate for a critical effect is the use of increased relative liver weight change as a surrogate for acute liver toxicity. The justification for treating a liver weight change as an adverse effect is the belief that the change in liver size is reflective of a toxic process. Typically, therefore, relative liver weight changes are not considered a critical effect unless they are supported by findings of associated pathology. Relative liver weight has also been used as a precursor, in the case of the chronic liver toxicity of trichloroethylene (Barton and Clewell, 2000). In this case, the acute change in liver weight after short-term dosing was assumed to reflect an early effect of the compound, proliferation of peroxisomes, which in turn was assumed to be a precursor for the chronic liver toxicity. It was also assumed that the precursor effect and the chronic toxicity would occur at similar doses.

An elegant example of the use of a mode-of-action analysis to justify the use of a precursor for a critical effect in a noncancer risk assessment is provided in the USEPA (2002c) risk assessment for perchlorate. In this case, the critical effect is disruption of thyroid function and associated thyroid-hormone-related effects on neurodevelopment. The precursor used as the basis for cross-species dosimetry, however, is perchlorate's inhibition of thyroid iodine uptake.

The new USEPA (2003a) cancer guidelines provide a detailed discussion of the use of data on precursor events to inform the dose–response below the level of the observation of the toxic sequelae. Evaluations of the potential for using precursor data in this way have been performed for butadiene, vinyl chloride, and benzene (Albertini et al., 2003), but to date there are no instances of a cancer risk assessment based on precursor data.

The major difficulties limiting the usefulness of precursor events to extend the dose–response in cancer risk assessment are:

- The possibility that the event is not actually an obligatory precursor to the lesion of concern. That is, an event may be associated with exposure in a similar fashion to the observed tumors, and may appear to be plausibly linked with the formation of tumors in that tissue, but may in fact not reside on the chain of events leading to the tumors of concern. For example, in the case of the cancer risk assessment for vinyl chloride (USEPA, 2000a), dose–response data on preneoplastic lesions in the liver were available, but were not used because they were determined to be precursors to hepatocellular carcinoma, while liver angiosarcomas, the tumor type of greatest relevance to human risk assessment, are derived from sinusoidal cells.
- The possibility that the event is a precursor in time, but not in dose. That is, the precursor event may occur sooner than the tumor but there may be no margin of safety between doses at which the precursor is observed and doses associated with the development of tumors.
- The possibility that the quantitative relationship between the incidence of the precursor event and the incidence of the tumor is difficult to establish. For example, in the case of the cancer risk assessment for vinyl chloride (USEPA, 2000a), substantial

dose–response data were available for a number of DNA adducts, but could not be used due to uncertainty regarding their quantitative implications.[6]

3.5. Harmonization of risk assessment across cancer and noncancer endpoints

The draft risk assessment for perchlorate discussed earlier (USEPA, 2002c) provides an excellent example of the use of mode-of-action information to support a harmonization of the cancer and noncancer risk assessments for a chemical. In this case, no cancer risk assessment was performed. Instead, the document presents a solid case for the hypothesis that disruption of thyroid hormone homeostasis by perchlorate inhibition of iodine uptake is a common factor that serves as the key event leading to a variety of observed effects, both cancer and noncancer. The most sensitive noncancer endpoint, neurodevelopmental effects, and then serves as the basis for the dose–response assessment.

In several ways, the draft USEPA (2002c) risk characterization for perchlorate represents a significant advance over previous agency risk characterizations for other chemicals. A notable feature of the draft perchlorate risk characterization is the unparalleled use of mode of action to drive the structure and assumptions of the risk assessment approach, in the spirit of the agency's new carcinogen risk assessment guidelines (USEPA, 2003a). In particular, the perchlorate risk assessment makes effective use of the mode-of-action information relevant to perchlorate to provide the foundation for a scientifically sound, harmonized assessment.

Another recent example of a harmonized risk assessment based on common mode of action is the risk assessment for chloroform (USEPA, 2001b). In this case the harmonizing assumption in the risk assessment is that the carcinogenicity of chloroform is secondary to sustained or repeated cytotoxicity and associated regenerative hyperplasia. In contrast to the perchlorate risk assessment, cancer dose–response analyses are performed, but rather than deriving a linear risk estimate, the point of departure (LED_{10})[7] for tumors is compared with the noncancer RfD to obtain a margin of exposure (MOE). The adequacy of the MOE is then evaluated.

The USEPA is currently reviewing harmonized risk assessments for three additional chemicals: vinyl acetate, acetaldehyde, and formaldehyde. The common harmonizing mode-of-action assumption for these chemicals is cytotoxicity in the upper respiratory tract, with a variable potential for a genotoxic contribution (Bogdanffy et al., 2001b; Conolly et al., 1992).

3.6. Selection of dosimetry approach and metric

One of the key uses of mode-of-action information is to support the selection of the appropriate dose metric and dosimetry approach. For example, one of the important features of the USEPA's recent risk assessment for TCDD (USEPA, 2003b) is the use of body burden (ng/kg) as the dose metric in contrast to the traditional use of daily intake, ng/kg/day. The use of body burden is consistent with the receptor-mediated mode of action for the effects of TCDD and incorporates differences between species in the half-life for TCDD elimination (USEPA, 2003b).

The use of mode-of-action information to drive the selection the appropriate dose metric is of particular importance for the application of PBPK modeling in a risk assessment. The first instance in which a PBPK model was applied in an agency risk assessment was the use of a model of methylene chloride (Andersen et al., 1987) in the USEPA (1987) inhalation cancer risk assessment. In this case, there were two competing mode-of-action hypotheses, reflecting the possibility that reactive species from either the oxidative or conjugative metabolism of methylene chloride could be responsible for its carcinogenicity. The PBPK model was used to resolve this uncertainty by comparing the predicted dose–response of the appropriate dose metrics for each of the alternative mode-of-action hypotheses with the observed dose–response for tumors. The conclusions of the PBPK evaluation, indicating that the conjugation pathway produced the ultimate carcinogenic species, were subsequently supported by directed experiments that further illuminated the mode of action (Clewell, 1995).

Two additional risk assessments have been conducted in which mode-of-action information was used to identify the appropriate dose metric for PBPK-model-based dosimetry: a noncancer risk assessment for ethylene glycol monobutyl ether (USEPA, 1999), using the model of Corley et al. (1994, 1997), and both the cancer and noncancer risk assessments for vinyl chloride (USEPA, 2000a), using the model of Clewell et al. (2001). The perchlorate risk assessment (USEPA, 2002c) also makes use of extensive PBPK modeling. In addition, the agency is currently in the process of evaluating a proposed PBPK risk assessment for isopropanol (Clark et al., 2004; Gentry et al., 2002).

[6] The toxicological review of vinyl chloride (USEPA, 2000a) states that: "adduct levels normally cannot be used directly to extend tumor dose–response data to lower doses, since tumor formation from adducts depends on many factors, including the consequences of adduct repair or failure to be repaired. Thus, although a quantitative analysis of the relationship between VC metabolism, adduct formation, and tumor formation is likely to be a fruitful area for additional research, it is premature to attempt to establish a quantitative link between the tissue concentrations of a specific adduct and the risk of cancer in that tissue."

[7] The LED_{10} in a cancer dose–response assessment is the equivalent of the $BMDL_{10}$ in a noncancer dose–response assessment, and represents the 95% lower bound estimate of the dose associated with a 10% increase in an effect over controls.

3.7. Support for derivation of chemical-specific adjustment factors

Recent guidance from the International Programme on Chemical Safety (IPCS, 2001) addresses the data needs for replacing default uncertainty factors with chemical-specific adjustment factors (CSAFs). The CSAF approach breaks the interspecies and intraspecies uncertainty factors into toxicokinetic and toxicodynamic components, each of which can be replaced by a CSAF if data are available. Mode-of-action information plays a key role in the identification of appropriate dose metrics and biomarkers that can be used to estimate the toxicokinetic and toxicodynamic components, respectively.

The use of mode-of-action information to identify an appropriate dose metric for the evaluation of the toxicokinetic component is similar to its use to support PBPK dosimetry, as discussed above, The use of the mode of action to identify biomarkers for the toxicodynamic component is clearly addressed in the guidance document (IPCS, 2001): "CSAFs for interspecies [toxicodynamic] differences and human [toxicodynamic] variability may be derived from comparative response data for the toxic effect itself in the target organ (e.g., haemolysis as in Case B [EGBE]) or for a point in the chain of events that is considered critical to the toxic response (i.e., a relevant end-point, sometimes referred to as a "biomarker" of effect) based on understanding of mode of action, under experimental conditions where toxicokinetic variations have been precluded." The USEPA (2002d) is currently in the process of developing a risk assessment for boron and compounds that includes the derivation of CSAFs.

3.8. Support for BBDR modeling

In essence, a biologically based model represents an attempt to provide a mathematical representation of the mode of action for the chemical being described. Both the structure and the parameters in the model should, to the extent possible, be derived from the mode-of-action information available for that chemical. The ongoing risk assessment process for TCDD (USEPA, 2003b) has been marked by the development of a number of sophisticated pharmacokinetic and pharmacodynamic models that have explored the implications of different mode-of-action hypotheses. However, none of these models can be employed as a BBDR in the fashion envisioned in the USEPA (2003a) cancer guidelines. In the case of a carcinogenic effect, such a BBDR model would take the tissue dosimetry from a PBPK model as input and predict the resulting tumor incidence over time in both rodents and humans, in place of empirical (e.g., benchmark) dose–response modeling. It is important to note that, as defined by the USEPA cancer guidelines, a BBDR model cannot, in the end, be essentially empirical; that is, it cannot merely represent a statistical fit to bioassay tumor incidence data, no matter how sophisticated the biological constructs in the model. Instead, the parameters in the model must have direct biological correspondence (mutation rates, cell division rates, etc.), similar to the requirements for the parameters in the PBPK model, and must have been determined on the basis of experiments apart from the animal bioassays themselves. To date, the only risk assessment for which a BBDR model meeting these criteria has been proposed for use in both the rodent and human is the inhalation cancer risk assessment for formaldehyde, which is currently under internal review. The proposed mode of action and the implementing BBDR model have been described by the developers, Rory Conolly and co-workers (CIIT, 1999; Conolly et al., 2003, 2004).

3.9. Challenges for the use of mode-of-action information in risk assessment

The expansion in the use of the mode-of-action concept in risk assessment has not been without controversy. On the contrary, several recent risk assessments have been marked by heated debate, centering on whether the agency's risk assessment approach was appropriate for the mode of action of the toxic chemical. Perhaps the Agency's newly stated willingness to consider mode of action raises unwarranted stakeholder expectations regarding the possibility of departing from default risk assessment approaches. The debate on use of mode of action can be categorized into three areas:

- What should be used as the criteria for acceptance of a postulated mode of action?
- How much detail must a mode of action provide regarding the specifics of the toxic process before it can be used to direct risk assessment decisions?
- To what extent should the mode of action be reflected in the alternative approaches used for the quantitative risk assessment

The nature of these issues can be illustrated by examples from two recent Agency risk assessments: trichloroethylene and arsenic.

3.10. Trichloroethylene

The USEPA (2001c) draft risk assessment for trichloroethylene (TCE) serves as an example of the practical difficulties associated with attempting to use mode of action to direct the risk assessment approach. This assessment was conducted using a novel process involving substantive input from recognized outside experts.

Consensus was fostered through a joint USEPA/stakeholder effort that included two "Williamsburg meetings" at which the opinions of experts on TCE and risk assessment were solicited. Subsequently, the Agency commissioned papers by several EPA scientists and outside experts, most of whom had been involved in the consensus process, that were published together as the "state-of-the-science" in a special issue of the Environmental Health Perspectives Supplement in May 2000 (Volume 108, Supplement 2).

Despite this considerable effort, the USEPA (2001c) draft risk assessment was widely perceived as representing a departure from the scientific consensus in several key respects. In particular, one of the major points of scientific agreement documented in the proceedings of the second Williamsburg meeting (Clewell and Andersen, 1997) was that, based on mode-of-action considerations, only the margin of exposure (MOE) approach should be used for the mouse liver carcinogenicity of TCE, rather than the default linear extrapolation approach. This consensus position was also reflected in the USEPA-commissioned state-of-the-science chapter on mode of action in the liver (Bull, 2000). Nevertheless, the USEPA (2001c) draft TCE risk assessment featured linear risk estimates based on the mouse liver tumors. In fact, these linear risk estimates based on mouse liver tumors provided the highest risk estimates from animal studies and served as a center-point of the Agency's risk assessment for TCE.

In support of their decision to calculate linear risks for mouse liver tumors, the USEPA (2001c) described what they viewed as critical inadequacies in the existing mode-of-action information: "at present, however, the extensive mode-of-action information still lacks identification of the sequence of key events and a quantitative description of the doses at which those key events begin to occur." This decision was subsequently called into question by the Agency's own Scientific Advisory Board (SAB); in its review of the draft TCE risk characterization, the SAB (USEPA SAB, 2002) called on the Agency to "provide a more detailed explanation for the Agency's treating cancer mode of action in a linear way." In the face of widespread criticism on a number of issues by both stakeholders and independent scientists who had participated in the consensus process, the USEPA Office of Research and Development announced that they no longer supported the draft TCE risk characterization, and were launching a new, multi-year risk assessment effort for TCE. The problems encountered by the agency in this case should be viewed as a measure of the practical difficulty of satisfying the expectations of stakeholders for incorporation of state-of-the-art methods for dosimetry and mode-of-action evaluation, on the one hand, while maintaining a public health protective posture in the face of uncertainty, on the other.

3.11. Arsenic

The USEPA has published a maximum contaminant level (MCL) for arsenic in drinking water of 0.01 mg/L (USEPA, 2001d), replacing the former value of 0.05 mg/L. This MCL was derived on the basis of the estimated dose–response for bladder and lung cancer in a population in Taiwan chronically exposed to concentrations of arsenic in drinking water ranging as high as 1.75 mg/L. These dose–response calculations were performed under the standard default assumption of linearity, despite growing evidence of a nonlinear mode of action for the carcinogenicity of arsenic (Abernathy et al., 1996; Clewell et al., 1998; Snow et al., 2001). As in the case of TCE, the USEPA (2001c) felt unable to depart from the linear default in the case of arsenic due to the lack of definitive data on a specific nonlinear mode of action for its carcinogenicity. Unfortunately, the result of the use of a linear dose–response calculation in this case is a highly conservative drinking water standard, which may entail very significant costs to many local communities in the United States (USEPA, 2001d).

Of course, it is entirely possible that even if the mode of action for the carcinogenicity of arsenic is highly nonlinear, the nonlinearity could occur at concentrations well below those of concern for environmental exposure, and the dose–response in the range of the MCL and above could actually be linear. While there is currently no completely satisfying description of a specific mode of action for arsenic carcinogenicity, it almost certainly involves the binding of trivalent inorganic arsenic to key cellular proteins involved in cell cycle control (Salnikow and Cohen, 2002). In general, interactions of trivalent inorganic arsenic with cellular proteins have been observed to occur at cellular concentrations on the order of 0.1 μM (Hu et al., 2002). Based on a combination of in vitro experiments and PBPK modeling of in vivo kinetics, the cellular concentrations at which this "threshold" occurs have been suggested to be associated with drinking water exposures on the order of the MCL (Lee, 1999).

More problematic than the impact of a nonlinear arsenic dose–response on the MCL are its implications for an ambient water quality criterion (AWQC) for arsenic. The current AWQC for arsenic (0.000018 mg/L) was based on a linear estimate of a water concentration associated with a risk of one in a million for skin cancer from water consumption of 2 L/day. The AWQC for arsenic is now being revisited in view of the recent decrease in the MCL. If one is willing to believe that a nonlinearity in the dose–response for arsenic carcinogenicity occurs in the general vicinity of the MCL, then the actual risks in the range of the AWQC could be much lower than the linear estimates, and even zero. Due to variability in the sensitivity of individuals across a population, which has been estimated to typically be greater

that an order of magnitude (Hattis et al., 1987), it can be expected that the dose at which such a nonlinearity would occur varies from one individual to another. As a result, the nonlinearity in the cancer dose–response curve for the population would perhaps extend over roughly an order of magnitude, with risks similar to those estimated from the Taiwanese study at the high end (above the MCL), and essentially zero risk at the low end (well above the AWQC).

Fig. 3 attempts to describe the nature of the impact of a nonlinear mode of action for the carcinogenicity of arsenic on the expected dose–response. In Fig. 3 a linear risk estimate for arsenic carcinogenicity such as that used for the arsenic MCL is depicted by the dashed straight line. The heavy solid curve provides an example of a more plausible (but highly speculative) nonlinear dose–response for arsenic in an average individual. The strongly nonlinear nature of this curve reflects the sharp transition that would be expected to occur in a particular individual's cells, from concentrations of arsenic with little effect to those at which inhibition of a key cellular protein becomes evident. The factors listed at the right of Fig. 3 have been suggested to alter the sensitivity of an individual to the carcinogenic effects of arsenic in drinking water (NRC, 1999). For example, dietary intake of inorganic arsenic would add to the risk from drinking water exposure, while deficiencies in key nutrients, such as selenium and choline, could potentially increase the sensitivity of an individual to the effects of arsenic. Similarly, individual differences in the metabolism and clearance of arsenic could alter the relationship between drinking water intake and tissue arsenic concentrations. An example of a potentially important source of individual variation is the polymorphism for glutathione transferase Pi (GST-Pi), a key enzyme in the metabolism of arsenic. Other risk factors could include genetic predispositions, as well as alterations in key genes (e.g., the P53 tumor suppressor gene) due to exposures to other environmental carcinogens. The narrow solid curves suggest how variation in these risk factors across a population could shift the dose–response curve for specific individuals in either direction from the population mean.

The dose–response for the population would therefore be a curve with a shallower slope, transitioning smoothly between the curves for the more and less sensitive individuals. This "true" population risk is illustrated by the heavy dashed curve in Fig. 3. Thus the slope of the dose–response associated with the nonlinearity would depend on both the magnitude of the overall risk reduction in an individual due to the nonlinearity in the mode of action, and the breadth of the population transition resulting from inter-individual variability in sensitivity. The net result, as illustrated by comparing the curved and straight dashed lines, would be that lower arsenic exposures would result in a much greater reduction in the population risk than would be predicted by linear estimates.

The problem faced by the Agency at this point is the lack of a quantitative approach for estimating low-dose risks in the case of a nonlinear dose–response. Therefore, the only current alternative to calculating a linear risk estimate is the MOE approach, which does not actually yield a risk estimate. In the case of arsenic, the MOE of interest would be between the lowest ED_{01} for cancer and the RfD. The arsenic RfD is currently 0.0003 mg/kg/day, which corresponds to a drinking water concentration of 0.01 mg/L, while the lowest ED_{01} is on the order of 0.2 mg/L (NRC, 1999). This MOE of 20 would probably not be considered an adequate MOE to justify the use of the current RfD as the basis for an AWQC for arsenic. The problem then becomes one of providing an objective basis for determining an adequate MOE.

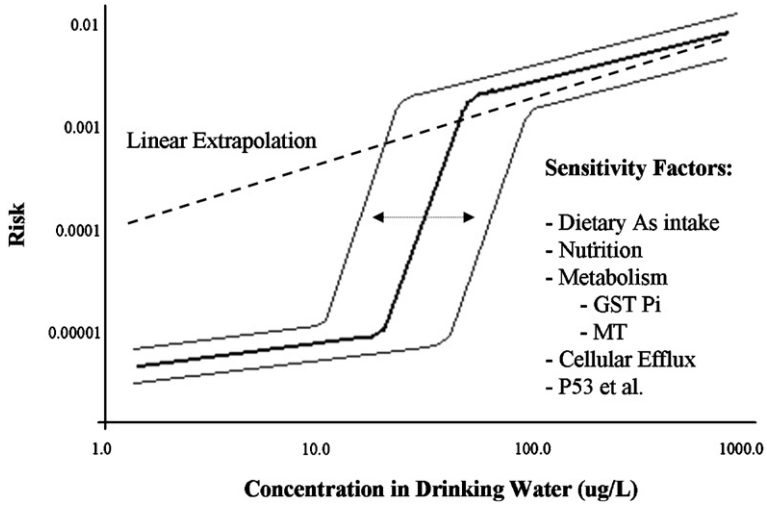

Fig. 3. Conceptual illustration of alternative dose–response curves for the carcinogenicity of arsenic in a population exposed to arsenic in drinking water (from Clewell, 2001, with permission).

Ultimately, a preferable approach would be the use of a biologically based dose–response model for arsenic to perform low-dose extrapolation based on in vitro data on the interactions of arsenic with critical cellular control proteins (Andersen et al., 1999).

4. Summary

Mode of action has clearly been demonstrated to be a useful concept, serving as an organizing principal for the incorporation of chemical-specific information in risk assessment. Its utility stems to a large extent from its flexibility. That is, the mode of action for a chemical can be described at almost any level of complexity, reflecting the extent of chemical-specific information available and the needs of the risk assessment.[8]

At one extreme, a very simple description of the mode of action can often suffice to provide the basis for a more accurate risk assessment alternative to replace a generic default. For example, the brief mode-of-action statement, "the liver carcinogenicity of vinyl chloride results from the production of a short-lived, reactive epoxide metabolite that forms DNA adducts, leading to mistranscription and neoplasia," is all that is needed to support the PBPK-based risk assessment for vinyl chloride (USEPA, 2000a). Similarly the mode of action underlying the USEPA (2001b) harmonized risk assessment for chloroform can be described as simply as "the chronic cancer and noncancer effects of chloroform in the liver and kidney result from the local metabolism of chloroform to reactive species, leading to cytotoxicity with an associated regenerative hyperplasia and, if exposure is continued, the eventual production of tumors." At the other extreme, the mode of action can be described at a level of detail adequate to support the development and evaluation of a sophisticated BBDR model, such as in the case of formaldehyde (Conolly et al., 2003, 2004).

This flexibility of the definition of the mode-of-action concept, however, is also its chief drawback. A discussion of the desired elements of a mode of action, and the kinds of data that inform its development is provided in the new USEPA (2003a) carcinogen risk assessment guidelines, along with a conceptual framework for mode-of-action evaluation, which has also been adopted by IPCS (Sonich-Mullin et al., 2001). Nevertheless, it is still difficult to determine when a mode-of-action hypothesis contains an adequate level of detail for a particular application. Cases in point are the ongoing controversy regarding the risk assessments for trichloroethylene (USEPA, 2001c), dioxin (USEPA, 2003b), and arsenic (USEPA, 2001d), where much of the debate appears to reflect different understandings of the necessary elements of a mode of action and, in particular, different criteria for its acceptance, as reflected in the nature and extent of its application to actually tailor the risk assessment approach. More importantly, uncertainty regarding the necessary elements of a mode of action may hinder the process of identifying the experimental data that would be most useful to refine it. In view of the difficulties and extensive resources that may be required to establish a mode of action, every effort must be made to clarify the criteria that will be used to evaluate it.

The most important challenge for improving the use of mode-of-action information in risk assessment is the need to develop methods for evaluating the potential quantitative impact of a nonlinear mode of action, short of the development of a fully validated BBDR model. As difficult as it may be to firmly establish a nonlinear mode of action, it is even more difficult to develop a quantitative description of the nonlinear dose–response for such a mode of action. As in the example of arsenic, until such quantitative alternatives can be described, it will continue to be necessary for agencies to resort to the default linear approach to obtain quantitative risk estimates, even in cases where the qualitative evidence for the existence of a nonlinear dose–response is compelling.

[8] The pragmatic use of flexible definitions was suggested by Lewis Carroll (in "Through the Looking Glass and What Alice Found There."): "'When I use a word,' Humpty Dumpty said in a rather a scornful tone, 'it means just what I choose it to mean—neither more nor less.'"

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