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# On the incorporation of chemical-specific information in risk assessment

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ABSTRACT

This paper describes the evolution of chemical risk assessment from its early dependence on generic default approaches to the current situation in which mechanistic and biokinetic data are routinely incorporated to support a more chemical-specific approach. Two methodologies that have played an important role in this evolution are described: mode-of-action evaluation and physiologically based biokinetic (PBBK) modelling. When used together, these techniques greatly increase the opportunity for the incorporation of biokinetic and mechanistic data in risk assessment. The resulting risk assessment approaches are more appropriately tailored to the specific chemical and are more likely to provide an accurate assessment of the potential hazards associated with human exposures. The appropriate application of PBBK models in risk assessment demands well-formulated statements about the chemical mode of action. It is this requirement for an explicit, mechanistic hypothesis that gives biologically motivated models their power, but at the same time serves as the greatest impediment to the acceptance of a chemical-specific risk assessment approach by regulators. The chief impediment to the regulatory acceptance and application of PBBK models in risk assessment is concern about uncertainties associated with their use. To some extent such concerns can be addressed by the development of generally accepted approaches for model evaluation and quantitative uncertainty analysis. In order to assure the protection of public health while limiting the economic and social consequences of over-regulation, greater dialogue between researchers and regulators is crucially needed to foster an increased use of emerging scientific information and innovative methods in chemical risk assessments.



## 1. Introduction

Until quite recently, approaches for quantitative chemical risk assessment relied almost exclusively on default approaches that could be applied across a wide variety of chemicals and effects. These default methods were easy to use because they required little to no information about the chemical or the manner in which it caused toxicity. However, it was recognized that a number of chemical-specific factors, such as biokinetics[1] and mechanism of toxicity, that could greatly impact the relative risks for different chemicals were ignored by the default approaches.

To compensate for the uncertainty associated with the lack of chemical-specific information, the default approaches made heavy use of health-conservative assumptions and/or safety (uncertainty) factors to assure the protection of public health. The evolution of chemical risk assessment in recent years has been characterized by a steady movement away from these default approaches and toward approaches that attempt to tailor the risk assessment to the chemical being evaluated. This has primarily been accomplished by the incorporation of three types of chemical-specific data: (1) data on the dose–response for the effects of the chemical, (2) data on the mechanism by which the chemical causes toxicity, and (3) data on the uptake, distribution, metabolism and elimination of the chemical.

This paper provides an overview of the evolution of risk assessment from its early reliance on generic default approaches to the current situation in which mechanistic and biokinetic data are routinely incorporated to support a more chemical-specific approach. Two methodologies that have played an important role in this evolution are described: mode-of-action evaluation and physiologically based biokinetic (PBBK) modelling. Impediments to the greater acceptance of these methodologies are then discussed, and

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[1] The time-course of drugs in biological systems has traditionally been referred to as pharmacokinetics. On the other hand, it has become popular to use the term toxicokinetics when dealing with chemicals that are toxic. This, of course, ignores the wisdom of Paracelsus: only the dose differentiates a poison and a remedy. To avoid this false distinction, the term biokinetic will be used in this paper.

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possible steps that could be taken to overcome these impediments are suggested.

## 2. Historical development of chemical risk assessment

It could be said that chemical risk assessment is both a new discipline and an old one. A well-known toxicologist, Dr. John Doull of the University of Kansas, has suggested that although the beginnings of modern risk assessment practice took place during the latter half of the previous century, risk assessment is likely to have been the second oldest profession (Doull, 1991). Certainly, our most ancient ancestors found it beneficial to categorize their environment into foods, poisons and remedies. In what was perhaps the first chemical risk management decision, King Louis XIV issued a royal decree forbidding apothecaries to sell arsenic or other poisonous substances, except to persons known to them (Gilbert, 2007).

The earliest documented concerns related to chemical hazards in the environment were prompted by the association of chemical exposure with occupational illness, as recorded in 1713 by Bernardino Ramazzini in his classic work, "*Diseases of Workers*." Commenting on the "harvest of diseases reaped by certain workers from their crafts and trades," Ramazzini describes the principle cause as ". . .the harmful character of the materials that they handle, for these emit noxious vapors and very fine particles inimical to human beings and induce particular diseases. . .." In his studies, Ramazzini identified chemical hazards ranging from heavy metals to tobacco smoke, although with regard to the latter he was quick to add:

> *"However, let no one suppose that I wish to speak ill of a plant so celebrated that it has been dignified with the title 'Queen', a plant so agreeable to all Europeans, above all in those realms where the use of tobacco is reckoned a profitable source of revenue."*

Several episodes of "killer smog" – the most famous of which, in December 1952, in London, England, caused over 8000 deaths (Stone, 2002) – and the 1962 publication of Rachel Carson's "*Silent Spring*", raised the awareness of hazardous chemicals as a threat to the environment in the minds of the public. In the U.S. in particular, there was a rapid downhill spiral to public chemophobia fueled by a succession of highly publicized toxic chemical concerns: DDT, saccharin, FD&C Red No. 2, cyclamates, ethylene dibromide, dioxin, and Alar (on apples).

Up to this time, the focus of chemical risk assessment was essentially qualitative—describing pathological changes observed after the exposure of laboratory animals, often in large numbers. Despite the accumulation of a large volume of animal data, there has been a growing question of its usefulness due to the perceived difficulty of interpreting the significance of the animal results for humans. One of the more critical areas of uncertainty in the relationship between the results of laboratory animal experiments and the likely human risk from a chemical relates to the nature of the dose–response for the effects of the chemical; that is, how effects at frankly toxic doses in animals can be extrapolated to predict risks at the generally much lower doses to which humans may be exposed.

An understanding of the importance of dose–response in the effects of toxic chemicals dates at least as far back as the 16th century, with the famous statement of Paracelsus: "Solely the dose determines that a thing is not a poison" (Binswanger and Smith, 2000). This principal has guided chemical risk assessment to the present day. The overarching goal of human health risk assessment is simply to estimate a level of chemical exposure (dose) that is unlikely to be associated with adverse effects in the population of concern (workers, the public, children, etc.). This estimate is usually based on data from experimental animal studies or human epidemiological studies that differentiate exposures with and without adverse effects.

In the U.S., the increasing concern about the risk of cancer from exposure to environmental chemicals has driven the development of low-dose extrapolation methods to estimate cancer risks at doses that are orders of magnitude below those at which tumors are seen in animal bioassays. The first instance of a U.S. regulatory agency conducting a formal quantitative risk assessment (i.e., the calculation of a probability of harm) occurred in 1973, when a U.S. Food and Drug Administration regulatory document, "*Compounds Used in Food-Producing Animals*" (38 Fed. Reg. 19226, 1973), specified the required sensitivity of methods for measuring trace levels of carcinogens in meat products on the basis of the "maximum exposure resulting in a minimal probability of risk to an individual (e.g., 1/100,000,000). . .." A few years later, the 1980 U.S. Supreme Court decision on benzene provided the first clear mandate for quantitative low-dose extrapolation. Referring to OSHA's responsibility to protect workers from significant risk, the Court stated:

> *"It is the Agency's responsibility to determine in the first instance what it considers to be a "significant" risk. Some risks are plainly acceptable and others are plainly unacceptable. If, for example, the odds are one in a billion that a person will die from cancer by taking a drink of chlorinated water, the risk could clearly not be considered significant. On the other hand, if the odds are one in a thousand that regular inhalation of gasoline vapors that are 2% benzene will be fatal a reasonable person might well consider the risk significant and take the appropriate steps to decrease or eliminate it."* (I.U.D. v. A.P.I., 448 U.S. at 655)

A few years later, following widespread criticism of several risk assessment decisions made by health regulatory agencies, the U.S Congress commissioned a report by the National Academy of Science, "*Risk Assessment in the Federal Government: Managing the Process*," (NAS, 1983) that laid a formal foundation for modern chemical risk assessment. More recently, a comprehensive review of risk assessment for chemicals in food and the diet was conducted under the auspices of the European Commission. This concerted action, known as the Food Safety in Europe (FOSIE) initiative, resulted in a series of important publications, by experts in the field, describing the various aspects of chemical risk assessment:

- Hazard identification, by methods of animal-based toxicology (Barlow et al., 2002), *in vitro* toxicology (Eisenbrand et al., 2002), and epidemiology (van den Brandt et al., 2002)
- Dose–response assessment (Dybing et al., 2002; Edler et al., 2002)
- Exposure assessment (Kroes et al., 2002)
- Risk characterization (Renwick et al., 2003)

These references provide a valuable resource for chemical risk assessments, not only for chemicals in food, but also for environmental and occupational exposure to chemicals.

## 3. Recent innovations in chemical-specific risk assessment

To a large extent, the increased use of chemical-specific data has been catalyzed by the development and application of a number of relatively new quantitative methodologies, as described in an excellent review by Edler et al. (2002). The application of these mathematical methods has benefited in turn from the harmonization of approaches for the evaluation of mechanism of toxicity (Sonich-Mullin et al., 2001). This discussion will focus on two methodologies that, together, provide a highly effective basis for

incorporating chemical-specific data in risk assessments: mode-of-action evaluation and PBBK modelling.

### 3.1. Mode-of-action evaluation

Mechanism of toxicity has occasionally been considered in risk assessments in the past, either to help in the determination of whether a particular carcinogenic effect seen in animals was relevant to humans or to support the use of a threshold approach for estimating safe human exposures, such cases served as exceptions to a standard approach that was applied across chemicals regardless of differences in mechanism of action. A concept that has proved useful for the incorporation of data on the mechanism by which a chemical causes a toxic effect is the 'mode of action', a term coined by the USEPA during the development of their new guidelines for carcinogen risk assessment (USEPA, 2005). In the USEPA (2005) guidelines, 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."*

The guidelines provide a discussion of the desired elements of a mode of action and a description of the kinds of data that can inform its development, using a conceptual framework for mode-of-action evaluation that was developed by the International Programme on Chemical Safety (IPCS) (Sonich-Mullin et al., 2001). The IPCS mode-of-action evaluation framework is an extension of the criteria of causation originally presented by Bradford Hill to aid in the interpretation of epidemiological data (Hill, 1965). The IPCS framework extends the Hill criteria to include the evaluation of experimental animal data. The key elements of the framework are listed below.

1. Description of tumor endpoint of concern
2. Postulated mode of action (sequence of events leading to tumor outcome)
3. Description of key events critical to the induction of tumors
4. Dose–response relationships between the key events and the tumor outcome
5. Temporal associations between the key events and the tumor outcome
6. Strength, consistency and specificity of association of tumor response with key events
7. Biological plausibility and coherence with data on other effects of the chemical
8. Discussion of alternative modes of action
9. Assessment of confidence in the postulated mode of action
10. Discussion of uncertainties, inconsistencies, and data gaps

This process must be carried out for each toxic endpoint, since it is possible for different endpoints to be mediated by different modes of action. For each endpoint, the associated mode of action has important implications for the risk assessment approach, including

- the likelihood that a toxic effect observed in animal studies is relevant to humans,
- whether human exposures by routes not tested in animals are of concern,
- the most appropriate method (linear or nonlinear) to use for extrapolation below the experimentally observed dose range,
- on what basis cross-species and cross-route equivalence of exposure should be determined (parent chemical concentration, metabolite concentration, production of a reactive metabolite, etc.).

Once the overall evaluation has been completed, one or more concise mode-of-action statements are sometimes developed that summarize the key aspects of the proposed mode(s) of action for the effects of the chemical (see text box).

Determining the implications of mode-of-action information for the likely human relevance of an animal outcome is particularly problematic and has frequently been a source of controversy. In order to promote transparent, harmonized approaches for such evaluations the IPCS has recently extended its mode-of-action framework to address consideration of human relevance for both cancer and noncancer effects observed in animal studies (Boobis et al., 2006, 2008).

**Examples of a Mode-of-Action Statement**

Although a complete description of a mode of action requires the use of the full framework, it is often possible to provide a brief statement that conveys, in a general way, the key elements of the process. For example, the carcinogenic modes of action of vinyl chloride and chloroform can be contrasted as follows:

*Vinyl Chloride*: The liver carcinogenicity of vinyl chloride results from its metabolism to a reactive metabolite, chlorovinylepoxide, that can enter the nucleus and form adducts with DNA that lead to mistranscription, mutation, and eventually tumors.

*Chloroform*: The liver carcinogenicity of chloroform results from its metabolism to a reactive metabolite, phosgene, that reacts with cellular macromolecules resulting in cytotoxicity and compensatory hyperplasia. When sustained, the combination of cytotoxicity and increased proliferation can eventually lead to tumors by increasing the frequency of spontaneous mutations in the absence of direct DNA-reactivity.

Although developed in the context of cancer risk assessment, mode-of-action evaluation can also be useful in risk assessments for noncancer effects. Understanding of the mode-of-action of a chemical effect provides important insights for the proper application of quantitative noncancer methodologies. For example, if there is evidence that the mode of action of a chemical changes significantly between the low and high doses in a study, then the dose–response at higher doses may not appropriately inform the behavior at lower doses and it may be preferable to censor the data from the higher doses when conducting dose–response analysis. The proper incorporation of chemical-specific biokinetic data also requires knowledge of the mode of action, e.g., whether the toxicity results directly from tissue exposure to the chemical itself, or whether it is mediated by the production of toxic metabolites.

### 3.2. Physiologically based biokinetic modelling

Biokinetics is the study of the time-course for the absorption, distribution, metabolism, and excretion of a chemical substance in a biological system. In biokinetic modelling, established descriptions

of chemical transport and metabolism are employed to simulate observed biokinetics *in silico* (Andersen et al., 1995a). Implicit in any application of biokinetics to toxicology or risk assessment is the assumption that the toxic effects in a particular tissue can be related in some way to the concentration time-course of an active form of the substance in that tissue. Moreover, in the absence of evidence for differences between species in the nature or extent of the tissue response, it is assumed that similar responses will be produced at equivalent tissue exposures regardless of species, exposure route, or experimental regimen (Andersen, 1981; Andersen et al., 1995a,b). Of course the actual nature of the relationship between tissue exposure and response, particularly across species, may be quite complex.

Classic compartmental modelling is largely an empirical exercise, where data on the time-course of the chemical of interest in blood (and perhaps other tissues) are collected. Based on the behavior of the data, a mathematical model is selected which possesses a sufficient number of compartments (and therefore parameters) to describe the data. The compartments do not generally correspond to identifiable physiological entities but rather are abstract concepts with meaning only in terms of a particular calculation. The advantage of this modelling approach is that there is no limitation to fitting the model to the experimental data. If a particular model is unable to describe the behavior of a particular data set, additional compartments can be added until a successful fit is obtained. Since the model parameters do not possess any intrinsic meaning, they can be freely varied to obtain the best possible fit, and different parameter values can be used for each data set in a related series of experiments.

Once developed, these models are useful for interpolation and limited extrapolation of the concentration profiles which can be expected as experimental conditions are varied. They are also useful for statistical evaluation of a chemical's apparent biokinetic complexity (O'Flaherty, 1987). However, since the compartmental model does not possess a physiological structure, it is often not possible to incorporate a description of these non-linear biochemical processes in a biologically appropriate context. For example, without a physiological structure it is not possible to correctly describe the interaction between blood-transport of the chemical to the metabolizing organ and the intrinsic clearance of the chemical by the organ.

PBBK models differ from the conventional compartmental biokinetic models in that they are based to a large extent on the actual physiology of the organism (Teorell, 1937a, 1937b). A number of excellent reviews on the subject are available (Himmelstein and Lutz, 1979; Gerlowski and Jain, 1983; Fiserova-Bergerova, 1983; Bischoff, 1987; Leung, 1991).

Instead of compartments defined solely by mathematical analysis of the experimental biokinetic data, compartments in a PBBK model are based on realistic organ and tissue groups, with weights and blood flows obtained from the literature. Moreover, instead of compartmental rate constants determined solely by fitting data, actual physical–chemical and biochemical properties of the compound, that can be experimentally measured or estimated by quantitative structure–property relationships, are used to define parameters in the model. To the extent that the structure of the model reflects the important determinants of the biokinetics of the chemical, the result of this approach is a model which can predict the qualitative and quantitative behavior of an experimental time-course without having been based directly on it. Fig. 1 illustrates the structure of a simple PBBK model for styrene, a volatile lipophilic compound.

The basic approach to PBBK model development is illustrated in Fig. 2. The process begins with the definition of the chemical exposure and toxic effect of concern, as well as the species and target tissue in which it is observed. Literature evaluation involves the integration of available information about the mechanism of toxicity, the pathways of chemical metabolism, the nature of the toxic chemical species (i.e., whether the parent chemical, a stable metabolite, or a reactive intermediate produced during metabolism is responsible for the toxicity), the processes involved in absorption, transport and excretion, the tissue partitioning and binding characteristics of the chemical and its metabolites, and the physiological parameters (i.e., tissue weights and blood flow rates) for the species of concern (i.e., the experimental species and the human). Using this information, the investigator develops a PBBK model which expresses mathematically a conception of the animal-chemical system. In the model, the various time-dependent chemical transport and metabolic processes are described as a system of simultaneous differential equations.

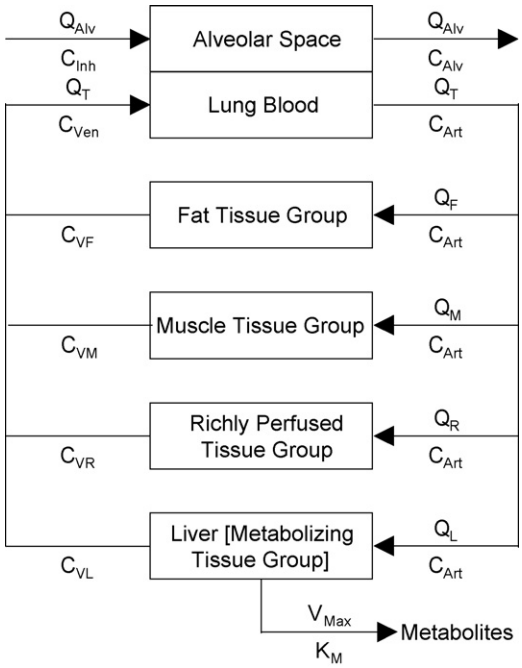


**Fig. 1.** Diagram of a PBBK model for styrene. In this description, groups of tissues are defined with respect to their volumes, blood flows ($Q$), and partition coefficients for the chemical. The uptake of vapor is determined by the alveolar ventilation ($Q_{ALV}$), cardiac output ($Q_T$), blood:air partition coefficient, and the concentration gradient between arterial and venous pulmonary blood ($C_{ART}$ and $C_{VEN}$). Metabolism is described in the liver with a saturable pathway defined by a maximum velocity ($V_{MAX}$) and affinity ($K_M$). The mathematical description assumes equilibration between arterial blood and alveolar air as well as between each of the tissues and the venous blood exiting from that tissue. (Adapted from Ramsey and Andersen, 1984)

The specific structure of a particular model is driven by the need to estimate the appropriate measure of tissue dose under the various exposure conditions of concern in both the experimental animal and the human. Before the model can be used in risk assessment it has to be validated against biokinetic, metabolic, and toxicity data and, in many cases, refined based on comparison with the experimental results. Importantly, the model itself can frequently be used to help design critical experiments to collect data needed for its own validation. Perhaps the most desirable feature of a PBBK model is that it provides a conceptual framework for employing the scientific method: hypotheses can be described in terms of biological processes, quantitative predictions can be made on the basis of the mathematical description, and the model (hypothesis) can be revised on the basis of comparison with targeted experimental data.

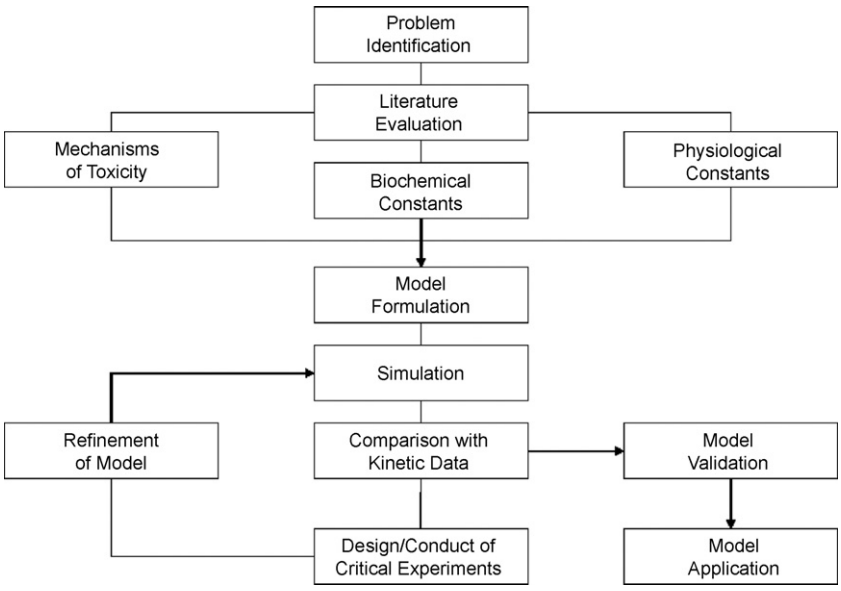


**Fig. 2.** Flow chart of the PBBK modelling process, showing the iterative process of model development in which the discrepancies between data and model predictions drive refinement of the model through the design of informative studies. (Adapted from Clewell and Andersen, 1989)

Refinement of the model to incorporate additional insights gained from comparison with experimental data yields a model which can be used for quantitative extrapolation well beyond the range of experimental conditions on which it was based. In particular, a properly validated PBBK model can be used to perform the high-to-low dose, dose-route, and interspecies extrapolations necessary for estimating human risk on the basis of animal toxicology studies (Clewell and Andersen, 1985, 1987, 1989; Andersen et al., 1987, 1991; O'Flaherty, 1989; Reitz et al., 1990; Johanson and Filser, 1993). The physiological structure of PBBK models is particularly useful for examining early life exposure (Fisher et al., 1989, 1990; Barton, 2005; Clewell et al., 2007), and the target tissue dosimetry provided by PBBK modelling is a essential component in models of toxicodynamics, such as acetylcholinesterase inhibition (Gearhart et al., 1994) or mixture interactions (el-Masri et al., 1995), as well as in biologically based dose–response models of cancer (Clewell and Andersen, 1989).

### 3.3. *Chemical-specific adjustment factors*

Risk assessments have typically applied default factors to account for uncertainty regarding animal to human extrapolation and human variability. Significant progress has been made in recent years in refining this approach beyond the use of default uncertainty factors (e.g., Renwick, 1993; USEPA, 1994; Renwick and Lazarus, 1998; Renwick and Walton, 2001). An important step forward in the development of approaches for incorporating chemical-specific data in risk assessment is the recent guidance from the IPCS (2005) addressing the data requirements for replacing default uncertainty factors with chemical-specific adjustment factors (CSAFs). The IPCS (2005) CSAF approach breaks the inter- and intra-species uncertainty factors into toxicokinetic and toxicodynamic components, each of which can be replaced by a CSAF if adequate chemical-specific data are available. IPCS (2005) defines toxicokinetics as "the process of the uptake of potentially toxic substances by the body, the biotransformation they undergo, the distribution of the substances and their metabolites in the tissues, and the elimination of the substances and their metabolites from the body." Toxicodynamics is defined as "the process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects."

The toxicokinetic factor for interspecies differences ($AK_{UF}$) represents the ratio of the external exposures in humans and animals that would produce the identical internal (target tissue) exposures. Similarly, the toxicokinetic factor for human variability ($HK_{UF}$) represents the ratio of the doses in average and sensitive individuals that would produce the identical internal (target tissue) exposure. Depending on the data available for the chemical, the magnitude of the adjustment factor for toxicokinetics may be calculated based on a variety of biokinetic factors, such as the clearance of the chemical or the area under the blood concentration–time curve (AUC) for the chemical. For example, a cross-species toxicokinetic adjustment factor for boric acid has been estimated on the basis of the ratio of glomerular filtration rates in the animal and human (USEPA, 2002). PBBK models can also be used to estimate the adjustment factors for toxicokinetics, as is described in the example in the text box.

**Example of the calculation of a CSAF**

The calculation of a CSAF for interspecies differences, $AK_{UF}$, for 2-butoxyethanol provides a good example of the approach and considerations required for the IPCS methodology. In the case of 2-butoxyethanol, several PBBK models have been developed that could be used to determine the cross-species adjustment for toxicokinetics (Corley et al., 1994, 1997; Lee et al., 1998). In fact, it would be difficult to determine the $AK_{UF}$ in this case without a PBBK model (Health Canada, 2003). This is because the best animal data available to support the calculation of an $AK_{UF}$ consist of AUCs of 2-butoxyacetic acid in the blood of rats exposed to 2-butoxyethanol by inhalation for 6 h (Dill et al., 1998); however, the AUCs were reported for the post-exposure period only. Therefore, it is necessary to estimate the total AUC using a PBBK model, by integrating the predicted concentration of 2-butoxyacetic acid in venous blood both during and following an inhalation exposure of 6 h. Using the rodent PBBK model of Lee et al. (1998), Health Canada (2003) determined that the AUC during the exposure period was

actually on the same order as the AUC reported for the post-exposure period. Thus, use of the reported AUCs would result in an error factor of two in estimating the $AK_{UF}$. Use of the human data (Johanson and Johnsson, 1991) in the calculation of an $AK_{UF}$ is also problematic, because the exposures were conducted under exercising conditions. Analyses performed with the human PBBK model (Corley et al., 1994, 1997) indicate that the uptake of the parent compound is linearly related to the ventilation rate. Therefore, the AUC value in this study must be adjusted to account for working *versus* resting conditions using the results of the human PBBK model. (Note that the effect of ventilation rate on the highly soluble 2-butoxyethanol contrasts with the case of poorly soluble, lipophilic compounds, where ventilation rate has little impact on uptake.)

The toxicodynamic factor for interspecies differences ($AD_{UF}$) represents the ratio of the internal (target tissue) exposures in humans and animals that would produce the identical response. Similarly, the toxicodynamic factor for human variability ($HD_{UF}$) represents the ratio of the internal (target tissue) exposures in average and sensitive individuals that would produce the identical response. Toxicodynamic factors may frequently be determined from *in vitro* studies. For example, the toxicity of 2-butoxyethanol is due to the hemolytic effects of its metabolite 2-butoxyacetic acid on red blood cells. Therefore, a comparison of the concentrations of 2-butoxyacetic acid that result in lysis of red blood cells *in vitro* has been used as a basis for the toxicodynamic CSAFs for 2-butoxyethanol (Health Canada, 2003). An important source of uncertainty in the use of short-term *in vitro* studies as the basis for toxicodynamic adjustments is the extent to which the *in vitro* responses can provide a dependable surrogate for *in vivo* responses, particularly those for which there is a potential for evolution of the response over time, due to processes such as

- accumulation of damage (e.g., due to slow repair)
- induction of repair
- changes in cell population over time
- multi-organ feedback signaling

For example, in a study of hemolysis by 2-butoxyethanol, exposure for 12 days was associated with a smaller decreases in rat erythrocyte counts than 3 days of exposure, apparently due to increased erythrocyte production leading to an increase in the fraction of cells that were young, and therefore less susceptible to hemolysis (Ghanayem et al., 1992). Any quantitative species differences in this *in vivo* response could result in a relationship of the responses for chronic *in vivo* exposure that was different from the relationship observed *in vitro*.

These toxicodynamic CSAFs relate effect to internal dose, not external dose. Therefore, they cannot be applied independently of the toxicokinetic factors. That is, while a toxicokinetic factor could be applied together with a default toxicodynamic factor, the reverse is not true. In the example for 2-butoxyethanol, the toxicodynamic factors, which relate hemolysis to blood concentration of 2-butoxyacetic acid, cannot be applied in place of the default $AD_{UF}$ and $HD_{UF}$ unless there is adequate data to determine the relationship of 2-butoxyethanol exposure to blood concentration of 2-butoxyacetic acid.

## 4. Building bridges between researchers and regulators

The growing use of mode-of-action evaluation and biokinetic modelling has greatly increased the use of biokinetic and mechanistic data in risk assessment, resulting in approaches that are more appropriately tailored to the specific chemical and therefore provide a more accurate assessment of the potential hazards associated with human exposures. This progress in incorporating chemical-specific data in risk assessment has been marked by a productive, although sometimes contentious, interaction in which the evolution of scientific understanding has driven improvements in the risk assessment process, and perspective drawn from the risk assessment process has focused scientific research on key areas of inquiry.

The occasional discord in this interaction reflects the fact that the discipline of chemical risk assessment encompasses both elements of natural science, including toxicology, biochemistry, epidemiology, and veterinary science, as well as elements of public health protection policy and quantitative decision analysis. This admixture of science, which moves steadily forward, and policy, which can be somewhat intransigent in the face of change, creates an inevitable tension. However, the interaction also provides a unique opportunity to focus scientific research in directions that can be of immediate benefit to the public.

There is a fundamental difference between research and risk assessment, with highly significant implications. The scientific method is an intentionally iterative process in which hypotheses are generated, tested, and revised in the light of contradictory data. It has been said that "If we knew what to do when we started we'd call it search, not research." This exploratory process necessarily entails a likelihood of false steps.

In contrast to scientific research, risk assessment is a process in which there are considerable potential costs associated with an erroneous conclusion. Therefore, the level of certainty in a hypothesis required to embark on a new research effort or publish a paper documenting it is not the same as the level of certainty required to embrace a new risk estimate, particularly when the cost of being mistaken may be reduced protection of human health. As a result, many scientific researchers feel regulatory scientists are overly cautious, while regulators complain that researchers focus only on their primary hypothesis and are not sufficiently concerned about alternatives. Therefore, risk assessments based on less than optimal approaches tend to be retained until the research on the chemical of concern provides the regulator with an adequate level of (subjective) confidence in an alternative approach.

The challenge of gaining regulatory acceptance for alternative approaches is exacerbated by the rapidity with which the state of the art for chemical risk assessment is changing. For example, the Health Council of the Netherlands has recommended adoption of a more efficient risk assessment approach that puts greater emphasis on the mechanism of action and makes use of new technologies ranging from structure–activity relationships to genomics (HCN, 2001). As a result of the rapid evolution of modern science and technology, state-of-the-art risk assessments can no longer be accomplished by single individuals; they require a team of scientists with expertise in a variety of disciplines, ranging from biology and chemistry to mathematics and statistics. Some examples of the disparate skills and experience that can be required in a major risk assessment are listed in Table 1.

The accuracy of future health risk assessments will benefit not only from exciting new experimental methods such as genomics, but also from highly sophisticated quantitative methodologies such as biologically based dose–response modelling. To assure that these emerging techniques fulfill their intended purpose (i.e., to increase the accuracy of the risk assessment), they must be applied correctly and adequately documented.

Two suggestions can be made in this regard. First, there is a crucial need for rigorous quality assurance in all aspects of a complex human health risk assessment. Regulatory agencies need to adhere

**Table 1**
Some of the scientific skills and experience potentially required for a major risk assessment

| |
|---|
| Mode of action |
| Evaluation of biological plausibility of the proposed sequence of events |
| Qualitative and quantitative analysis of *in vitro* and *in vivo* data on genotoxicity and other endpoints |
| Interpretation of genomics data |
| Analysis of affected signaling pathways |
| Biokinetic modelling |
| Evaluation of biological plausibility of model structure and parameters |
| Verification of the mathematical equations defining the model |
| Verification of the computer code implementing the model |
| Monte Carlo analysis of model uncertainty and global sensitivity |
| Calibration and validation of the model using hierarchical Bayesian analysis |

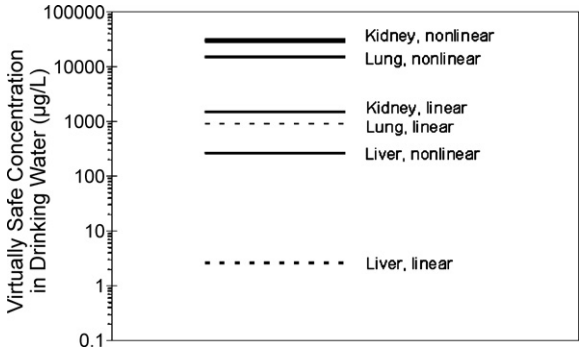


**Fig. 3.** Comparison of virtually safe concentrations for trichloroethylene in drinking water. Dashed lines indicate approaches that are not recommended. Broader solid line identifies the preferred approach based on biological plausibility. (Adapted from Clewell and Andersen, 2004)

to formal quality assurance procedures for health risk assessments, including independent verification of all key quantitative analyses. The Information Quality Act in the U.S. provides an example of an attempt to assure the quality of regulatory analyses (OMB, 2002).

Second, assuring the quality and accuracy of major health risk assessments is too important a matter to be left solely to the usual cadre of internal agency reviews, short-term external peer reviews, and public comment periods. The successful completion of a complex health risk assessment absolutely depends upon continuity of the quality assurance process throughout its development. Accordingly, regulatory agencies will need to seek continuing participation and review by external experts in each of the relevant methodologies. An example of such a relationship is one maintained by the USEPA Office of Pesticide Programs with its Scientific Advisory Panel (SAP). For major risk assessments, the SAP is maintained throughout the risk assessment, beginning early in the planning and scoping phase. Expertise on the panel is tailored to the requirements of each risk assessment.

In summary, the incorporation of chemical-specific data and modelling in risk assessment requires a continuing interaction between research scientists and regulatory scientists. Regulators, on the one hand, need to maintain a dialog with researchers and other experts in the wide variety of disciplines and specialties that are relied upon in the practice of risk assessment. In order to assure that best possible risk assessment practice is maintained, regulators should make use of this dialog to keep abreast of emerging scientific information and new methods of analysis. They also need to regularly draw support from experts in the field to assure that complex and technically demanding methodologies are correctly applied. Regulators cannot be expected to be experts in the many and various quantitative techniques that can be required in a modern risk assessment.

On the other hand, researchers performing studies on chemicals with potential human health implications should design their studies with the goal of assuring their relevance for the quantitative risk assessment process. Doing this requires an understanding of the methods of quantitative risk assessment as well as familiarity with the needs and current concerns of regulators. It has truthfully been said: "If you don't have a target, they're just arrows on the wall."

## 5. Research to foster more rapid acceptance of new data and models

The chief impediment to the regulatory acceptance and application of new data and biokinetic models in risk assessment is concern about uncertainties associated with their use. To some extent such concerns can be addressed by the development of accepted approaches for model evaluation (Clark et al., 2004). Another important response to these concerns is the development and demonstration of methods for quantitative uncertainty analysis. Methods that have been used in the past include parameter sensitivity analysis (Clewell et al., 1994) and Monte Carlo uncertainty analysis (Clewell, 1995; Allen et al., 1996). More recently, a hierarchical Bayesian analytical method, known as Markov chain Monte Carlo analysis has been used to combine parameter estimation with uncertainty analysis (Covington et al., 2007).

However, these methods only provide information regarding quantitative uncertainties associated with a single model structure and a single risk assessment approach. They do not help to objectively characterize the quantitative implications of uncertainties regarding the correct model structure and the appropriate application of the model to support alternative mode-of-action hypotheses. Research on this broader question is critically needed to provide regulators with tools for quantitatively characterizing the range of plausible risk estimates and objectively identifying preferred estimates.

As an example, Fig. 3 shows an attempt to summarize the results of the quantitative risk assessment for trichloroethylene in a fashion that conveys, to some extent, the scientific judgment of the risk assessors (the authors, in this case) regarding the relative scientific plausibility of the various risk estimates. This manner of presentation, which provides a sense of the range of plausible risk estimates while highlighting the most scientifically plausible values, was first suggested by Rodricks et al. (1987). The dotted lines represent approaches that are not recommended (linear approach for liver and lung tumors), and the broader solid line identifies the preferred approach based on biological plausibility (nonlinear approach for the kidney).

Of course, the presentation of alternative risks in Fig. 3 is fairly subjective. At least one research effort has been performed to investigate an approach for objectifying this kind of analysis using a methodology referred to as decision tree analysis (Clement and Tatman, 1990). In this approach the risk assessment is decomposed into its decision elements, which are then represented using a tree diagram (Fig. 4).

The risk assessment used in this case was the cancer risk assessment for methylene chloride, and the principal decisions involved were related to the alternative approaches for applying (or not applying) a PBBK model for that chemical (Andersen et al., 1987) in the risk assessment. These decisions, as illustrated in the tree diagram in Fig. 4, included whether to use the PBBK (PB-PK in Fig. 4) model or applied dose in the animal, whether to scale from animal

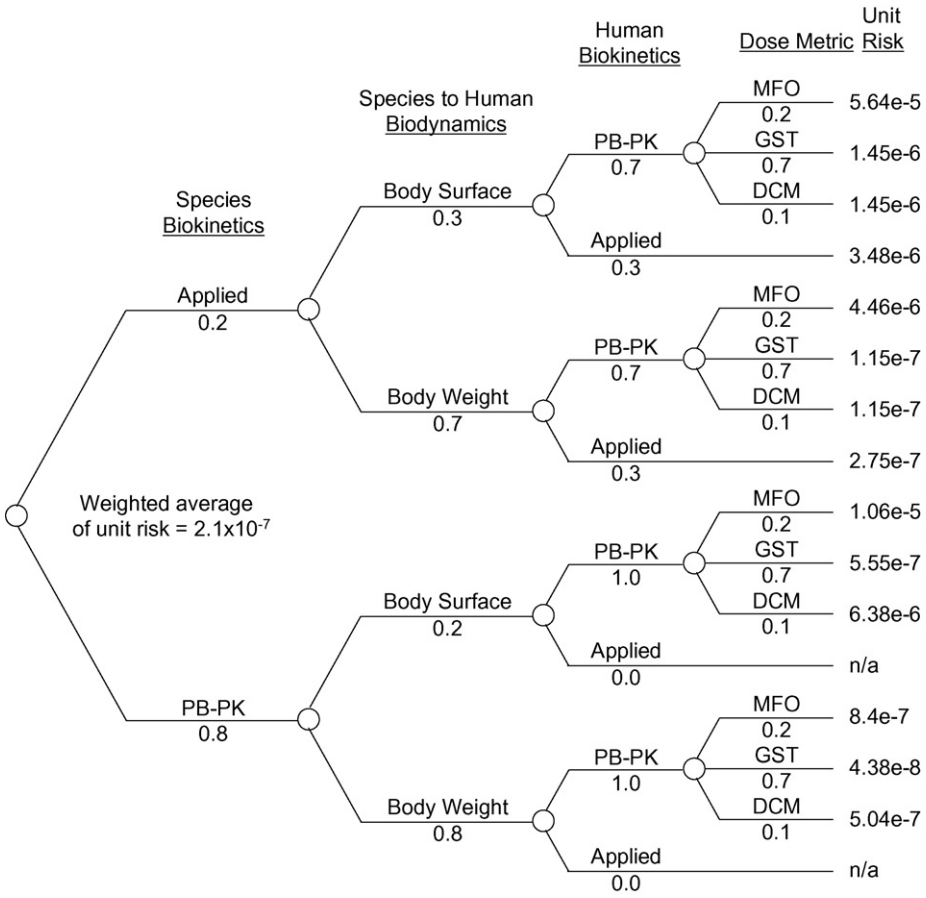


**Fig. 4.** Decision tree diagram for the cancer risk assessment for methylene chloride. Each branch of the tree is annotated with the probability assigned to that alternative (i.e., the level of belief that it is the correct alternative). See text for explanation of terms and abbreviations. (Adapted from Clement and Tatman, 1990)

to human on the basis of body weight or body surface area, whether to use the PBBK model or applied dose in the human, and which mode of action to assume: effect of parent chemical (DCM), effect of glutathione conjugation pathway (GST), or effect of oxidative metabolism pathway (MFO).[2]

The probabilities for each of the alternative decisions in the tree must be obtained by expert elicitation. In this case they were obtained from a single expert, the principal author on the methylene chloride PBBK model publication. In any more formal approach of this kind, expert opinion would naturally be gathered from a broader group of individuals (Hawkins and Graham, 1988).

Once the decision tree has been constructed and probabilities assigned, it is possible to determine the distribution of unit risks (estimated increase in lifetime risk of cancer associated with continuous exposure at 1 $\mu g/m^3$) associated with the selected weighting for the alternative approaches (Fig. 5).

A more formal decision tree approach similar to that described here would seem to be a highly promising possibility for answering the need of regulators for quantitative characterization of uncertainty in the application of new data and modelling in a risk assessment. Further development and application of this methodology and other techniques from the field of decision analysis, such as value of information, is a critical area of research.

## 6. Research to expand the application of mathematical modelling

The chemical-specific risk assessment approaches described in this paper focused primarily on the application of PBBK modelling. There are, of course, a number of other mathematical and statistical

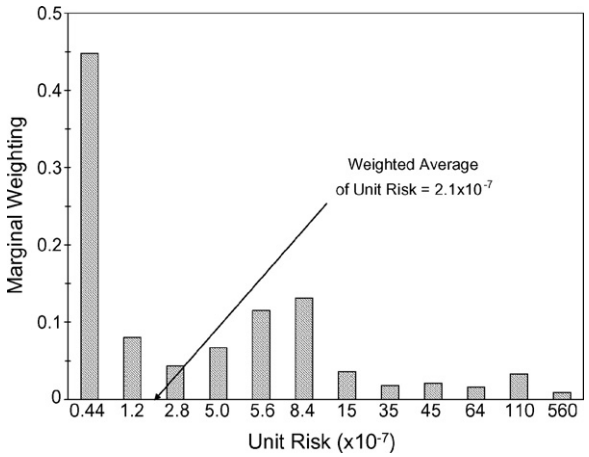


**Fig. 5.** Distribution of unit risk estimates associated with decision tree diagram for the for the cancer risk assessment for methylene chloride. Unit risk in this case is defined as the increased lifetime risk associated with continuous exposure at 1 $\mu g/m^3$. (Adapted from Clement and Tatman, 1990)

[2] MFO, mixed function oxidase, is an old name for Cytochrome P-450-mediated oxidation. With methylene chloride the active isoform is CYP2E1.

**Table 2**
Research directions for expanding the application of biologically based modelling

| |
|---|
| Incorporation of *in vitro* toxicity data in risk assessment (Blaauboer, 2003) |
| Assessment of the impact of genetic polymorphisms on risk (Gentry et al., 2002) |
| Interpretation of human biomonitoring data (Tan et al., 2006) |
| Biokinetic modelling of essential or endogenous chemicals (Andersen et al., 1999; Nong et al., 2007) |
| Simulation of cellular dosimetry and transport (Hack et al., 2007) |
| Extension of biokinetic modelling to cellular dynamics (Tan et al., 2003) |
| Biologically based dose–response modelling (DeWoskin et al., 2001) |

modelling approaches that can be used in risk assessment (Edler et al., 2002), but a useful research agenda could be defined solely on the basis of expanding the applications of biologically based modelling. A few of the potentially fruitful areas of investigation are listed in Table 2.

From the viewpoint of advancing the state of the art for the incorporation of chemical-specific data in risk assessment, perhaps the most important area of research is on the extension of biologically based modelling approaches to the initial interactions of chemicals with tissues and the resulting coordinated cellular responses. The relatively new field of systems biology has provided a framework for understanding and describing cellular response that is fundamentally changing the science of toxicology. The toxic effects of a chemical can now be understood as a perturbation of normal cellular function, leading to predictable alterations in cell signaling and regulation (Andersen et al., 2005). Quantitative description of these cellular responses has the potential to revolutionize risk assessment to an even greater extent than the progress that has resulted from the modelling of biokinetics (NAS, 2007).

## 7. Final words

The desire to increase the biological basis of chemical risk assessments has driven the development of new methodologies, such as PBBK modelling (Reddy et al., 2005). The development and application of PBBK models in turn demands well-formulated statements about the chemical mode of action. The requirement for an explicit, mechanistic hypothesis gives biologically based models their power, but at the same time serves as the greatest impediment to their acceptance by regulators. Biologically based models also serve to make other uncertainties in the risk assessment more visible, such as cross-species and inter-individual variation. In some cases the increased visibility given to these uncertainties has lead to improvements in the default risk assessment process (USEPA, 1994). By replacing poorly characterized uncertainties (in the default approach) with definable model uncertainties, biologically based models have spurred the development and application of the sophisticated uncertainty analysis techniques, such as Monte Carlo analysis and hierarchical Bayesian analysis, that are now used to provide a better understanding of the range of risk estimates consistent with the information available on a given chemical. It is crucial that this parallel development of biologically motivated descriptions of dosimetry and tissue response and methods for their quantitative evaluation continues as the emphasis inexorably shifts from modelling of biokinetics to tissue response.

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