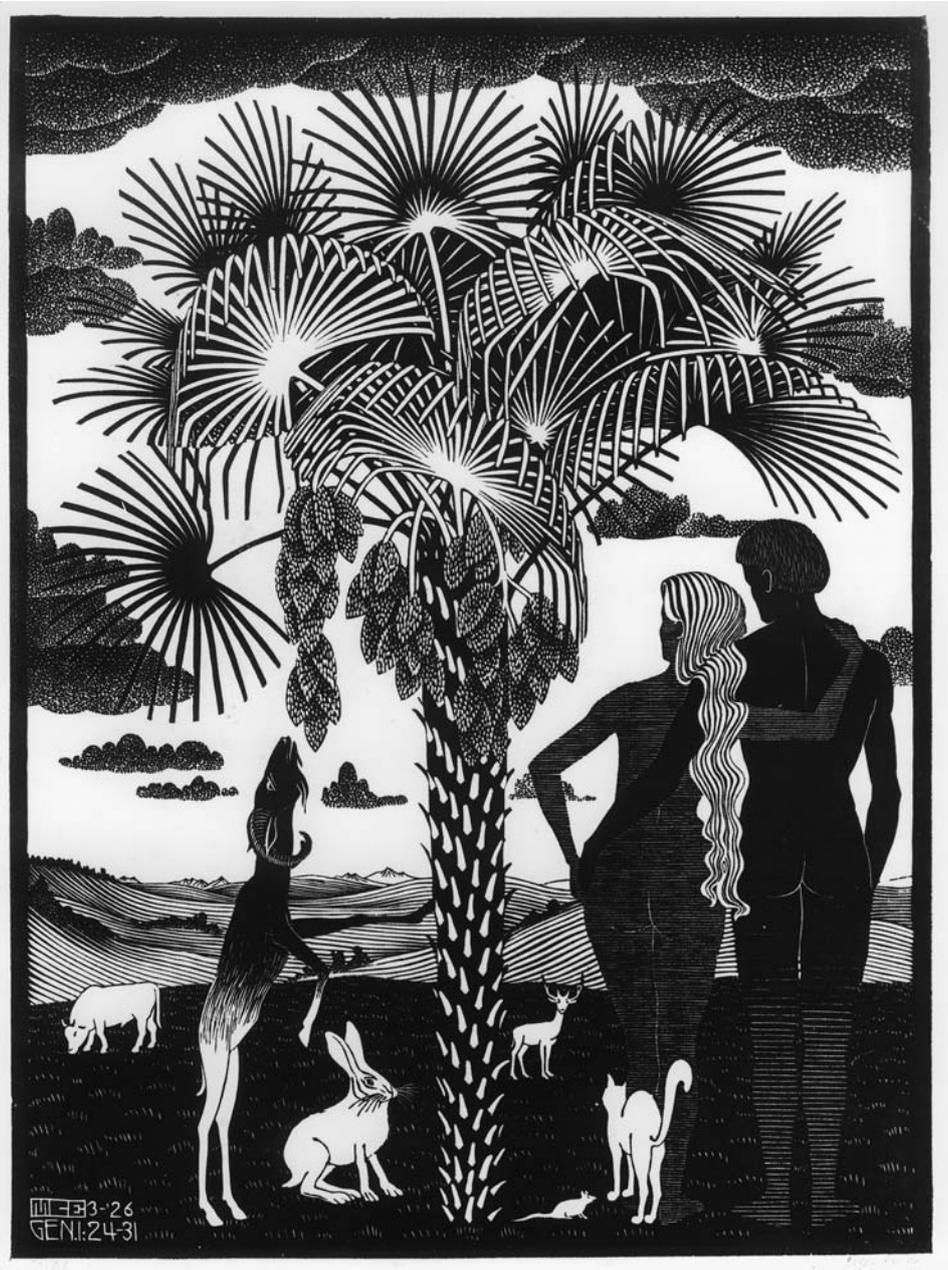


On the incorporation of biokinetic and mechanistic data in modeling for risk assessment



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Universiteit Utrecht

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On the incorporation of biokinetic and mechanistic data
in modeling for risk assessment

Harvey J. Clewell III

On the incorporation of biokinetic and mechanistic data
in modeling for risk assessment

De toepassing van gegevens over de biokinetiek en het mechanisme
van toxiciteit bij het modelleren voor risicobeoordeling van stoffen

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

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"We're tired of all this modelin', gonna take us a sea cruise."

-- *PBPK Blues*, Howlin' Harv and Met'l Plate Mel

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(In which we see that the first human risk assessment decision did not turn out well.)

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Chapter 1

On the Incorporation of Biokinetic and Mechanistic Data in Modeling for Risk Assessment: Introduction

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Chemical risk assessment encompasses elements of natural science, including toxicology, biochemistry, epidemiology, and veterinary science, together with 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 between the two disciplines. However, the interaction also provides a unique opportunity to focus scientific research in directions that can be of immediate benefit to the public. The goal of the research described in this thesis is to develop approaches for fostering the increased use of emerging scientific information and innovative methods in chemical risk assessments, in order to assure the protection of public-health while limiting the economic and social consequences of over-regulation.

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 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)..." (Yes, that's one in 100 million.) 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.

Recent Innovations in Quantitative Chemical Risk Assessment

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 (see text box below) and mechanism of toxicity, that could greatly impact the relative risks for different chemicals were ignored by the default approaches.

Biokinetics, Pharmacokinetics, or Toxicokinetics?

The time-course of drugs in biological systems has traditionally been referred to as pharmacokinetics, while data on tissue response has been referred to as pharmacodynamics. On the other hand, it has become popular to use the terms toxicokinetics and toxicodynamics 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 terms biokinetic and biodynamic will be used in the introduction and discussion of this thesis. The intervening chapters, which have already been published, will use the less appropriate terms in order to be consistent with the publications.

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 mechanism by which the chemical causes toxicity, (2) data on the dose-response for the effects of the chemical, and (3) data on the uptake, distribution, metabolism and elimination of the chemical.

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). To provide the necessary background for the rest of the thesis, a number of these methodologies will be described here: mode of action evaluation, benchmark dose modeling, physiologically based biokinetic modeling, and chemical-specific adjustment factors.

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 (2001) 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 the animal toxicity is relevant to humans,
- whether human exposures by routes not tested in animals are of concern,
- the most appropriate method to use for extrapolation below the experimentally observed dose range (linear or nonlinear),
- on what basis to determine cross-species and cross-route equivalence of exposure (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 on next page).

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, chlorovinylepoxyde, 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; if sustained, the combination of cytotoxicity and increased proliferation can eventually lead to tumors.

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 and biodynamic 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.

Benchmark Dose Modeling

Risk assessments have traditionally been based on the identification of a No-Observed-Adverse-Effect-Level (NOAEL) for the critical effect of a chemical. This NOAEL is then modified by adjustments and uncertainty factors to obtain an estimate of an acceptable exposure. However, in recent years the reliability of the NOAEL has been questioned, due to its dependence on the design of the experiment from which it is obtained, particularly the dose-spacing and number of animals per dose (Crump 1984). The benchmark methodology (Crump 1984, 1995; Gaylor and Slikker 1990; AIHC 1993) provides an alternative to the NOAEL approach for determining an acceptable exposure.

The benchmark dose (BMD) is defined as the dose (or exposure concentration) predicted to result in a specified amount of increased risk (called the "benchmark risk", or BMR). Benchmarks based on concentrations are sometimes referred to as BMCs. The BMD is calculated using a statistical dose-response model applied to either experimental or epidemiological data. A BMD or its statistical lower bound (BMDL) can be used as the point of departure for

the determination of an exposure guideline (USEPA, 1994; 2005; Edler et al. 2002). An illustration of the benchmark modeling approach is provided in Figure 1.

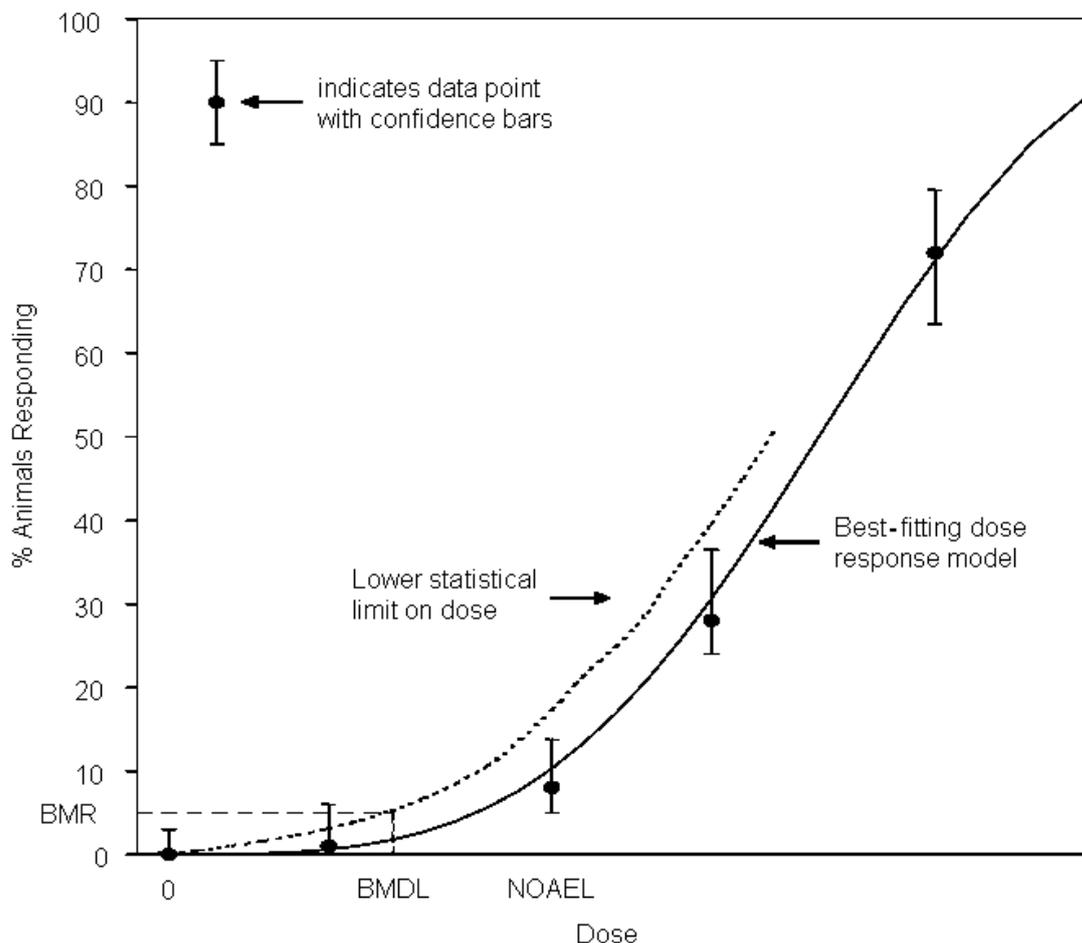


Figure 1. Example of Benchmark Dose Modeling. Abbreviations: BMDL = benchmark dose lower bound; BMR = benchmark risk; NOAEL = no-observed-adverse-effect-level. (Adapted from USEPA, 2005)

The benchmark approach has several advantages over the traditional NOAEL approach (Crump, 1984; Clewell et al., 2003): (1) the benchmark approach makes better use of the dose-response information available from multiple-dose animal studies, (2) the benchmark approach appropriately reflects the study sample size (smaller studies tend to result in smaller BMDLs, whereas the opposite is true for NOAELs), and (3) the benchmark approach does not require the arbitrary categorization of the data in epidemiological studies that is necessary to obtain a NOAEL.

Different types dose response models can be used to implement the benchmark approach depending upon whether the data are quantal (yes-no) or continuous (can assume a continuum of values in a range). In the case of

quantal data, the Weibull dose response model shown below is one of the alternative models that can be used:

$$P(d) = 1 - \exp[-e^{\alpha} - (\beta * d)^k],$$

where d is the dose or exposure concentration, $P(d)$ is the probability of an adverse response from an exposure d , α determines the probability of an adverse response in an unexposed population, $\beta \geq 0$ is a "potency parameter", and $k \geq 1$ permits the response to be non-linear. Each of α , β , and k are estimated by fitting the model to dose response data. Covariates can be included in the model by replacing α with $\alpha_0 + \alpha_1 X_i$, where X_i is the value of the covariate. The BMD is defined as the exposure that causes the probability of an adverse response to increase by the BMR, i.e., for a BMR of 10%, the BMD is defined as the solution to the equation:

$$P(\text{BMD}) - P(0) = 0.1.$$

As an example of the modeling of continuous data, in the k-power model (Crump 1995) an individual's test score is assumed to be normally distributed with mean

$$\alpha + (\beta * d)^k$$

and variance σ^2 . To define the BMD in a manner that is consistent with the way it was defined above for quantal data, an adverse response can be defined on the basis of the assumption that the most severe 5% of responses among individuals not exposed to the chemical are adverse. This approach is consistent with the convention that the "normal range" in clinical tests is generally assumed to encompass test results of 95% of the population. This determines a cutoff, x_0 , between adverse and normal, defined by

$$P(0) \equiv 0.05 = 1 - N[(x_0 - \alpha)/\sigma],$$

where N is the standard normal distribution. (This expression assumes that larger responses are more adverse; a similar expression applies when smaller responses are more adverse.) The probability of an adverse response at an exposure d is given by

$$P(d) = 1 - N\{([x_0 - \alpha - (\beta * d)^k]/\sigma)\}.$$

Then, as in the case of discrete data, the BMD is defined by the expression

$$P(\text{BMD}) - P(0) = 0.1.$$

In fitting models to either continuous or quantal data, the maximum likelihood estimating procedure can be applied. The BMDL is typically determined as a 95% statistical lower bound to the BMD. These statistical confidence limits can be calculated using the profile likelihood method (Cox and Oakes, 1984).

One of the controversies associated with the use of benchmark dose modeling is the selection of the level of increased risk (BMR) for which the benchmark should be calculated. There are three schools of thought in this regard:

1. The BMR should be selected to provide comparability of the benchmark dose (BMDL) with the traditional NOAEL
2. The BMR should be selected to provide protection for an appropriate fraction of the population (e.g., use of a 1% BMR to protect 99% of the population)
3. The BMR should be selected on the basis of the biological significance of the effect

In the case of the first suggestion, in order to be able to use the benchmark dose in place of a NOAEL it is first necessary to understand how they relate to each other. To this end, a study (Allen *et al.* 1994) was conducted that compared benchmark doses with NOAELs for 424 sets of dichotomous (quantal) data from developmental studies. Benchmark doses at three different levels of increased risk (1%, 5%, and 10%) were computed using a Weibull dose response model and a 95% level of statistical significance. This study found that BMDLs based on an additional risk of 0.1 (10% increase) were smaller than the corresponding NOAEL for between 75% and 90% of the data sets and were less than the NOAEL by an average factor of 2.9; BMDLs based on an additional risk of 0.05 (5% increase) were smaller than the corresponding NOAEL for between 90% and 95% of the data sets and were less than the NOAEL by an average factor of 5.9; and BMDLs based on an additional risk of 0.01 (1% increase) were smaller than the corresponding NOAEL for more than 95% of the data sets and were less than the NOAEL by an average factor of 29.

The results of these analyses suggest that use of a 0.1 additional risk would increase the conservatism in the determination of exposure guidelines by a factor of about 2 to 3 (i.e., would decrease guidelines by a factor of 2 to 3 on average). Based on this study and other similar analyses of both quantal and continuous data, agencies have typically used 0.1 or 0.05 additional risk as the basis for calculating BMDLs. BMDLs corresponding to an additional risk of 0.1 have the advantage that they are likely to be closer to the lower end of the dose-response data in a typical study, and therefore less dependent upon the dose response model selected, than benchmark doses corresponding to lower additional risk (Crump 1984).

An important advantage of the benchmark method is that it provides an estimate of an exposure that can be expected to entail a specified increase in risk. In contrast, an exposure identified as the NOAEL for a chemical could be associated with a wide range of increased risks, depending on the inherent dose-response of the chemical and the specifics of the experimental design. The results of the comparison described above (Allen *et al.*, 1994) indicate that, even for studies using roughly the same design, NOAELs for different chemicals can be

associated with a wide range of increased risks, from less than 5% to more than 50%. Thus the use of a NOAEL is associated with a great deal of uncertainty regarding the level of protection provided. The use of a BMDL can reduce this uncertainty, but not completely, due to limitations in the dose-response data and the necessity of extrapolating from the conditions of the study from which the dose-response was derived (e.g., from animals to humans).

For a quantal endpoint (incidence), the selection of a BMR on the basis of the fraction of the population it is intended to protect makes intuitive sense, even if it cannot easily be related to the traditional NOAEL. The question of whether the effect is biologically significant is taken up during the selection of the critical effect, not in the selection of the BMR. Of course, if a surrogate endpoint for a critical effect is used in the dose-response analysis, it is necessary to evaluate the dose-relationship of the surrogate and critical endpoints.

The situation is different for the selection of a BMR for a continuous endpoint. In this case, the selection of the BMR must be based on a scientific judgment regarding the amount of change in the endpoint measure that is considered adverse. The method of Crump (1995) assumes, based on common practice in clinical testing, that abnormal values of the endpoint measure are those that exceed the 95th percentile of the distribution of the values observed in the normal population. It has been suggested that the BMR for a continuous endpoint should alternatively be selected on the basis of scientific evidence regarding the amount of change that is adverse. The problem in this case, of course, is gaining consensus regarding, for example, the percent decrease in red blood cell count that should be considered adverse (Edler et al. 2002).

Although originally developed as a technique for use in a noncancer (NOAEL) risk assessment approach, benchmark modeling can also be used in quantitative cancer risk assessment, either as part of a nonlinear (margin of exposure) dose-response analysis for a non-genotoxic carcinogen, or in a low-dose linear risk extrapolation for a genotoxic carcinogen (USEPA, 2005).

Physiologically Based Biokinetic Modeling

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 modeling, 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). Of course the actual nature of the relationship between tissue exposure and response, particularly across species, may be quite complex.

Classic compartmental modeling 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 modeling 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.

Physiologically based biokinetic (PBBK) models differ from the conventional compartmental pharmacokinetic models in that they are based to a large extent on the actual physiology of the organism (Teorell, 1937a,b). 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. Figure 2 illustrates the structure of a simple PBBK model for styrene, a volatile lipophilic compound.

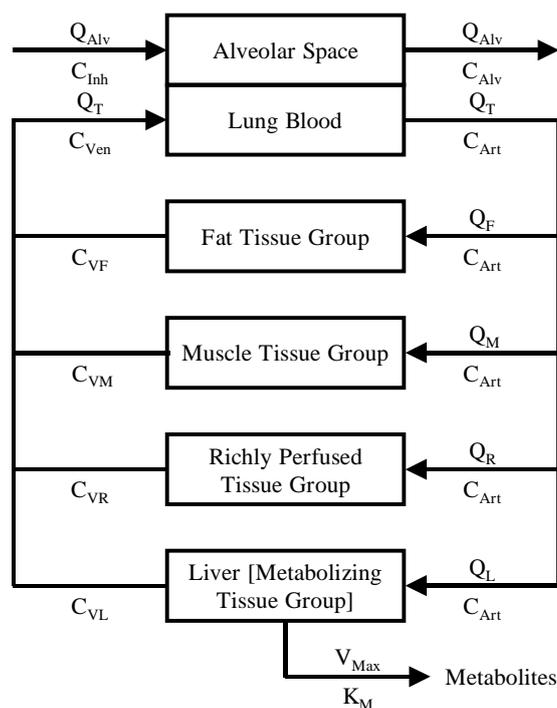


Figure 2. Diagram of a physiologically-based biokinetic (PBBK) model for styrene. In this description, groups of tissues are defined with respect to their volumes, blood flows (Q), and partition coefficients for the chemical. The uptake of vapor is determined by the alveolar ventilation (Q_{ALV}), cardiac 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 basic approach to PBBK model development is illustrated in Figure 3. 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.

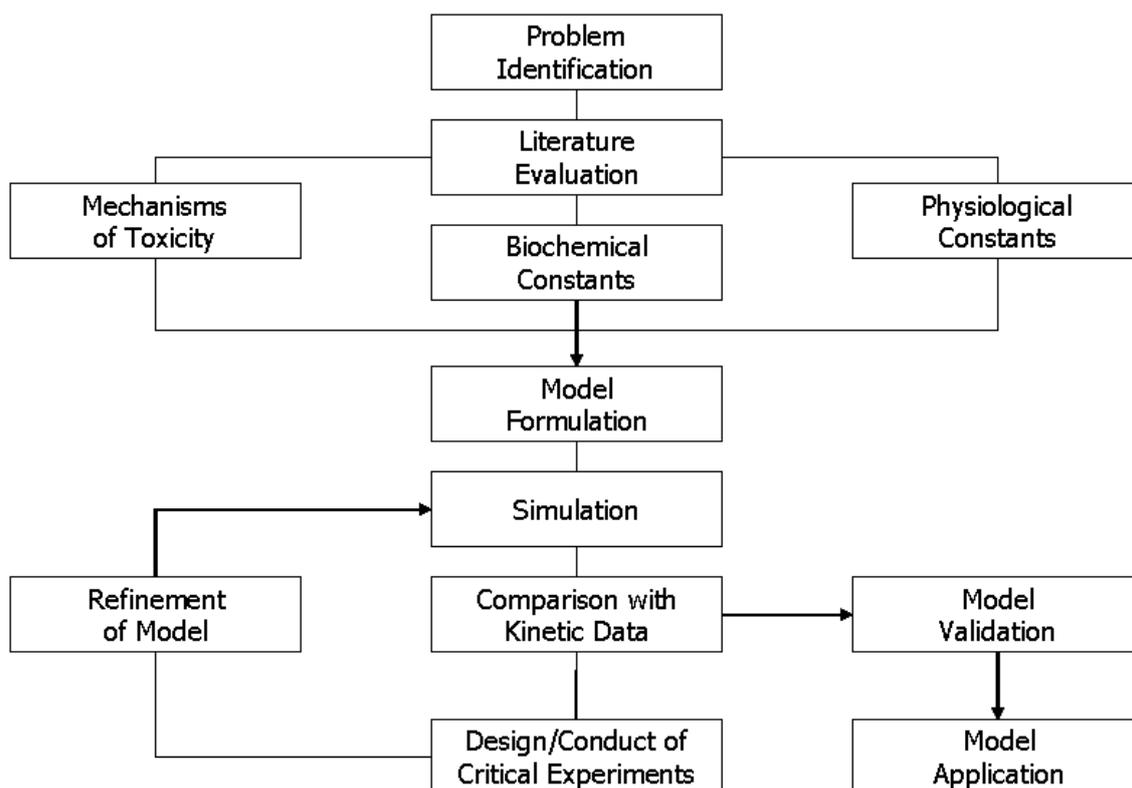


Figure 3. Flow-chart of the PBBK modeling process, showing the iterative process of model development in which the discrepancies between data and model predictions drive refinement of the model through the design of informative studies. (Adapted from Clewell and Andersen, 1989)

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.

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; 1991; Barton, 2005; Clewell et al., 2007), and the target tissue dosimetry provided by PBBK modeling is an essential component in models of pharmacodynamics, 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).

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 biokinetic and biodynamic components, each of which can be replaced by a CSAF if adequate chemical-specific data are available. IPCS (2005) defines biokinetics 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." Biodynamics is defined as "the process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects."

The biokinetic 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 biokinetic 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 biokinetics 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 biokinetic 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 biokinetics, as is described in the example in the text box on the next page.

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 biokinetics (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 hours (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 hours. 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 a factor of two error 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 biodynamic 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 biodynamic 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. Biodynamic 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 biodynamic 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 biodynamic 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
- *In vivo* biodynamic processes (e.g., multi-organ signaling)

For example, in a study of hemolysis by 2-butoxyethanol, it was observed that exposure for 12 days was associated with a smaller decrease 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*.

It is also important to note that the biodynamic CSAFs relate effect to internal dose, not external dose. Therefore, they cannot be applied independently of the biokinetic factors. That is, while a biokinetic factor could be applied together with a default biodynamic factor, the reverse is not true. In the example for 2-butoxyethanol, the biodynamic 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.

Overview of This Thesis

This thesis is comprised of a number of chapters representing individually published studies that have in common the fact that they were conducted with the goal of advancing the state of the art for the incorporation of biokinetic and mechanistic data in modeling for risk assessment. The chapters are divided into three sections that reflect the different nature of the research described.

The first section describes three studies that were performed to foster the broader use of biokinetic and mechanistic data and mathematic modeling approaches in risk assessment. *Chapter 2* provides a review of the potential uses of mode of action evaluation in risk assessments and highlights successful examples from the literature to demonstrate the value of the mode of action concept. It then presents a more detailed analysis of two case studies, on risk assessments for trichloroethylene and inorganic arsenic, and identifies a number of key issues that need to be addressed in order to assure optimal use of mode of action evaluation in future risk assessments. *Chapter 3* argues that quantitative dose-response analysis of noncancer endpoints can be of value for putting noncancer risks in perspective and to support cost-benefit analysis. Quantitative dose-response analysis has been used for many years in the case of cancer endpoints, but it has not yet been generally accepted for use with noncancer effects (Crump et al., 1997). *Chapter 4* describes an approach for the incorporation of biokinetic and metabolic data in risk assessment using PBBK modeling. The approach makes use of mode of action analysis and can be applied across both cancer and noncancer endpoints.

The second section describes three studies that were performed to examine the extent to which chemical-specific properties and human variability

interact to produce different risks across individuals in a population. *Chapter 5* describes a study that combined Monte Carlo uncertainty analysis with PBBK modeling to estimate the population distribution of safe doses for the effects of methylmercury. The toxicity of concern was the potential for neurodevelopmental effects from fetal exposure to methylmercury associated with maternal exposure to methylmercury from the ingestion of contaminated fish. *Chapter 6* provides a critical review of the available data on the age- and gender-dependence of human physiology, metabolism, and clearance. It then performs an evaluation of the potential impact of these differences on tissue dosimetry, and hence risk. *Chapter 7* describes a PBBK modeling effort that builds on the data and analysis from Chapter 6 and examines the extent to which chemical-specific properties, physiology, and age-dependent biochemical processes interact to produce different risks across individuals in a population at different ages. The PBBK model developed for this study includes fully age-dependent descriptions of all of the physiological and biochemical parameters, and can be used to estimate an HK_{UF} for children or the elderly using chemical-specific data on partitioning and metabolism.

The final section describes three studies that attempt to make optimal use of the available biokinetic and mechanistic data in cancer risk assessments for three chloroethylenes using mode of action evaluation, PBBK modeling, and dose-response modeling. *Chapter 8* describes a cancer risk assessment performed for vinyl chloride, a chemical with a well-documented, genotoxic mode of action. The analysis provides a comparison of predicted human risks using data from different species and different routes of exposure. *Chapter 9* presents a cancer risk assessment conducted for trichloroethylene, a chemical with poorly-understood, non-genotoxic modes of action for several different cancer endpoints. This analysis required an extensive mode-of-action evaluation and the development of a fairly complex PBBK model. *Chapter 10* demonstrates an approach for conducting a critical evaluation of alternative PBBK models, using perchloroethylene as a case study. A modification of the most successful model, which provides improved fits to the available data, is then used to conduct a cancer risk assessment for perchloroethylene.

The final chapter in the thesis provides a discussion of the key points derived from the research described in the thesis and suggests possible future directions for research on the incorporation of biokinetic and mechanistic data and mathematical modeling approaches in risk assessment.

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Part I

Development of Methods for the Incorporation of Biokinetic and Mechanistic Data in Modeling for Risk Assessment

Chapter 2

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.

Role of Mode-of-Action Information in Risk Assessment

The flow of the risk assessment process is depicted in Figure 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 quantitative toxicokinetic and toxicodynamic information into the approach.

¹ In the USEPA's Draft Final Guidelines for Carcinogen Risk Assessment (USEPA, 2003), 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."

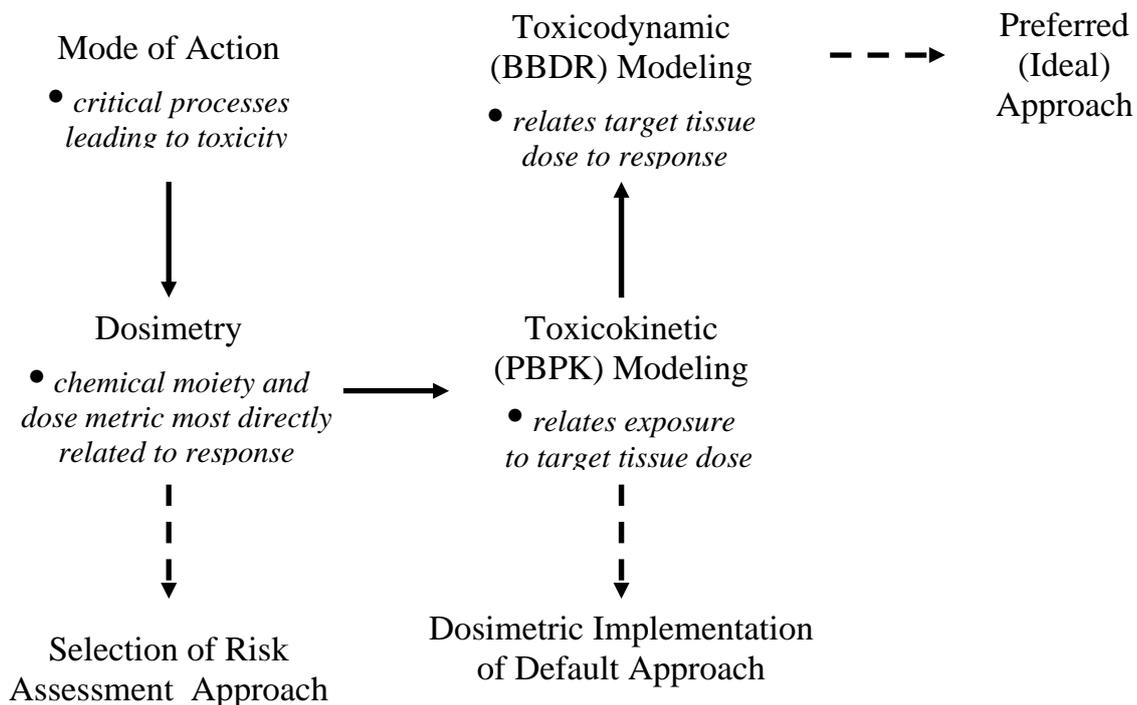
Qualitative InformationQuantitative Tools

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

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

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."

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).

History of the Application of Mode-of-Action Information in Risk Assessment

In the earliest formal risk assessment methodology (Lehman and Fitzhugh, 1954; Dourson and Stara, 1983), a NOAEL³ 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 (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).

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.

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

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., 2001).

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).

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 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 Figures 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.

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)

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).

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 is 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).

Cumulative Risk Assessment

As with TEFs, one of the criteria for the performance of a cumulative risk assessment across multiple chemicals is that 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).

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 pre-neoplastic 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

⁶ The Toxicological Review of Vinyl Chloride (USEPA 2000) states that: "Adduct levels normally cannot be used directly to extend tumor dose-response data to lower doses, since tumor

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, 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).

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.

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 (Gentry et al., 2002; Clark et al., 2004).

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.

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 coworkers (CIIT, 1999; Conolly et al., 2003; 2004).

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.

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.

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 micromolar (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 2L/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 non-linearity 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 than 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).

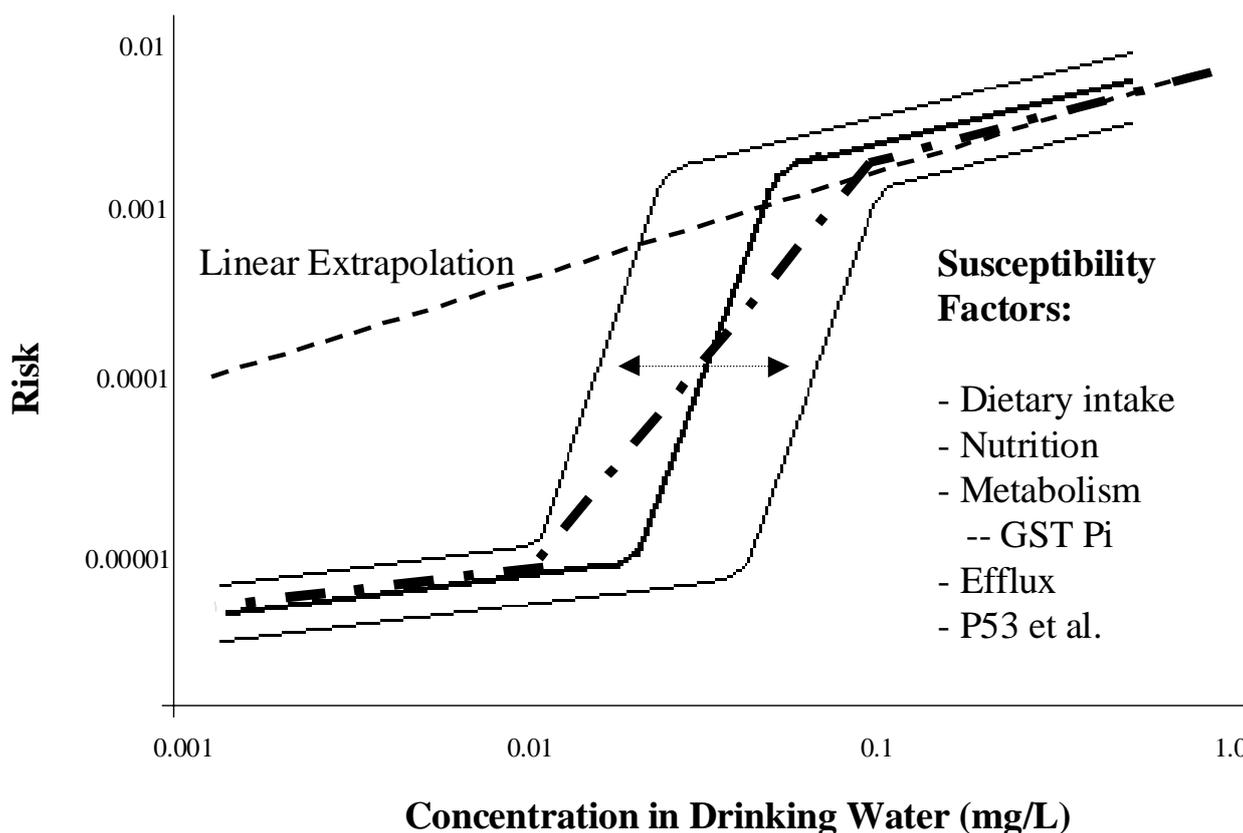


Figure 3: Conceptual illustration of alternative dose-response curves for the carcinogenicity of arsenic in a population exposed to arsenic in drinking water (from Clewell, 2001, with permission).

Figure 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

Figure 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 Figure 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 Figure 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. 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).

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

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.'"

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 in order to obtain quantitative risk estimates, even in cases where the qualitative evidence for the existence of a nonlinear dose response is compelling.

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Chapter 3

Quantitative Estimates of Risk for Non-Cancer Endpoints

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Commentary

While quantitative estimates of risk have been a standard practice in cancer risk assessment for many years, no similar practice is evident in noncancer risk assessment. We use two recent examples involving methylmercury and arsenic to illustrate the negative impact of this discrepancy on risk communication and cost-benefit analysis. We argue for a more balanced treatment of cancer and non-cancer risks and suggest an approach for reaching this goal.

23. INTRODUCTION

For many years there has been a fundamental dichotomy in risk assessment. Since the 1970's, the potential carcinogenic hazards of chemicals have generally been characterized by quantitative estimates of the increased lifetime risk of cancer associated with a given exposure. On the other hand, the potential hazards of chemicals for toxic endpoints other than cancer have generally been characterized only by the specification of an exposure level considered to be without appreciable risk, as exemplified by the USEPA's reference dose (RfD). Despite recent efforts to harmonize cancer and non-cancer risk assessment approaches,⁽¹⁾ this fundamental difference has to a large extent remained undiminished. The negative impact of this dichotomy can be seen in two areas: risk communication and cost-benefit analysis.

Lacking quantitative estimates of risk for non-cancer effects, communication of non-cancer risk has often reduced to characterizing persons above a regulatory exposure guideline simply as being "at risk for health problems." This approach inappropriately interprets an exposure guideline as a bright line between "safe" and "unsafe." Also, unlike cancer, the severity of many non-cancer health effects increases with exposure. A simple "safe – unsafe" dichotomy gives no indication of the severity of the health effects that may be expected at different exposures.

Cost-benefit analysis is used by regulatory agencies to identify cost-effective regulatory strategies that provide adequate health protection. A crucial component of this process is the quantification of health risks under various regulatory options. Unfortunately, without readily available means for quantifying non-cancer risks, the focus is often solely on cancer risks, while non-cancer health risks are ignored. This practice clearly has the potential for being misleading and producing inappropriate regulatory decisions.

We present examples below of risk communication and cost-benefit analyses that illustrate these problems. Following these examples we argue further for a balanced treatment of cancer and non-cancer risks and suggest an approach for reaching this goal.

23. COMMUNICATION OF RISK FROM METHYLMERCURY (MeHg)

In its review of the USEPA RfD for MeHg (0.1 $\mu\text{g}/\text{kg}/\text{day}$), the National Academy of Science⁽²⁾ stated that: "The committee estimates that over 60,000 children are born each year at risk for adverse \square euron-developmental effects due to *in utero* exposure to MeHg." When asked by the FDA to clarify the nature of their estimate, the committee⁽³⁾ explained that their statement was based on the assumption that all children born of mothers with an exposure to MeHg greater than the RfD could be characterized as "at risk", and that their estimate "should not be interpreted as an estimate of the annual number of cases of adverse \square euron-developmental effects."⁹ Unfortunately, this figure has been widely cited in the media without benefit of this clarification.

Characterizing children exposed above the RfD as "at risk" carries the implicit but unwarranted assumption that the RfD represents a bright line delineating safe and harmful exposures. In fact, based on our current knowledge, children exposed at levels substantially above the RfD could be risk-free; on the other hand, children exposed below the RfD could be at "increased risk", depending upon how one defines "risk". Therefore simply to state that exposures above the RfD places children "at risk", without any further attempt to explain the risk, is overly simplistic, and apt to be misleading.

In this particular case, the dose-response model relied upon by the NRC committee for recommending the methylmercury RfD was readily available for putting a perspective on the risk. This model predicts that expected scores of all exposed children (not just those whose mothers were exposed above the RfD) on a test of \square euron-psychological function (the Boston naming test) administered at seven years of age are shifted downward by an amount proportional to the concentration of methylmercury in the children's cord blood. The top panel in Figure 1 shows the distribution of test scores predicted by this model in children of mothers unexposed to methylmercury, compared to children whose mothers were exposed at the RfD. The difference in these distributions, which is at most barely discernable in Figure 1, corresponds to a change in the mean score on a standardized test of 0.25%. In NHANES,⁽⁴⁾ the 99.8 percentile of blood mercury levels among U.S. women of child-bearing age (2nd highest value among 1588 measurements from such women) was 22 $\mu\text{g}/\text{L}$, corresponding to a mercury intake of 3.8 times the RfD. We estimate that 8000 children (out of 3,900,000,

⁹ Based on the recently available blood mercury data from NHANES,⁽⁴⁾ we estimated 300,000, rather than 60,000, children born each year to mothers exposed above EPA's RfD, based on 60,000,000 women of childbearing age (15-45 yr), 7.5% of whom have mercury blood levels that exceed 5.8 $\mu\text{g}/\text{L}$,⁽⁴⁾ and an annual birth rate of 0.065. A recent claim that "630,000 U.S. newborns had unsafe levels of mercury in their blood in 1999-2000" (Washington Post, Feb 6, 2004) is apparently not an estimate of the number of children born to mothers exposed above the RfD, but rather an estimate of the number of children whose cord blood mercury blood level exceeded the maternal blood level ($\sim 5.8 \mu\text{g}/\text{L}$) resulting from exposure at the RfD. Equivalently, since mercury in cord blood is estimated as 1.7 times mercury in maternal blood,⁽⁵⁾ this figure is an estimate of the number of children whose mothers were exposed to the RfD/1.7, and whose blood levels are estimated to be $\sim 5.8/1.7 = 3.5 \mu\text{g}/\text{L}$.

or 0.2%) are born in the U.S. every year to mothers exposed above this level, which corresponds to exposures among women in the Faroe Islands who were high consumers (3 or more dinners per week) of pilot whale with a high content of methylmercury.⁽⁶⁾ These 8,000 children represent only 2.7% of the 300,000 estimated as being born yearly to mothers exposed above the RfD. The bottom panel of Figure 1 shows the increment of expected change in the test score distribution resulting from this exposure level, which corresponds to a change in the mean score on a standardized test of 1.6%.

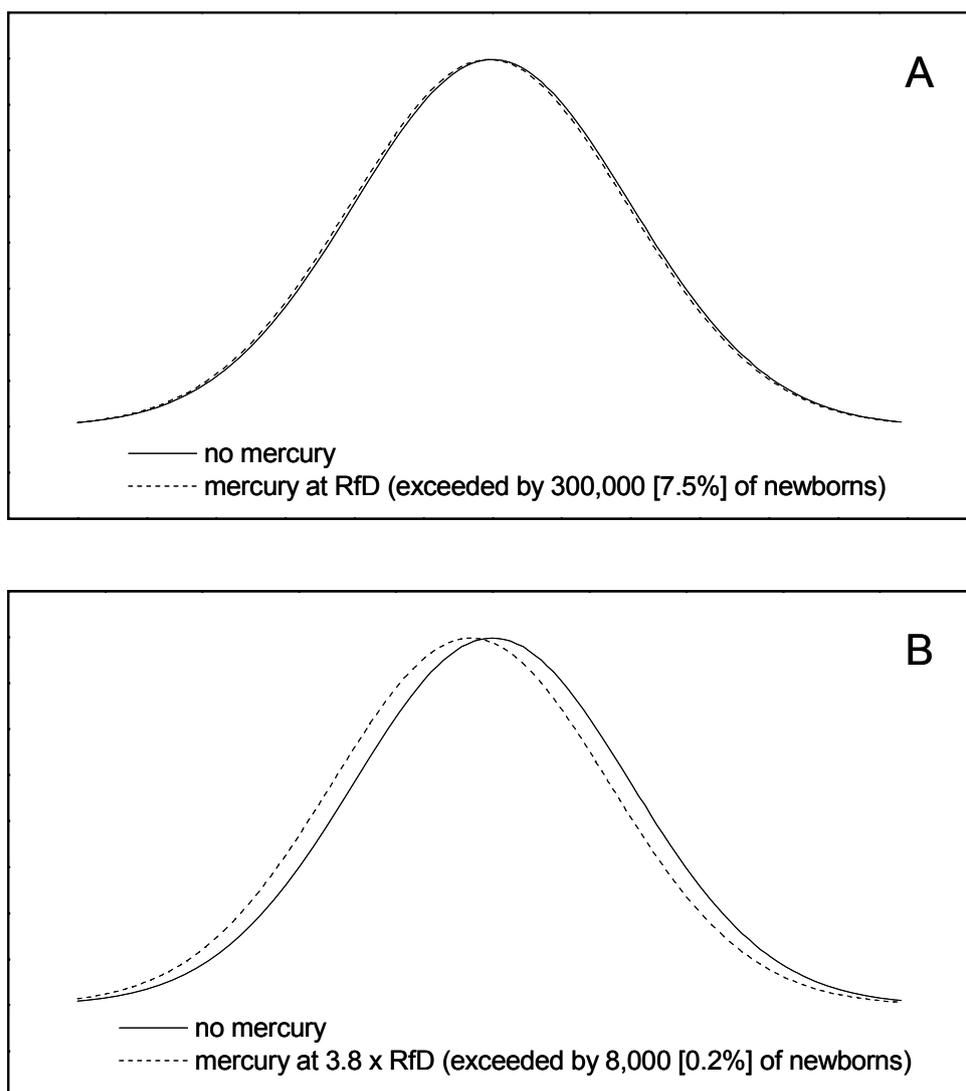


Fig. 1. Predicted distribution of performance in children not exposed to methylmercury and in children whose mothers were exposed at the RfD (A) or at 3.8 times the RfD (B), using the NRC model.

Although these calculations involved extrapolation beyond the range of the data and are consequently uncertain, we believe they are no more so than estimates of cancer risk from low exposures, which are often used to inform the public. The NRC model used in developing Figure 1 assumes no threshold and that the

risk varies linearly with exposure. As such, most scientists would probably consider that risks illustrated by this figure are more likely to be overestimates than underestimates.

The purpose of this discussion is not to minimize or trivialize risk from exposure to methylmercury; any decrease in neuropsychological health should be taken very seriously. At the same time it is important for the public to have as clear an understanding as possible of the nature of potential risks from exposure to methylmercury, as well as an understanding of the health benefits that are derived from eating fish. We believe the type of analysis presented above would be of value in informing the public beyond the oversimplification that persons exposed above the RfD are "at risk."

23. COST-BENEFIT ANALYSIS FOR ARSENIC

The need for an accepted practice for quantifying non-cancer risk can also be seen in cost-benefit analysis. The cost-benefit analysis performed in support of the MCL for arsenic⁽⁷⁾ provides an informative example in this case. In its rulemaking, the agency used the results of a cost-benefit analysis to justify the adoption of an MCL of 10 ug/L, rather than the Practical Quantitation Limit (PQL) of 3 ug/L, because the costs of treatment to reach the PQL would exceed the health benefits derived. The health benefits included in this analysis were avoidance of cancers, based on increased lung and bladder tumors observed in a Southwest Taiwanese population exposed to arsenic in their drinking water.⁽⁸⁾ Life-threatening non-cancer effects of arsenic, including death from ischemic heart disease (ISHD), were also observed in this same population.⁽⁹⁾ While the agency recognized the need to consider noncancer health benefits in their analysis, they concluded⁽⁷⁾ that the non-cancer benefits could not be quantified because, "while they may be substantial, the extent to which these impacts occur at levels below 50 ug/L is unknown." This decision not to quantify the non-cancer effects may have substantially altered the outcome of the cost-benefit analysis. Our own analyses of these two studies indicate that the mortality risk of ISHD from drinking water may be greater than the mortality risk from cancer.⁽¹⁰⁾

The agency's reluctance to perform low-dose extrapolation for the non-cancer effects of arsenic stands in marked contrast with their willingness to rely on linearly extrapolated low-dose risk estimates for arsenic carcinogenicity. This is particularly ironic in view of the substantial evidence that the carcinogenicity of arsenic is likely to exhibit a non-linear cancer dose-response.⁽¹¹⁾ The agency's failure to provide a quantitative estimate for the non-cancer benefits in this case does not result from a limitation in the methods of cost-benefit analysis. The estimation of health benefits from reductions in ambient air pollution provides a relatively mature example of the ability to quantify non-cancer effects.⁽¹²⁾ Rather, the failure to quantify the non-cancer effects of arsenic appears to result from the fact that there is not a generally accepted practice, or expectation, of performing low-dose extrapolation for non-cancer endpoints.

23. DISCUSSION

These examples highlight an important conceptual problem regarding the nature of dose-response relationships for chemical toxicity. The fundamental problem is the commonly accepted notion that there is a biological justification for the use of low-dose risk estimates for cancer, but not non-cancer, risk assessment. One justification that has historically been given for this distinction is that cancer is a fundamentally stochastic process, such that even a single molecule of a chemical could in principle produce a mutation leading to a tumor, while non-cancer toxicity is inherently non-linear, exhibiting a threshold. Thus one motivation for the development of quantitative cancer risk assessment was a concern that, due to the stochastic nature of the cancer process, even doses of a carcinogen well below the No-Observed-Adverse-Effect Level (NOAEL) for tumors in animals could entail an unacceptable risk of cancer to humans. On the basis of this understanding of the carcinogenic process, low-dose risk extrapolation approaches were developed for cancer risk assessment.⁽¹³⁾ However, as understanding of chemical carcinogenesis has advanced, there has been growing recognition that the application of this approach to all carcinogens is an over-generalization. As a result, the latest USEPA guidelines for cancer risk assessment⁽¹⁴⁾ now provide for multiple dose-response options for carcinogen risk assessment, driven by mode-of-action considerations. Specifically, mode of action considerations dictate whether a linear dose-response assessment should be performed to derive a risk estimate, or whether there is sufficient evidence of a nonlinear dose-response to justify the use of a threshold (margin of exposure) approach.

Linear low-dose risk extrapolation has also been defended when the effects of a chemical are additive to an existing process.⁽¹³⁾ This justification, however, can be applied equally well to the case of a background incidence of non-cancer toxicities as it can to a background incidence of cancer. Examples of non-cancer effects with an existing background in the general population include cardiovascular events, pulmonary insufficiency, male reproductive deficits, and developmental defects. Another factor that militates against a simplistic threshold assumption for non-cancer risk assessments is the impact of human inter-individual variability on the dose of a chemical that could cause an adverse effect in some portion of a population.^(15,16) Thus, in contrast to the historical view, it can be argued that there is no more, or less, justification for the use of low-dose risk extrapolation in the case of cancer risk assessment than non-cancer,⁽¹⁷⁾ and that low-dose risk extrapolation is as useful, and no less certain, for non-cancer risk assessment as for cancer.⁽¹⁶⁾ In both cases, a linear risk estimate is likely to provide a conservative basis for risk management, with uncertainty that increases as the extrapolation is extended further below the range of the data.

A number of approaches have been suggested for estimating the risks associated with the non-cancer effects of chemicals. Many of these approaches make use of the benchmark dose methodology^(18,19) or categorical regression,⁽²⁰⁾ with low-dose extrapolation based on a linear model^(21,22) or by considering population

variability.^(16,23) It has been suggested that the single most important uncertainty in non-cancer risk estimates is the extent of human inter-individual variability in the doses of specific chemicals that cause adverse responses. This conclusion is likely to be just as relevant to cancer risk assessment as to non-cancer, although population variability has historically received little attention in cancer risk assessment.

23. RECOMMENDATIONS

Estimation of low dose risks is generally a very uncertain process. For this reason we believe it should be undertaken only when there is an imperative reason for doing so. However, when there are overriding needs for such estimates (such as in cost-benefit analysis) a balanced treatment of cancer and non-cancer is needed. We believe that the time has come to begin the process for building a consensus regarding an accepted practice for estimating the risks of non-cancer effects. A joint effort of government, academia, and industry is needed, with active participation of the appropriate scientific societies, building on the efforts of EPA and other groups⁽¹⁾ that have already taken place to promote the harmonization of cancer and non-cancer risk assessment. Until this consensus is reached, however, we have the following suggestions regarding appropriate methods of non-cancer risk quantification:

- Cost-benefit analyses should consider the fatal and life-threatening non-cancer effects of a chemical in the same fashion as the cancer effects. In both cases, it should be recognized that risk estimates obtained by linear extrapolation below the range of the data may greatly over-estimate the actual risk at low doses, which could even be zero.
- Treating an exposure guideline such as the RfD as a bright line and equating exposures above the guideline with being "at risk" is apt to be misleading and should be replaced with more informative indications of both the severity and frequency of health effects that may occur in an exposed population.

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Chapter 4

A Consistent Approach for the Application of Pharmacokinetic Modeling in Cancer and Noncancer Risk Assessment

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ABSTRACT

Physiologically based pharmacokinetic (PBPK) modeling provides important capabilities for improving the reliability of the extrapolations across dose, species, and exposure route that are generally required in chemical risk assessment regardless of the toxic endpoint being considered. Recently, there has been an increasing focus on harmonization of the cancer and noncancer risk assessment approaches used by regulatory agencies. Although the specific details of applying pharmacokinetic modeling within these two paradigms may differ, it is possible to identify important elements common to both. These elements expand on a four-part framework for describing the development of toxicity: i) exposure, ii) tissue dosimetry/pharmacokinetics, iii) toxicity process/pharmacodynamics, and iv) response. The middle two components constitute the mode of action. In particular, the approach described in this paper provides a common template for incorporating pharmacokinetic modeling to estimate tissue dosimetry into chemical risk assessment, whether for cancer or noncancer endpoints. Chemical risk assessments typically depend upon comparisons across species that often simplify to ratios reflecting the differences; uses of this ratio concept are described. The advantages of this pharmacokinetic-based approach as compared to the use of default dosimetry are discussed.

INTRODUCTION

The process of assessing the health risks associated with human exposure to toxic environmental chemicals inevitably relies on a number of assumptions, estimates, and rationalizations. Some of the greatest challenges result from the necessity to extrapolate from the conditions in the studies providing evidence of the toxicity of the chemical to the anticipated conditions of exposure in the environment or workplace. For risk assessments based on animal data, the most obvious extrapolation which must be performed is from the tested animal species to humans; however, others are also generally required: from high dose to low dose, from one exposure route to another, and from one exposure time frame to another. Physiologically based pharmacokinetic (PBPK) modeling provides a powerful method for increasing the reliability of these extrapolations (1-3). The inherent capabilities of PBPK modeling are particularly advantageous for cross-species extrapolation: the physiological and biochemical parameters in the model can be changed from those for the test species to those which are appropriate for humans to provide a biologically meaningful animal-to-human extrapolation. However, it is important to recognize that a full PBPK model may not always be necessary to support a pharmacokinetic risk assessment; in some cases only a simple compartmental pharmacokinetic description is needed; an excellent example has been published for the case of cadmium (4-5).

Simple pharmacokinetic approaches have occasionally been used by regulatory agencies in cancer risk assessment; for example, the use of metabolized dose for trichloroethylene (6-7). The first case in which an agency has used a full PBPK approach was in the U.S. Environmental Protection Agency's (EPA) latest revision of its inhalation risk assessment for methylene chloride (8).

The decision to use the PBPK approach in this case was made only after a period of considerable controversy, including a workshop sponsored by the National Academy of Sciences at which the usefulness of PBPK modeling for chemical risk assessment was discussed. The scientific consensus following the workshop was that "relevant PBPK data can be used to reduce uncertainty in extrapolation and risk assessment ." (9) In 1989, after a detailed multi-agency evaluation of the available PBPK information and a review by the EPA Scientific Advisory Board, the EPA revised the inhalation unit risk and risk-specific air concentrations for methylene chloride in its Integrated Risk Information System (IRIS) database, citing the PBPK model developed by Andersen and colleagues (10). The resulting risk estimates were lower than those obtained by the default approach by nearly a factor of ten. This difference was driven by the lower rate of metabolism in humans, compared to mice, giving rise to the reactive intermediate associated with the tumors. Subsequently, an adaptation of the same PBPK model was used by OSHA in their rulemaking for methylene chloride (11). More recently, the EPA has used PBPK models for vinyl chloride (12) and 2-butoxyethanol (13) in its risk assessments for these chemicals.

The advantages of applying PBPK modeling in risk assessment have been discussed both for cancer (9, 14-17) and noncancer endpoints (18-21). In addition, the use of PBPK modeling has been recommended to improve route-to-route extrapolation (22) and the estimation of risk for chemical mixtures (23). Recently, there has been an increasing focus on harmonization of the cancer and noncancer risk assessment paradigms used by regulatory agencies in the United States.

The specific details of applying PBPK modeling within these two paradigms may differ. For example, lifetime average daily dose is used for cancer risk assessment while average daily dose during exposure is used for noncancer. Nevertheless, it is possible to identify important elements common to both (24). The starting point for a harmonized approach is a four-part framework for organizing qualitative and quantitative analyses of data available for chemical risk assessment. The four elements are: exposure, tissue dosimetry, toxicity process, and response (Figure 1).

Tissue dosimetry information is obtained from quantitative pharmacokinetic analyses. Quantitative descriptions of the processes leading to toxicity are referred to as pharmacodynamic analyses. These two elements link the exposure with the response and are often described as the mode of action of a chemical (though it should be noted that mode of action has also been used synonymously with toxicity process). The four elements form the basis for analyzing toxicity information in animals or humans and carrying out extrapolations between the species. They represent the minimum elements necessary for risk assessment purposes; elaborated series of steps can also be given that more completely describe the biological processes. In the following discussion, an attempt is made to describe the key elements of the approach for applying pharmacokinetic modeling of tissue dosimetry to dose-response assessment in a format equally applicable to both cancer and noncancer endpoints.

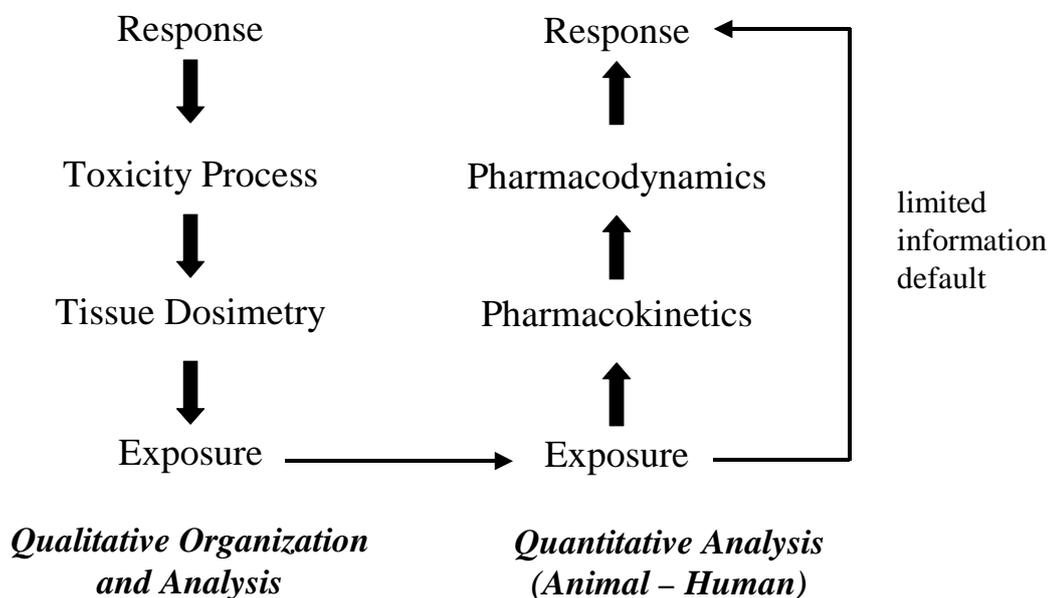


Figure 1: Framework for Using Mode of Action Information in Dose-Response Analysis

DOSE METRIC SELECTION

The ultimate aim of using pharmacokinetic modeling in risk assessment is to provide a measure of dose which better represents the "biologically effective dose"; that is, the dose which causally relates to the toxic outcome. The improved dose metric can then be used in place of traditional dose metrics (such as concentration or exposure dose) in an appropriate dose-response model to provide a more accurate extrapolation to the human exposure conditions of concern. Implicit in any application of pharmacokinetics to risk assessment is the assumption that the toxic effects can be related to the concentration of an active form of the substance in the mechanistically relevant tissue. Often the tissue where the chemical is active and the toxic effects occur is the same; it is referred to as the target tissue. Sometimes, the target for the chemical's effects and the organ in which the toxicity are observed are different (e.g., effects on brain may alter hormonal signaling observed as toxicity in a reproductive organ). In this case, the term target tissue must be used with care as the concept is altered from its traditional usage. It is expected that similar responses will be produced at equivalent tissue exposures regardless of species, exposure route, or experimental regimen unless there are pharmacodynamic differences between animal species (3, 25, 26). The motivation for applying pharmacokinetics in risk assessment is the expectation that the observed effects of a chemical will be

more simply and directly related to a measure of target tissue exposure than to a measure of administered dose (1, 27).

The specific nature of the relationship between target tissue exposure and response depends on the chemical mechanism of toxicity, or mode of action, involved. Many short-term, rapidly-reversible toxic effects, such as acute skin irritation or acute neurological effects, may result primarily from the current concentration of the chemical in the tissue. In such cases, the likelihood of toxicity from a particular exposure scenario can be conservatively estimated by the maximum concentration (C_{MAX}) achieved in the target tissue (28-30). On the other hand, the acute toxicity of highly reactive chemicals, as well as many longer-term toxic effects such as tissue necrosis and cancer, may be cumulative in nature, depending on both the concentration and duration of the exposure. A simple metric for such cases is the area under the concentration curve (AUC) in the tissue, which is defined mathematically as the integral of the concentration over time (27, 31, 32). This mathematical form implicitly assumes that the effect of the chemical on the tissue is linear over both concentration and time. The use of the AUC represents an extension of the concept, developed from observations of the effects of chemical warfare gases (33), that toxicity is proportional to the product of the concentration and time of exposure ($C \times T$). For developmental effects, the chemical time course may also have to be viewed in the context of with the window of susceptibility for a particular gestational event (34).

An important factor in selecting an appropriate dose metric is to determine the toxicologically active form of the chemical. In some cases, a chemical may produce a toxic effect directly, either through its reaction with tissue constituents (e.g., ethylene oxide) or its binding to cellular control elements (e.g., dioxin). Often, however, it is metabolism of the chemical that leads to its toxicity. In this case, toxicity may result primarily from reactive intermediates produced during the process of metabolism (e.g., chlorovinyl epoxide produced from the metabolism of vinyl chloride) or from the toxic effects of stable metabolites (e.g., trichloroacetic acid produced from the metabolism of trichloroethylene). The selection of the dose metric, that is, the active chemical form for which tissue exposure should be determined and the nature of the measure to be used, e.g., peak concentration (C_{MAX}) or area under the concentration-time profile (AUC), is the most important step in applying pharmacokinetics in risk assessment.

Dose metrics must be selected to be consistent with the modes of action for the chemical being evaluated. No single dose metric will always be appropriate for a given effect, though consistency is expected for chemicals acting via the same mechanism. The EPA (1992) (35), in a joint effort with scientists from several other agencies, prepared a review paper on cross-species extrapolation in cancer risk assessment, which concluded that "...tissues experiencing equal average concentrations of the carcinogenic moiety over a full lifetime should be presumed to have equal lifetime cancer risk." The use of the term "carcinogenic moiety" in this statement reflects the concern that the dose metric should be representative of the active form of the chemical. For example, the use of the lifetime average daily concentration for the parent chemical would

be appropriate for a directly genotoxic chemical such as ethylene oxide, which is detoxified by metabolism; however, it would not be appropriate for a chemical like vinyl chloride, which requires metabolic activation to be genotoxic. In the latter case, increasing metabolism would increase the exposure to the genotoxic species but would decrease a dose metric based on parent chemical concentration. In such a case, where a reactive species produced during the metabolism of a chemical is responsible for its carcinogenicity, an appropriate cancer dose metric would be the lifetime average daily production of metabolite in the target tissue divided by the volume of the tissue, as was used in the pharmacokinetic risk assessment for methylene chloride (10). Similar considerations apply in the case of noncancer risk assessment, except that the dose metrics are only averaged over the duration of the exposure (acute, subchronic, or chronic) or the critical developmental window, not over a full lifetime (28).

Finally, it should be noted that although C_{MAX} and AUC are the most commonly applied metrics for tissue exposure, other dose metrics might sometimes be more appropriate, particularly for chemicals with a mode of action related to some aspect of their interaction with a receptor. In such cases, time above a critical concentration (TACC) or average receptor occupancy might be more appropriate (36, 37). Unfortunately, the more an attempt is made to include pharmacodynamic processes into a dose metric (e.g., receptor occupancy), the more difficult it usually becomes to collect the data necessary for its use in each of the relevant species. Of the many possible dose metrics, typically only C_{MAX} , AUC, and TACC can be estimated from the kinds of data currently available on chemicals. While we have discussed these dose metrics in terms of the target tissue, often there is a simple proportional relationship between the blood level and the tissue level so the dose metrics used are in blood rather than the tissue. Typically, data on blood concentrations are more often available, particularly in humans, making it possible to validate model predictions.

THE RATIO CONCEPT IN RISK ASSESSMENT

Although it is crucial that the dose metric properly represent the essential nature of the biologically effective dose, as described above, it is often possible to simplify the actual dose metric calculation by recognizing that quantitative risk assessment is fundamentally based on a ratio: specifically, the ratio of the dose metric value for the exposure of concern to the value for the exposure (or exposures) defining the toxicity. Typically, the exposures defining the toxicity might be the no observed adverse effect level (NOAEL) in an animal experiment or the doses in a cancer bioassay, while the exposure of concern might be a lifetime continuous human exposure. Any factors which do not change across the conditions of these exposures will not effect the ratio of the dose metrics, and thus will not impact the risk assessment.

For example, the ultimate dose metric for a particular toxicity might be based on the chemical's concentration in the target tissue. However, an acceptable dose metric might be based on the chemical's blood concentration as

long as the relationship between the blood concentration and target tissue concentration could be expected to be the same in both the animal toxicity study and the human exposure. In fact, this is probably a reasonable assumption across different exposure conditions in a given species: namely, that the concentrations would be related by the tissue:blood partition coefficient. However, while tissue:air partition coefficients for volatile lipophilic chemicals appear to be similar in dog, monkey, and man (38), human blood:air partition coefficients appear to be roughly half of those in rodents (39). Therefore, the human tissue:blood partition would probably be about twice that in the rodent. Thus, if the model were to be used for extrapolation from rodents to humans, this 2-fold difference could be factored into the analysis as an adjustment to the blood concentration dose metric.

The dose metric just described for a reactive metabolite provides another example of the use of this ratio concept: the amount of metabolism divided by the volume of the tissue is used as a surrogate for the average concentration of the reactive species, on the assumption that other factors remain constant. That is, it is assumed that the stoichiometric yield of the reactive species and its reaction rate are invariant across species and over the exposure conditions being modeled.

Use of the ratio concept can greatly simplify risk assessment applications of pharmacokinetic modeling for developmental toxicity or teratogenicity studies. While it might seem necessary to use a model that includes compartments for the developing fetus, this might not always be the case. For some chemicals, the maternal blood or plasma concentration profile can provide an adequate surrogate for the fetal exposure (40, 41). An important point to be aware of in this regard is the fact that symmetric diffusion limitation, such as might be expected for placental transport of many environmental contaminants, does not affect the AUC in the tissue. That is, while diffusion limited transport across the placenta might delay the achievement of the maximum concentration in the fetus as compared to the maternal blood, the AUC in the fetus will bear the same relationship to the AUC in the blood as it would in the case of flow-limited transport. Moreover, for exposures of sufficient duration to reach steady state, the steady-state fetal concentration will be completely determined by partitioning and will not be affected by diffusion-limited transport. For many chemicals, the only important pharmacokinetic complication associated with fetal development is the resulting increase in the total volume of distribution for the chemical. This complication may be ignored for risk assessment purposes since the effect can be similar in both the toxicity study and the human exposure of concern.

IMPACT OF PHARMACOKINETICS IN RISK ASSESSMENT

Pharmacokinetics have been addressed differently in the default noncancer and cancer approaches. The standard paradigm for non-cancer risk assessment rarely considers chemical-specific pharmacokinetic information; typically, a NOAEL or lowest observed adverse effect level (LOAEL) derived from data for the exposure route of interest is simply adjusted by the application of generic uncertainty factors (UFs) to obtain reference concentrations (RfCs) or reference

doses (RfDs). Individual UFs of from 1 to 10 are applied for various potential sources of uncertainty including use of a LOAEL, extrapolation to a longer exposure duration, extrapolation from animals to humans, human variability and database limitations. Typically the total UF (the product of the individual UFs) is restricted to a maximum of 3000 (42).

In this paradigm, little attention has been given to incorporating knowledge of the mode of action or the dosimetry of the active chemical form in target tissues in these calculations. The selection of UFs has also generally failed to consider chemical-specific mechanistic information or pharmacokinetic data. One exception is the focus in the revised RfC process on delivered dose adjustments for inhaled materials (42).

Allometric Dose Scaling

Allometric scaling of a toxic dose can be defined as follows:

$$\mathbf{TD = a \times BW^b} \quad (1)$$

$$\mathbf{TD_A = a \times BW_A^b} \quad (2)$$

$$\mathbf{TD_H = a \times BW_H^b} \quad (3)$$

Where:

TD – toxic dose (mg)	A – animal (e.g., rat or mouse)
BW – body weight (kg)	H – human
a – constant	
b – scaling exponent (e.g., 2/3 or 3/4)	

By substituting $a = TD_A/BW_A^b$ (rearranged eq. 2) into eq. 3 the conversion of the animal dose expressed in mg to the human dose in the same units is obtained:

$$\mathbf{TD_H = (BW_H/BW_A)^b \times TD_A} \quad (4)$$

To obtain the relationship in the **mg/kg** units more typically reported in toxicology:

$$\mathbf{TD/BW = a \times BW^b/BW = a \times BW^{b-1}} \quad (5)$$

Rearranging this equation for animals:

$$\mathbf{a = TD_A / (BW_A \times BW_A^{b-1})} \quad (6)$$

Substituting Eq. 6 into Eq. 5 (for humans) obtains the conversion of the animal dose expressed in mg/kg to the human dose in the same units:

$$\mathbf{TD_H/BW_H = (BW_H/BW_A)^{b-1} \times TD_A/BW_A} \quad (7)$$

Equivalently the equation may be written as:

$$\mathbf{TD_H/BW_H = (BW_A/BW_H)^{1-b} \times TD_A/BW_A} \quad (8)$$

Figure 2: Allometric Scaling for Interspecies Extrapolation

In the traditional paradigm for cancer risk assessment in the United States, dose-response modeling was used to calculate a carcinogenic potency based on

tumors observed in animal bioassays or human epidemiology studies. In the case of animal studies, "body surface area" scaling (multiplying by the cube root of the ratio of the animal and human body weights) was used to obtain a human equivalent dose (HED) (Figure 2, Table 1).

Table 1. Examples of Interspecies Scaling Based Upon Body Weight Ratios*

	BW^b	Avg. Daily Dose for Species (ADD)	Animal/ Human Body Weight Ratio	Human/ Animal Body Weight Ratio	Human Equivalent Dose (HED) mg/kg/day
b = 1					
	BW¹		(A/H)⁰	(H/A)⁰	
Human	70	1	1	1	1
Rat	0.25	1	1	1	1
Mouse	0.030	1	1	1	1
b = 3/4					
	BW^{3/4}		(A/H)^{1/4}	(H/A)^{1/4}	
Human	24.20	1	1.0	1.0	1
Rat	0.354	1	0.244	4.1	0.244
Mouse	0.0721	1	0.144	7.0	0.144
b = 2/3					
	BW^{2/3}		(A/H)^{1/3}	(H/A)^{1/3}	
Human	16.5	1	1.0	1.0	1
Rat	0.401	1	0.153	6.5	0.153
Mouse	0.0988	1	0.075	13.3	0.075

*Representative values of body weight for different species have been assumed. The average daily dose (ADD) was assumed to be 1 mg/kg/day. The human dose equivalent to each animal species' ADD (i.e. the HED) was calculated using equation 8 in Figure 2 and the assumed values of BW and ADD in this table. Abbreviations: BW – body weight, A – Animal body weight, H – Human body weight

Dose-response modeling was then performed on the HEDs using the linearized multistage model (43). For inhalation studies, conversion from inhaled concentration to "absorbed dose" was performed by a rudimentary calculation involving the ventilation rate, body weight, and "fraction absorbed". As mentioned earlier, chemical-specific pharmacokinetic information has occasionally been used in this process, with the calculation of metabolized dose in the risk assessment for trichloroethylene (6, 7) and the use of a PBPK model in the risk assessment for methylene chloride (8) serving as examples. The guidelines for carcinogen risk assessment recently proposed by the EPA (1996) (44) would appear to provide the flexibility necessary to move forward in this area. Under the new guidelines, multiple options are available for performing a carcinogenic dose-response assessment: (1) a linear approach similar to the traditional cancer paradigm, and (2) a margin of exposure (MOE) approach more similar to the noncancer paradigm. The selection of the dose-response approach to be used with a particular chemical is determined on the basis of the

information available on the carcinogenic mode of action of the chemical, which considers both pharmacokinetic and mechanistic information.

Cross-Species Extrapolation

In the traditional default risk assessment approaches, all chemicals are implicitly treated as if the observed toxicity is produced directly by the parent chemical itself (3). This implicit assumption that the parent chemical is directly toxic is true even in the new RfC dosimetry guidelines (42), which differentiate respiratory effects from extra-respiratory effects and include different defaults for chemicals based on their solubility and reactivity. However, a risk assessment that considers pharmacokinetics must necessarily also consider the mode of action, at least to the extent of identifying the active form of the chemical for which the dosimetry should be performed.

To demonstrate the importance of considering pharmacokinetics and mode of action in dose-response risk assessment, PBPK models for methylene chloride, trichloroethylene, and vinyl chloride were exercised to determine general expectations for the cross-species dosimetry for one class of chemicals, the volatile lipophilic solvents (21). All three of these chemicals would be considered Category 3 gases (relatively water-insoluble chemicals which achieve a steady state during inhalation exposure) in the EPA (1994) (42) dosimetry guidelines. In a standard risk assessment, the animal-to-human dosimetry adjustment for each of these chemicals would be performed in exactly the same way. For example, for noncancer analyses time-weighted average (TWA) exposure concentration for inhalation and mg/kg/day administered dose for oral exposure would be used, regardless of the nature of the toxic endpoint or the mechanism of toxicity.

In contrast to the simplicity and uniformity of the default approaches, a pharmacokinetic approach requires the application of scientific judgement to select the appropriate option for each toxic effect of a chemical. A comparison of the default and PBPK-based approaches, as a function of the type of toxicity and the exposure route based upon the analyses for methylene chloride, vinyl chloride, and trichloroethylene is provided in Table 2.

For the purpose of this comparison, the acute, reversible neurological effects of these chemicals were assumed to result from the direct toxicity of the parent chemical, and an appropriate dose metric would therefore be either the C_{MAX} or AUC of the parent chemical in the brain or, as discussed above, in the blood as a surrogate for the brain. For the calculations used to prepare Table 2, the AUC was selected, since it is more analogous to the TWA calculation typically used for duration adjustment. On the other hand, the production of reactive species during metabolism was assumed to be responsible for the chronic liver toxicity of methylene chloride and vinyl chloride, so the average daily amount of metabolism divided by the volume of the liver was chosen as the dose metric. Finally, the development of liver toxicity from trichloroethylene was assumed to result from the activity of the stable metabolite, trichloroacetic acid; therefore,

the average daily AUC for trichloroacetate in the liver was used as the dose metric.

Table 2. Alternative Metrics for Cross-Species Equivalence^a

Route	Basis	Metric	Human/Animal Dose Equivalence	
Inhalation	<u>Default:</u>			
	1986 EPA Cancer Guidance	"Inhaled dose" * (A/H) ^{1/3}	0.15 - 0.3	
	New EPA Cancer Guidance	TWA Conc. (PD:UF=1-10)	1	
	EPA Noncancer Guidance	TWA Conc. (PD:UF=3)	1	
	<u>Allometric:</u>			
	Parent Chemical	TWA Conc.	1	
	Reactive Metabolite	TWA Conc / (A/H) ^{1/4}	4 - 7	
	Stable Metabolite	TWA Conc.	1	
	<u>PBPK:^b</u>			
	Parent Chemical	Parent AUC	0.6 - 1.7	
	Reactive Metabolite	Tmet / Vt	5 - 25	
	Stable Metabolite	Metabolite AUC	0.1 - 1	
	Oral	<u>Default:</u>		
		1986 EPA Cancer Guidance	Dose * (A/H) ^{1/3}	0.08 - 0.16
		New EPA Cancer Guidance	Dose * (A/H) ^{1/4}	0.15 - 0.25
EPA Noncancer Guidance		Dose (PK+PD:UF=10)	0.3	
<u>Allometric:</u>				
Parent Chemical		Dose * (A/H) ^{1/4}	0.15 - 0.25	
Reactive Metabolite		Dose	1	
Stable Metabolite		Dose * (A/H) ^{1/4}	0.15 - 0.25	
<u>PBPK:^b</u>				
Parent Chemical		Parent AUC	0.01 - 0.1	
Reactive Metabolite		Tmet / Vt	1 - 2	
Stable Metabolite		Metabolite AUC	0.02 - 0.07	

^aAbbreviations – (A/H): ratio of animal to human body weights, AUC: area under the blood concentration curve, PD: pharmacodynamic, PK: pharmacokinetic, Tmet/Vt: total metabolite formed in tissue divided by tissue volume, TWA Conc.: time-weighted average concentration, UF: uncertainty factor

^bBased on physiologically based pharmacokinetic (PBPK) modeling of methylene chloride, trichloroethylene, and vinyl chloride

To obtain the comparisons shown in Table 2, the PBPK models were exercised for a typical exposure scenario in the mouse and rat. The models were then rerun for the same exposure scenario but with human parameters, and the concentration or dose was varied until the human dose metric was the same as that obtained for the mouse and rat. These two pharmacokinetically-determined human equivalent concentrations (HECs) or doses (HEDs), one for the mouse and one for the rat, were then compared to the corresponding HECs or HEDs obtained by the default methodology. No animal-to-human UFs were applied in this comparison.

As shown in Table 2, the correct relationship for cross-species dosimetry depends on whether the toxicity is due to the parent chemical or a metabolite, and in the case of toxicity from a metabolite, whether the metabolite is highly reactive or sufficiently stable to enter the circulation. Moreover, the nature of the cross-species relationship for each of these possibilities is different for oral exposure than for inhalation. Therefore, pharmacokinetic modeling is required to improve the reliability of cross-species extrapolation that considers the nature of the toxic entity.

Default noncancer risk assessments apply a UF of ten for uncertainty regarding animal-to-human extrapolation. This UF is applied to consider the possibility of both pharmacokinetic and pharmacodynamic differences between rodents and humans which could put the human at greater risk (*i.e.*, result in toxicity at lower exposures), and is reduced to three when inhalation dosimetry is used to consider pharmacokinetic differences (42). A reduced factor of three has also sometimes been applied for data from species that are considered physiologically "closer" to humans, such as dogs or monkeys. This UF plays the same role, and is roughly the same magnitude, as the traditional use of body surface area scaling in cancer risk assessment (which resulted in a factor of about 7 for rats and 13 for mice) (Table 1). In both cases the concern that the human might receive a relatively greater exposure, and hence be at relatively greater risk, than smaller animals receiving the same nominal dose reflects years of experience with data on chemical toxicities that, for the most part, arise from oral exposure to chemicals which are directly toxic (*i.e.* as the parent compound, without the need for metabolic activation) (3).

As can be seen from Table 2, the human is indeed predicted to be at greater risk for oral exposures to chemicals that are directly toxic due to the effect of pharmacokinetic scaling. For oral exposures to a toxic chemical that must be cleared by metabolism or urinary excretion, the internal exposure (AUC) in the human is greater than in smaller animals at the same administered dose because the clearance of chemicals, both metabolic and urinary, tends to decrease relative to body weight as the animal becomes larger (45). In fact, the allometric scaling of clearance appears to follow body weight raised to the three-quarters power, producing slightly less of a difference across species than that predicted by body surface area scaling, which is body weight raised to the two-thirds power (35). Indeed, based on a multiagency analysis of the evidence for cross-species scaling factors, the EPA (1992) (35) changed from its default body

surface area cross-species scaling for cancer to a new scaling approach based on body weight raised to the three-quarters power.

In both cancer and noncancer risk assessment, there is continuing controversy regarding the appropriate cross-species default for pharmacodynamics. The origin of the default scaling/UF actually applied in either case rests on observations of relationships across species, which are completely consistent with pharmacokinetics alone (3). Nevertheless, the use of pharmacokinetics in a cancer or noncancer risk assessment has never been considered to fully replace the default scaling/UF. It can be seen from Table 2 that, in the case of noncancer risk assessment, the default approach is not necessarily conservative in all cases, even with the application of the full animal-to-human UF of 10 (e.g., compare the Human/Animal Equivalence Ratio 0.1 for the default approach with ratios ranging from 0.01 to 0.1 predicted with pharmacokinetics for the parent compound AUC). That is, in some cases, the cross-species differences in pharmacokinetics alone may exceed the default factor applied for both pharmacokinetics and pharmacodynamics. A similar result would be obtained for cancer risk estimates. Particularly in the case of toxicity due to a stable metabolite, the default dosimetry and scaling/UF may sometimes underestimate the human risk (overestimate the HEC/HED).

Cross-Route Extrapolation

Another important use of pharmacokinetics in risk assessment is for extrapolation from one exposure route to another. In default noncancer and cancer risk assessment approaches, no provision is made for the use of toxicity data from a different route than the human exposure of concern. Thus, for example, in performing an inhalation risk assessment for a chemical, data from animal studies performed by the oral route could not be included in the quantitative dose-response calculations. Except in the case of exposure-route-specific portal-of-entry effects, the use of pharmacokinetic modeling makes it possible to combine data from different routes in a quantitative risk assessment. Specifically, the pharmacokinetic model can be used to predict the target tissue dose associated with an animal toxicity study conducted by one route, and then can be exercised to predict the equivalent human exposure by another route that would result in the same target tissue dose (22).

Dose Extrapolation

A third use of pharmacokinetics in risk assessment is to incorporate dose-dependent pharmacokinetics and metabolism into the dose-response calculations for a chemical. For example, the observed dose-response relationship between the exposure concentration and resulting toxicity of vinyl chloride in animal studies is highly nonlinear due to the saturation of metabolism. When a pharmacokinetic model is used, however, and the tissue dose is expressed in terms of total metabolism, the dose-response for toxicity becomes linear, improving the accuracy of dose-response modeling.

Time Extrapolation

In some cases, pharmacokinetic modeling provides a more accurate method for extrapolating across exposure time frames than default methods such as the use of the TWA exposure concentration or average daily dose. For example, exposure to vinyl chloride at 100 ppm for 8 hours will not be equivalent to exposure at 800 ppm for 1 hour, due to saturation of metabolism at the higher concentration and rapid post-exposure clearance of unmetabolized chemical by exhalation. Total metabolism in the latter exposure will be significantly lower than in the former, while the AUC for the parent chemical will be greater. However, for a highly lipophilic chemical with similar high-affinity, low-capacity metabolism, post-exposure metabolism of chemical stored in fat tissues could result in nearly the same amount of metabolism from an exposure of a few hours as from a continuous exposure at the same concentration. In cases such as these, a pharmacokinetic model, which incorporates a realistic description of the dose-response for metabolism, is necessary to determine the correspondence between exposures over different durations (2, 46).

The nature of time extrapolation performed by a pharmacokinetic model will also be determined to a large extent by the dose metric selected. For example, the two dose metrics described for acute toxicity, C_{MAX} and AUC, respond very differently to changes in exposure duration. In the case of a constant concentration inhalation exposure, the concentrations in the blood and tissue quickly rise to a steady-state and then remain constant until the exposure is terminated, at which time they rapidly return to zero (except, perhaps, in the fat). Thus C_{MAX} will be relatively invariant for exposures ranging from tens of minutes to years. In contrast, for the same exposure scenario the AUCs in the tissues will be roughly proportional to the length of exposure.

It should be recognized, however, that pharmacokinetic modeling is generally not very useful for extrapolating across widely different time frames, e.g., from acute to subchronic or from subchronic to chronic exposure durations. The principal determinants of the relationship between the effects of shorter-term and longer-term exposure are, to a large extent, pharmacodynamic factors such as fatigue, repair, and compensation. Therefore, the default approach in noncancer risk assessment, in which UFs are applied to account for uncertainty regarding the effect of significant differences in the duration of exposure, is still appropriate when pharmacokinetics is considered. For this reason, the dose metrics calculated with pharmacokinetic models for noncancer risk assessments chemicals are usually calculated as average daily values, where the average is taken over the total duration of the exposure. For example, instead of calculating the total AUC over a 90-day exposure, the average daily AUC (which is equivalent to a daily average concentration) is calculated by dividing the total AUC by the total duration of the exposure in days (90 in this case). Persistent chemicals remain in the body long after exposure so the period of concern for noncancer risk assessment may not be limited to the exposure period. During this time there can be extensive changes in body composition altering distribution and elimination. PBPK models are well suited for incorporating these aspects into the risk assessment.

APPLICATION OF PHARMACOKINETIC MODELING IN RISK ASSESSMENT

As should be apparent from the discussion thus far, the application of pharmacokinetic modeling in risk assessment is both chemical- and endpoint-specific. Therefore, it is not possible to completely describe the approach that should be taken under all possible circumstances. The application of pharmacokinetics in a particular case requires the use of scientific judgement and an understanding of the risk assessment process. Nevertheless, a number of steps can be described which will generally be required regardless of the details of the application. These common steps in the process are described in the following section (Figure 3).

<i>Step 1</i>	Selection of Potential Critical Studies and Organization of Mode of Action Literature
<i>Step 2</i>	Selection of Pharmacokinetic Model
<i>Step 3</i>	Calculation of Dose Metrics for Toxicity Studies and Analysis of the Potential Critical Studies
<i>Step 4</i>	Application of Uncertainty Factors
<i>Step 5</i>	Determination of Human Exposures
<i>Step 6</i>	Selection of Preferred Dose Metric/Study/Endpoint

Figure 3: Steps for Applying Pharmacokinetics in Noncancer and Cancer Dose-Response Assessment

Step 1: Selection of Potential Critical Studies and Organization of the Mode of Action Literature.

The first step in performing a risk assessment using pharmacokinetic modeling is essentially the same as in the default approach: evaluation of the available toxicological and mechanistic data for the chemical and selection of potential critical studies. The principal difference is that, since the cross-species equivalence for different toxicological endpoints may vary, as described above, it is not always possible to determine from a comparison of the animal exposure data which study will predict effects at the lowest human exposures. Another

major difference is the importance of organizing information regarding the mode of action of the chemical for the critical endpoints. Both qualitative and quantitative data help determine the appropriate methods (e.g., choice of dose metric) in later steps.

Step 2: Selection of Pharmacokinetic Model.

Once the exposure scenarios and endpoints of concern have been selected, the requirements for a pharmacokinetic model can be determined. The key elements of this determination are the animal species and exposure conditions that the model must be able to simulate, and the target tissue dose metrics that the model must be able to calculate. Of course, it is also necessary to determine whether the model has been adequately validated to ensure its reliability for the intended purpose (14). In particular, the reliability of the model predictions for each of the dose metrics should be carefully evaluated (47, 48).

Step 3: Calculation of Dose Metrics for Toxicity Studies and Analysis of the Potential Critical Studies.

For each study and endpoint selected in step 1, the pharmacokinetic model is used to calculate the appropriate dose metrics for the endpoint of concern. In some cases, it may be possible to postulate more than one reasonable dose metric. In such cases, all of the candidate metrics should be calculated. The final decision regarding which metric to use should be made only after the calculations have been completed for each metric, and should consider both the plausibility and conservatism of the various options, as will be discussed later. To calculate the dose metrics, the model parameters are set to those for the species represented in the study, whether an experimental animal study or a human study. In the case of developmental studies, it will be necessary to estimate parameters for a young animal or pregnant female rather than an average adult. To the extent possible, data from the study on animal strain, body weights, age, and activity should be used in selecting parameters for the model. The experimental parameters in the model are then set to reproduce the exposure scenario performed in the study, and the model is run for a sufficient period of time to characterize the animal exposure to the chemical and, if necessary, its metabolites.

There are often a number of options regarding the way in which the model should be run to characterize the exposure. These will depend upon the dose metric(s) selected based upon mode of action information as appropriate for the further analyses. Frequently, the desire is to estimate a daily average, although in some cases the total over the duration of the experiment is used. As mentioned earlier, while the averaging period in the case of cancer is typically taken to be the lifetime, the averaging period in the case of noncancer risk assessment is considered to be the duration of the exposure or, perhaps, the critical window.

For short-term exposures, the model must be run for an appropriate period reflecting the dose metric being used and the timing of the measurement of

toxicity in relation to the period of exposure. For short exposure, this is easily done by running the model for the total duration of the exposure (or exposures, for repeated exposure studies) to obtain dose metrics. If the animals were held for a post-exposure period before toxicity was evaluated, the model must be run either till the end of the post-exposure period or for a sufficient duration to ensure that the parent chemical has been completely cleared from the body or, for a dose metric based on a metabolite, a long enough time to ensure the complete clearance of the metabolite. The resulting dose metric obtained for the total duration of the exposure (including any post-exposure period) can then be divided by the number of days over which the experiment was conducted in order to derive the average daily value.

The same approach (running the model for the total duration of the study) can be used to calculate dose metrics for longer-term exposures. This approach would typically be necessary for models that describe changes in the physiology or chemical handling during different lifestages (e.g., adolescence, aged). However, an alternative approach, which is often attractive for modeling of chronic exposures with time-invariant model parameters, is to estimate the steady-state dose metric. There are two principal methods for calculating a steady-state estimate. In the first, the model is run until steady state is reached and then the dose metric is calculated by subtraction. For example, in the case of a chronic oral or inhalation exposure conducted 5 days per week, the model can be run consecutively for 1 week, 2 weeks, 3 weeks, and so on. To calculate the average daily AUC for a given week, the value at the end of the previous week is subtracted from the value at the end of the current week and the result is divided by 7. This process is repeated until the change in the dose metric from one week to the next is insignificant. For continuous exposures, the comparison can be made on a daily basis instead of weekly. The other method for estimating the steady-state dose metric is to estimate it from a single day exposure. The model is run for a single-day exposure plus an adequate post-exposure period to capture clearance of the parent compound or relevant metabolite. This value of the single-day dose metric is then modified by the necessary factors to obtain an average daily value (e.g., by multiplying by five-sevenths in the case of the 5-day per week exposure just described). This method, which is faster but only approximate, is sufficiently accurate for estimating average daily AUC in many cases. It can be checked against the first method described to determine its accuracy in a particular case.

The dose metric calculations needed are determined by the method to be used for the noncancer or cancer analysis. If the NOAEL/UF method is being used in a noncancer risk assessment, a dose metric only needs to be calculated for the NOAEL or LOAEL exposure for a particular study and endpoint. On the other hand, if dose-response modeling is going to be performed, such as in the Benchmark Dose approach (49, 50), dose-metrics must be calculated for all exposure groups. The dose metrics are then used in the dose-response model in place of the usual exposure concentrations or administered doses. It is important to remember that when this is done, the result of the dose-response modeling will also be in terms of a value of the dose metric rather than an exposure concentration or administered dose. Dose-response modeling is more

properly conducted on the dose metrics, since it is expected that the observed effects of a chemical will be more simply and directly related to a measure of target tissue exposure than to a measure of administered dose.

Step 4: Application of Uncertainty Factors

In the default noncancer risk assessment approach, animal exposure concentrations are converted to HECs before applying any necessary UFs. In a pharmacokinetic risk assessment approach, on the other hand, it is more proper to divide the dose metrics corresponding to the point of departure (for cancer MOE) or the noncancer equivalents (e.g., NOAEL or BMD) obtained from the toxicity studies by the necessary UFs rather than the HECs or HEDs. The rationale for applying the UFs to the dose metrics is the same as for using the dose metrics in dose-response modeling: it is expected that the observed effects of a chemical will be more simply and directly related to a measure of target tissue exposure than to a measure of administered dose. The dose metrics are specifically chosen to provide more useful measures of the biologically effective dose. Therefore, it is the dose metrics that should be adjusted to assure that the biologically effective dose is reduced to the extent desired. As a counter-example, consider the case where toxicity has been observed for exposure to a chemical at a concentration well above the point where saturation of the metabolism of the chemical occurs, and where the metabolism of the chemical is responsible for the toxicity. In this case, applying the UF to a dose metric based on total metabolism would assure that the extent of metabolism would be reduced by the same factor. However, if the concentration at which the effect was observed were sufficiently higher than the concentration at which metabolism is saturated, applying the UF to the exposure concentration might not actually reduce the extent of metabolism to any appreciable extent.

The selection of UFs in a pharmacokinetic risk assessment is essentially the same as for the default noncancer process, described earlier, except that the UF for uncertainty in animal-to-human extrapolation should be reduced to reflect the use of pharmacokinetic modeling. By analogy to the EPA (1992) (35) approach for inhalation dosimetry, reduction of the default animal-to-human UF from 10 to 3 would seem to be reasonable for both inhalation and oral risk assessments. The remaining factor of 3 is then considered to represent uncertainty regarding pharmacodynamic differences across species, and could be modified on the basis of other information for the chemical. The UFs will generally vary from one study to another, as well as from one endpoint to another, as dictated by the nature of the study (e.g., if only a LOAEL was identified) and the information associated with the endpoint (e.g., if there is evidence regarding the relative sensitivity of humans compared to the experimental species).

Step 5: Determination of Human Exposure

In order to convert a dose metric to an exposure concentration or administered dose, the pharmacokinetic model must be "run backwards"; that is, the model must be run repeatedly, varying the exposure concentration or administered dose until the desired dose metric value is obtained. In the case of calculating

the acceptable human exposure corresponding to a given toxicity study, the physiological, biochemical, and exposure parameters in the model are set to appropriate human values and the model is iterated until the dose metric obtained for the human exposure of concern, often continuous or daily lifetime exposure, is equal to the dose metric obtained for the toxicity study divided by the UFs. The dose metric should be calculated in an analogous way to the dose metric for the toxicity study; that is, if the dose metric in the toxicity study was expressed in terms of an average daily value, the dose metric used for calculating the associated human exposure should also represent an average daily value. For short-term exposures, where the model has been run for the total duration of the toxicity study and the total dose metric value has been calculated, the dose metric used for calculating the associated human exposure should usually be obtained for an exposure over the same time period. An exception to this rule is the case where it is anticipated that the short-term exposure of concern for the human may represent a short-term excursion against a background of chronic exposure. In this case, a more conservative approach may be preferred, in which a steady-state dose metric calculation is used for the human.

When a steady-state dose metric is used in both an experimental animal and the human, it should be noted that the calculation of a steady-state dose metric in the human generally requires running the model for a much longer period of time than in the animal. In fact, the time required to reach steady-state in the human can be estimated by multiplying the time to steady state in the animal by the ratio of human to animal body weights raised to the one-quarter power. This concept of the allometric scaling of equivalent times is sometimes referred to as "physiological time" (35, 51).

Step 6: Selection of Preferred Dose Metric/Study/Endpoint

After calculations for potential dose-response values (*i.e.*, RfD, RfC, cancer factors) have been performed for each of the candidate studies and endpoints, the most appropriate of the alternatives must be selected. There are two principal criteria for this selection: plausibility and conservatism. For each endpoint, priority should be given to the dose metric that, on the basis of the available evidence, appears to provide most plausible basis for estimating the biologically effective dose. The plausibility of a given dose metric is determined primarily by two factors: (1) its consistency with available information on the mode of action (mechanism of toxicity), and (2) the consistency of its dose-response with that of the endpoint of concern. The first factor has been discussed earlier; the second refers both to evaluating the dose metric's ability to linearize the dose-response for the associated endpoint within a study (internal consistency), as well as its ability to demonstrate a consistent quantitative relationship of dose metrics for positive vs. negative exposures, regardless of differences in exposure scenario, route, and species (external consistency).

To provide an illustration of these points, the dose metric used in the pharmacokinetic cancer risk assessment for vinyl chloride (12) demonstrated all of the attributes of an effective dose metric. First, the form of the metric (total

daily metabolism divided by the volume of the liver) was consistent with the mode of action for the endpoint of concern (liver tumors), which involves DNA adduct formation by a highly reactive chloroethylene epoxide produced from the metabolism of vinyl chloride. Secondly, while the dose-response for liver tumors vs. exposure concentration of vinyl chloride is highly nonlinear with a plateau at several hundred ppm, the dose-response for liver tumors vs. the metabolized-dose metric is essentially linear from 1 ppm to 6000 ppm. Finally, and most impressively, when the potency of vinyl chloride liver carcinogenicity was expressed in terms of the metabolized-dose metric, essentially the same potency was calculated from both inhalation and oral studies in the mouse and rat, as well as from occupational inhalation exposures in the human. While it is rare to find a case where there is such consistency across widely diverse studies, a dose metric that adequately represents the biologically effective dose should generally have lower values under exposure conditions with no effect and higher values for toxic exposures, regardless of differences in exposure scenario, route, or species.

The other criterion for the selection of the appropriate dose metric is conservatism. Where there is an inadequate basis for giving priority to one dose metric over another, the most conservative (the one producing the highest risk or lowest acceptable exposure) would be used in order to be health protective. After selecting the most appropriate dose metric for each endpoint, the results across the various studies and endpoints should be evaluated using the same criteria, plausibility (*i.e.*, internal and external consistency of dose-response) and conservatism, to arrive at the final recommendation. When a risk assessment is based on an endpoint in an animal experiment, it may sometimes be possible to evaluate data from human exposures as a test of the plausibility of the result, even though the data might not be adequate to serve as the basis for calculating an alternative value. Another useful exercise is to vary the physiological and biochemical parameters used in the model to determine the effect of human pharmacokinetic variability on the dose metric (52). In particular, the model can be used to evaluate whether selected groups may represent sensitive subpopulations (*e.g.*, pregnant women, children, the obese, etc.) due to differences in exposure or pharmacokinetic factors.

DISCUSSION

We have outlined a process for consistently using dosimetry information in a mode of action based dose-response analysis. The six steps described are summarized in Figure 3. In the first step, one evaluates potential critical studies and the relevant mode of action information for the effect observed in the critical studies. Because the appropriate dose metric is selected based upon the mode of action, different critical effects (*i.e.* effects that might form the basis for estimating acceptable exposures) may require different dose metrics. A PBPK model appropriately parameterized for the species in which the critical effects was observed is then selected (Step 2) and used to calculate the relevant dose metrics for the benchmark dose or NOAEL of the critical effects (Step 3). Uncertainty factors are then selected and applied to the dose metric description of the point of departure (Step 4). Next, a human parameterized model is used to estimate the exposure concentration that would produce the same value for

the dose metric as that obtained from the animal study modified by the uncertainty factors (Step 5). Finally, the values obtained for the different studies are compared and used as appropriate for the regulatory or analysis purpose under consideration (Step 6). Generally, the study giving the lowest allowable exposure would be used for establishing acceptable exposure levels assuming lifetime daily exposure. If one were evaluating the potential for a specific target organ toxicity to occur due to a mixture of chemicals, the value based upon the critical study with that endpoint would be selected.

Many of the issues described in this paper with regard to the PBPK-based approach are equally applicable to the default risk assessment process. For example, the selection of a dose metric which is appropriate for the chemical and toxicity of concern, while more evident in the PBPK-based approach, is just as important in the default approach. A case in point is the question of whether concentration or AUC should be used for short-term exposure guidelines (28). Unfortunately, with the exception of the RfC dosimetry guidelines (42), the selection of dose metric is typically described more as a matter of policy than of scientific judgment. In this regard, the spirit of the recently proposed cancer guidelines (44) represents an important departure from previous guidelines, which identified defaults as policy positions and required substantial justification for departure from the default approach. In contrast, defaults under the new cancer guidelines are described as no-information alternatives, the use of which must be defended on the basis of the lack of chemical-specific information to support a more scientifically appropriate approach.

The analyses described here have focused upon the use of pharmacokinetics with empirical analyses of the dose-response for the effect. This reflects the much greater extent of our knowledge and modeling pharmacokinetics as opposed to modeling the toxicity process, or pharmacodynamics. However, as pharmacodynamic models become available, they can be readily incorporated in the process described here. Pharmacodynamic models often currently only exist for the animal species in the toxicity study and human parameters values or the appropriateness of the model structure for humans may be unknown. Under these circumstances, the model would be used much as the empirical models are used for benchmark dose analysis (Step 3). That is the pharmacodynamic model would be used to better describe or predict the dose response in the animal study to obtain the point of departure for the subsequent analyses. This approach has largely been explored at this time in the area of cancer analysis with clonal growth models (53, 54).

The goal of research in pharmacodynamic modeling is to develop models that, like pharmacokinetic models, can be parameterized for both animals and humans. Then the chemical risk assessment process would be very similar to that described here except that both a pharmacokinetic and pharmacodynamic model would be used to analyze the animal study (Step 3) and then human versions of both models would be used to determine the human exposure that would be protective of that effect occurring in humans (Step 5). As noted earlier for pharmacokinetic analyses, this use of pharmacodynamic modeling would represent a significant change in the issue of cross-species concordance.

Biologically based analyses assume some degree of concordance in the mechanism of action, if not in the resulting toxicity. The current default position is that endpoints will be used with no assumption of concordance unless it is conclusively demonstrated to be an animal-specific mechanism. While this may be a health protective position, if unmodified it makes it impossible to use mechanistic toxicology or modeling in risk assessment and impossible to improve our ability to predict human consequences based upon animal studies.

There is a continuing interest on the part of regulatory agencies concerning the use of PBPK modeling in chemical risk assessment. Yet, while risk assessments using PBPK models have been proposed for a number of chemicals, for both cancer and noncancer endpoints, there are relatively few cases to date of the actual acceptance of PBPK-based risk assessments by the agencies. The slow progress of the application of PBPK modeling in risk assessment may be due in part to the lack of a common expectation regarding the necessary elements of a PBPK-based approach. The intent of this paper was to describe the essential elements of a preferred approach for applying PBPK modeling in a chemical risk assessment, whether for cancer or noncancer endpoints. It is hoped that to the extent that PBPK-based risk assessments can adhere to a similar protocol, the familiarity of the agencies with the process may increase and its acceptance may be fostered.

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Part II

Investigation of Chemical-Specific Properties and Physiological Variability as Determinants of Individual Risk

Chapter 5

Evaluation of the Uncertainty in an Oral Reference Dose for Methylmercury Due to Interindividual Variability in Pharmacokinetics

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ABSTRACT

An analysis of the uncertainty in guidelines for the ingestion of methylmercury (MeHg) due to human pharmacokinetic variability was conducted using a physiologically based pharmacokinetic (PBPK) model that describes MeHg kinetics in the pregnant human and fetus. Two alternative derivations of an ingestion guideline for MeHg were considered: the U.S. Environmental Protection Agency Reference Dose (RfD) of 0.1 $\mu\text{g}/\text{kg}/\text{day}$ derived from studies of an Iraqi grain poisoning episode, and the Agency for Toxic Substances and Disease Registry chronic oral Minimal Risk Level (MRL) of 0.5 $\mu\text{g}/\text{kg}/\text{day}$ based on studies of a fish-eating population in the Seychelles Islands. Calculation of an ingestion guideline for MeHg from either of these epidemiological studies requires calculation of a dose conversion factor (DCF) relating a hair mercury concentration to a chronic MeHg ingestion rate. To evaluate the uncertainty in this DCF across the population of U.S. women of child-bearing age, Monte Carlo analyses were performed in which distributions for each of the parameters in the PBPK model were randomly sampled 1000 times. The 1st and 5th percentiles of the resulting distribution of DCFs were a factor of 1.8 and 1.5 below the median, respectively. This estimate of variability is consistent with, but somewhat less than, previous analyses performed with empirical, one-compartment pharmacokinetic models. The use of a consistent factor in both guidelines of 1.5 for pharmacokinetic variability in the DCF, and keeping all other aspects of the derivations unchanged, would result in an RfD of 0.2 $\mu\text{g}/\text{kg}/\text{day}$ and an MRL of 0.3 $\mu\text{g}/\text{kg}/\text{day}$.

1. INTRODUCTION

The current Reference Dose (RfD) for MeHg (MeHg) developed by the U.S. Environmental Protection Agency (USEPA)⁽¹⁾ is based on a retrospective study of an acute poisoning incident in Iraq in which grain contaminated with a MeHg fungicide was inadvertently used in the baking of bread.⁽²⁾ The exposures, which were relatively high but lasted only a few months, were associated with neurological effects in both adults (primarily paresthesia) and infants (late walking, late talking). In particular, neurodevelopmental effects were observed in the offspring of mothers exposed to MeHg who themselves were asymptomatic. The USEPA derived an RfD of 0.1 $\mu\text{g}/\text{kg}/\text{day}$ based on a NOAEL of 11 ppm mercury in maternal hair estimated by Benchmark Dose modeling of the combined neurological endpoints reported for children exposed *in utero*. Due to the acute nature of the exposure, the maternal hair concentrations used in this analysis were the peak concentrations achieved during pregnancy. This RfD included an uncertainty factor of 10, consisting of a factor of 3 to consider pharmacokinetic variability and a factor of 3 for database limitations (lack of data on multigeneration effects or possible long-term sequelae of perinatal exposure). The USEPA⁽¹⁾ also conducted a Monte Carlo uncertainty analysis which estimated the impact of pharmacokinetic variability on the determination of the ingestion rate associated with the NOAEL hair concentration, but they did not explicitly use the results of this analysis in the derivation of the RfD.

A more recent study conducted on a population in the Seychelles Islands⁽³⁻⁴⁾ was selected as the critical study for a chronic oral Minimal Risk Level (MRL) by the Agency for Toxic Substances and Disease Registry (ATSDR). The exposures to MeHg in this population resulted from chronic, multigenerational ingestion of contaminated fish. This prospective study was carefully conducted and analyzed, included a large cohort of mother-infant pairs, and was relatively free of confounding factors. The results of this study were essentially negative, and a daily ingestion rate of 0.5 $\mu\text{g}/\text{kg}/\text{day}$, derived from the median of the distribution of maternal hair mercury concentrations in the studied population (5.9 ppm), was proposed by ATSDR as the chronic oral MRL for MeHg.⁽⁵⁾ In the case of this chronically exposed population, the average maternal hair concentration during pregnancy was used, although the authors found no evidence of significant temporal variation. Due to the large size of the study population, no uncertainty factor was considered necessary in the derivation of the MRL.

In their risk assessments for MeHg, both the USEPA⁽¹⁾ and ATSDR⁽⁵⁾ employed empirical, one-compartment pharmacokinetic models to describe the relationship between hair concentration and ingestion rate of MeHg. Parameters in these models were chosen on the basis of empirical data regarding the kinetics and partitioning of MeHg in human subjects. Based on the selected parameter values, the agencies then calculated "best estimates" of an average daily ingestion rate which would produce a given hair concentration. The USEPA⁽¹⁾ also evaluated the uncertainty in this Dose Conversion Factor (DCF) resulting from the potential variability in the pharmacokinetics of MeHg across a population, and used the results of this analysis to support their application of an uncertainty factor of 3 to address this concern in their derivation of the RfD. A more recent analysis by Stern⁽⁶⁾ also evaluated the pharmacokinetic uncertainty in the RfD. The analysis described in this paper differs from these previous analyses in that instead of an empirical, one-compartment pharmacokinetic model, a physiologically-based pharmacokinetic (PBPK) model was used. The resulting estimate of the variability in the relationship between ingestion rate and hair concentration (i.e., the DCF) in a population of U.S. women of child-bearing age was then used to define a reasonable uncertainty factor for pharmacokinetic variability which could be applied consistently in both of the guidelines.

2. METHODS

2.1 PBPK Model

The structure of the PBPK model of MeHg in the human used in this analysis (Figure 1) has been described previously.⁽⁷⁾

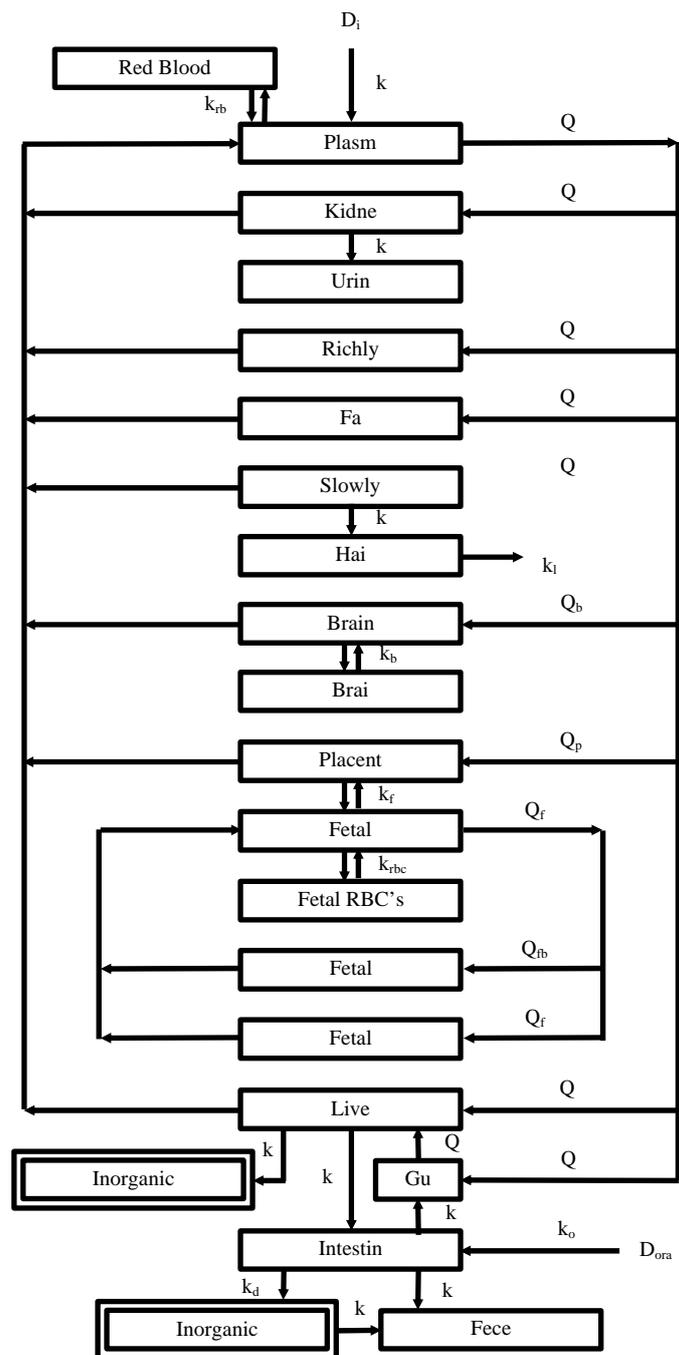


Figure 1. Physiologically based pharmacokinetic model for MeHg used in this analysis. Abbreviations are defined in Table I.

For the present study, the model was re-parameterized specifically for U.S. women of child-bearing age. Enterohepatic recirculation of MeHg is described by the excretion of MeHg in the bile (k_b) and its subsequent reabsorption into the gut tissue (k_r). Oral absorption is modeled as zero-order stomach emptying (k_0) followed by intestinal absorption (k_r). The transport of MeHg and its conversion to inorganic mercury (k_i) in the model is described by linear processes. Distribution in the blood is assumed to be plasma-flow limited, with the exception of transport across the placenta (k_{fe}), blood-brain barrier (k_{br}), and red cell membranes (k_{rbc} and k_{rbcf}), which are considered to be diffusion limited. The most important excretion mechanisms for mercury are excretion in hair (k_h) and conversion of MeHg to inorganic mercury by the gut flora (k_d), with subsequent excretion of inorganic mercury in the feces (k_f). Urinary excretion (k_u) only becomes important at the higher experimental doses used in animals, in which cases renal damage often occurs. Following the approach of Farris et al.,⁽⁸⁾ loss of hair (k_l) and (in the case of rodents) re-ingestion of hair by preening (k_{lr}) are also described.

The fetal portion of the model consists of four compartments which grow during the time of gestation: plasma, RBCs, brain and the remaining fetal tissue. Increases in maternal tissue during pregnancy are also described in the case of plasma, RBCs, richly perfused tissues (representing changes in the uterus and mammary glands), fat and fluid. The time-course for these physiological changes during pregnancy and gestation was taken from Hytten and Leitch.⁽⁹⁾ Maternal dietary intake in the model also increases over the course of pregnancy, based on data for U.S. women.⁽⁹⁾ The pre-pregnancy tissue-volumes (V 's) and blood-flows (Q 's) in the model are standard values taken from Brown et al.⁽¹⁰⁾ and ICRP,⁽¹¹⁾ while the tissue/blood partition coefficients (P 's) were based on tissue mercury data from Berlin et al.,⁽¹²⁾ Kitamura et al.,⁽¹³⁾ Kawasaki et al.,⁽¹⁴⁾ and Vahter et al.⁽¹⁵⁾ Tissue volumes are scaled in the model in proportion to body weight, while blood flows and kinetic parameters (in the form of clearances) are scaled in proportion to body weight raised to the three-quarters power.⁽¹⁶⁾ The kinetic parameters in the model were estimated either from the physiological literature or by simultaneously fitting data from a number of MeHg pharmacokinetic studies for a variety of dosing scenarios in both monkeys,⁽¹⁷⁻²³⁾ and humans.⁽²⁴⁻²⁸⁾

The resulting model is able to accurately describe both the uptake and clearance of MeHg in hair and blood for human volunteers ingesting various diets of MeHg in fish.⁽⁷⁾ For the analysis described here, the model was run at a constant daily dietary intake of MeHg (1 $\mu\text{g}/\text{kg}/\text{day}$) until steady-state was achieved in all maternal tissues. At this point (600 days into the exposure) the pregnancy was initiated and the dosing was continued until conception, at which time the average and peak maternal hair concentrations during pregnancy were calculated.

2.2 Monte Carlo

In order to provide an estimate of the distribution of ingestion rates in a population that could be associated with a given hair level, probability distributions for each of the model parameters were determined from the literature and used in a Monte Carlo analysis to generate a distribution of DCFs.

The parameter distributions used in the Monte Carlo analysis, expressed as means and coefficients of variation (CV's, where $CV = \text{std. dev.}/\text{mean}$), are defined in Table I. In most cases, the means of the distributions are the parameter values identified during the development and validation of the model described above. The exceptions are those for which data more relevant to the specific population of interest (U.S. women of child-bearing age) were available. The distribution of body weights was obtained from the NHANES III database,⁽²⁹⁾ and includes only women of child-bearing age (14–45 years, inclusive) in the U.S. Data from the human physiological literature was used to estimate variability for the plasma flows⁽³⁰⁻³⁵⁾ and tissue volumes,^(11,29,36-40) as well as the critical kinetic parameters, e.g., hair excretion rate constant, k_{hi} ,^(11,41-45) and fecal excretion rate constant, k_{fi} .^(11,46) The variability of tissue/blood partition coefficients was estimated from autopsy data.⁽⁴⁷⁾ In the case of the hair/blood partition coefficient, a global distribution was estimated (See Appendix) from data reported in nine independent studies.^(24,48-55) Normal distributions were used for plasma flows and tissue volumes, while lognormal distributions were used for partition coefficients and kinetic parameters. To avoid physiologically implausible values, most distributions were truncated at three standard deviations above and below the mean, and normal distributions were also truncated at 1% of the mean (to avoid negative or zero values). In the case of body weights, the extreme values were obtained directly from the NHANES III database.

To perform the Monte Carlo simulation, the probability distributions for each of the PBPK model parameters were repeatedly sampled, and the PBPK model was run using each chosen set of parameter values. Random sampling was performed with the Latin hypercube method, which provides a thorough coverage of the distributions using fewer iterations than the standard Monte Carlo method. It was found that 1000 iterations were adequate to ensure the reproducibility of the mean and standard deviation of the output distributions as well as the 1st through 99th percentiles. The output of the Monte Carlo simulation was a distribution of hair concentrations (peak and average during pregnancy) associated with an ingestion rate of 1 $\mu\text{g}/\text{kg}/\text{day}$. To obtain the ingestion rate distributions, the output distributions were inverted (to produce a distribution of DCFs in $\mu\text{g}/\text{kg}/\text{day}/\text{ppm}$) and multiplied by the NOAEL hair concentration.

Table I: Parameter Distributions Used in the Monte Carlo Analysis

	Parameters	Mean	CV	Upper Bound	Lower Bound	Distribution
Plasma Flows (fraction of cardiac output)						
QCC	Cardiac output (L/hr scaled by BW ^{3/4})	20.0	0.22	33.2	6.8	Normal
QBrBC	Brain	0.114	0.30	0.217	0.011	Normal
QFC	Fat	0.052	0.30	0.099	0.0052	
QGC	Gut	0.181	0.33	0.360	0.002	Normal
QKC	Kidney	0.175	0.30	0.333	0.018	Normal
QLC	Liver	0.046	0.32	0.090	0.01	Normal
QRC	Richly perfused tissues	0.183	0.30	0.348	0.018	Normal
QSC	Slowly perfused tissues	0.249	0.30	0.473	0.025	Normal
QPIM	Placenta (L/hr scaled by BW ^{3/4})	58.5	0.35	119.9	10.0	Normal
QFeC	Fetal (L/hr scaled by BW ^{3/4})	54.0	0.30	102.6	10.0	Normal
Tissue Volumes (fraction of body weight)						
BW	Body weight (kg)	67.77	0.26	139.9	30.81	Log-Normal
VBrC	Brain	0.02	0.30	0.038	0.002	Normal
VBrBC	Brain plasma	0.007	0.30	0.013	7.0e-4	Normal
VFC	Fat	0.273	0.24	0.47	0.076	
VGC	Gut	0.017	0.15	0.025	0.009	Normal
VHC	Hair	0.002	0.50	0.005	1.0e-4	Normal
VIC	Intestine	0.014	0.30	0.027	0.001	Normal
Tissue Volumes (continued)						

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VKC	Kidney	0.004	0.30	0.008	4.0e-4	Normal
VLC	Liver	0.026	0.25	0.046	0.006	Normal
VPC	Plasma	0.024	0.14	0.058	0.024	Normal
VRBCC	Red blood cells	0.024	0.25	0.046	0.006	Normal
VRC	Richly perfused tissues	0.10	0.30	0.190	0.01	Normal
VSC	Slowly perfused tissues	0.35	0.16	0.52	0.18	Normal
VRem	Remainder (non-perfused)	0.122	0.30	0.23	0.012	Normal
Partition Coefficients for MeHg						
PBr	Brain/blood	3.0	0.30	6.93	1.19	Log-Normal
PBrB	Brain blood/plasma	1.0	0.30	2.31	0.397	Log-Normal
PF	Fat/blood	0.15	0.30	0.347	0.060	
PFe	Fetal plasma/placenta	2.0	0.30	4.62	0.794	Log-Normal
PG	Gut/blood	1.0	0.70	5.45	0.123	Log-Normal
PHB	Hair/blood	248.7	0.70	1361.7	30.4	Log-Normal
PK	Kidney/blood	4.0	0.30	9.24	1.59	Log-Normal
PLiv	Liver/blood	5.0	0.30	11.6	1.99	Log-Normal
PPI	Placenta/blood	2.0	0.30	4.62	0.794	Log-Normal
PRBC	Red blood cell/plasma	12.0	0.30	27.7	4.76	Log-Normal
PRBCFe	RBC/plasma for fetus	14.0	0.30	32.4	5.56	Log-Normal
PR	Richly perfused tissues/blood	1.0	0.30	2.31	0.397	Log-Normal
PS	Slowly perfused tissues/blood	2.0	0.30	4.62	0.794	Log-Normal
Kinetic Parameters (L/hr scaled by BW ^{3/4})						
Kbrici	Incorp. of inorg. Hg in brain	5.0e-5	0			

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Kbrili	Loss of inorg. Hg from brain	0.001	0			
Kbrini	Brain MeHg to inorganic Hg	1.2e-5	0.30	2.77e-5	4.76e-6	Log-Normal
Kbi	Biliary clearance of MeHg	0.0001	0.30	2.31e-4	3.97e-5	Log-Normal
Kbri	Brain uptake	0.01	0.30	0.0231	3.97e-3	Log-Normal
Kdi	MeHg to inorg. Hg in intestine	0.0001	0.30	2.31e-4	3.97e-5	Log-Normal
Kfi	Fecal excretion of	0.0002	0.36	5.36e-4	6.60e-5	Log-Normal
Khi	Excretion into hair	7.0e-6	0.25	1.42e-5	3.25e-6	Log-Normal
Kii	Conversion to inorg. Hg	1.0e-5	0.30	2.31e-5	3.97e-6	Log-Normal
Krbci	RBC/plasma diffusion	1.5	0.30	3.47	0.596	Log-Normal
Kri	Intestinal reabsorption	0.005	0.30	0.012	1.99e-3	Log-Normal
Fetal Kinetic Parameters (L/hr)						
Kfe	Placenta/embryo diffusion	1.0	0.50	3.69	0.217	Log-Normal
Krbcfe	Fetal RBC/plasma diffusion	100.0	0.50	369.0	21.7	Log-Normal

In conjunction with the Monte Carlo analysis, sensitivity analysis was performed by two different methods. First, analytical sensitivity coefficients, defined as the ratio of the percent change in the DCF to the percent change in a model input parameter that produced it, were obtained by varying each of the parameters in turn by 1% and noting the resulting change in the DCF predicted by the PBPK model. Second, analysis of the correlation of the DCF with each of the input parameters was performed on the results of the Monte Carlo analysis. The analytical sensitivity coefficients most accurately represent the functional relationship of the output to the specific inputs under the conditions being modeled. The correlation coefficients, on the other hand, document the impact of interactions between the parameters during the Monte Carlo analysis.

In their Monte Carlo analysis for MeHg, the USEPA⁽¹⁾ considered three correlations between parameters: blood volume with body weight, fraction of MeHg in blood with body weight, and hair/blood partition with elimination half-life. The first and last of these correlations result naturally from the PBPK model's physiological structure, but the second does not. We reviewed the study⁽²⁷⁾ which was cited as evidence for the correlation between the fraction of MeHg in blood and body weight in the USEPA⁽¹⁾ analysis. It appears that the observed correlation actually reflects a higher ratio of men to women in the groups with the larger average body weights. Men have relatively less fat per kilogram body weight than women, and fat has a much lower partition for MeHg than the other tissues. Thus the negative correlation between fraction of MeHg in the blood and body weight observed by the USEPA⁽¹⁾ can be understood physiologically as a positive correlation between fraction of MeHg in blood and fraction of fat in the body. Therefore, the correlation of fat content and body weight in adult females⁽⁵⁶⁾ was included in the Monte Carlo analysis. There was no evidence that any of the other key (high sensitivity) input parameters in the PBPK model were significantly correlated. Of course, the structure of the model itself provides many physiological constraints on the parameters (e.g., sum of tissue blood flows equal to cardiac output, tissue/plasma partitions related to tissue/blood partitions by the hematocrit, etc.). A more complete description of the derivation of the parameter distributions and the details of the Monte Carlo analyses is available from the authors.⁽⁵⁷⁾

3. RESULTS

To validate the selection of parameter distributions for the Monte Carlo analysis, the distribution of biological half-lives of MeHg predicted by the model was compared with half-lives published in the literature. Reported half-lives from four studies of controlled exposures to MeHg^(25,27,28,49) ranged from 32 to 70 days, with a pooled mean of approximately 49 days (S.D. 7.45), while half-lives obtained from patients during the Iraqi grain poisoning incident⁽⁵⁸⁾ averaged 72 days (S.D. 27.9). In the study conducted by Sherlock et al.,⁽²⁷⁾ the mean half-life in males was 49.7 days (S.D. 7.47; n=14), while the mean half-life for women was 54.2 days (S.D. 3.62; n=6). The distribution of half-lives for women of child-bearing age output by the PBPK Monte Carlo analysis had a mean of 61

days, with a standard deviation of 35 days. Thus the central tendency of the distribution for this model output is reasonably consistent with observations, but the Monte Carlo analysis tends to somewhat overestimate the variability observed in most studies.

The result of the Monte Carlo analysis of hair concentrations is shown in Figure 2, which portrays the distribution of ingestion rates associated with the BMDL of 11 ppm MeHg in maternal hair calculated by USEPA from the Iraqi study.

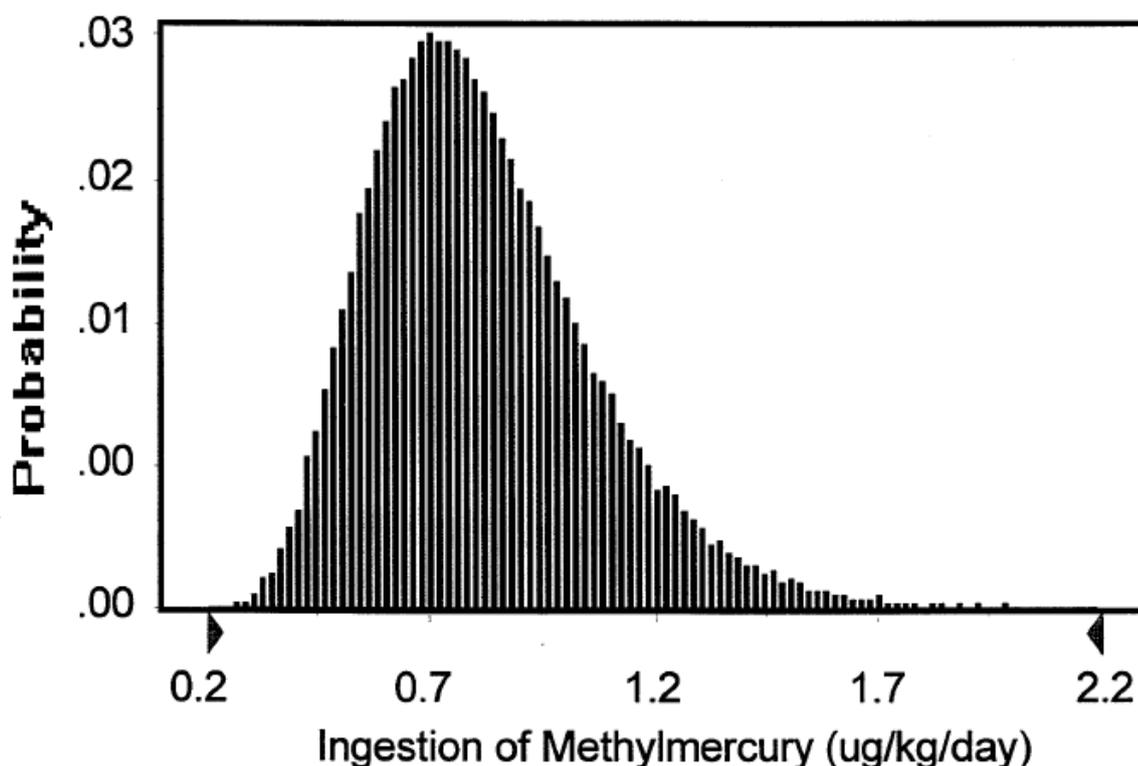


Figure 2. Distribution of daily ingestion rates in the U.S. population of women of child-bearing age associated with the NOAEL for MeHg in hair derived by USEPA from the study of the Iraqi poisoning incident.

The geometric mean (GM) for the distribution of daily ingestion rates in this case was 0.84 $\mu\text{g}/\text{kg}/\text{day}$ with a geometric standard deviation (GSD) of 1.33, and the percentiles for the daily ingestion rate are shown in Table II. The similar distribution of dietary MeHg ingestion rates corresponding to the hair mercury concentration identified by ATSDR as the NOAEL for the Seychelles study, 5.9 ppm, is shown in Table III. The GM and GSD for this distribution are 0.45 $\mu\text{g}/\text{kg}/\text{day}$ and 1.33. In both cases, the ratio of the 5th percentile of the distribution to the median is approximately 1.5, while the ratio of the 1st percentile to the median is approximately 1.8.

Table II

Percentiles in the Distribution of Ingestion Rates of MeHg Associated with the NOAEL in the Iraqi Population Identified by Benchmark Dose Modeling (11 ppm)

<u>Percentile</u>	<u>($\mu\text{g}/\text{kg}/\text{day}$)</u>
1%	0.45
5%	0.54
10%	0.60
25%	0.69
50%	0.83
75%	1.00
90%	1.20
95%	1.36
99%	1.73

Table III

Percentiles of the Distribution of Ingestion Rates of MeHg Associated with the Median Hair Concentration in the Seychelles Population (5.9 ppm)

<u>Percentile</u>	<u>($\mu\text{g}/\text{kg}/\text{day}$)</u>
1%	0.24
5%	0.29
10%	0.32
25%	0.37
50%	0.44
75%	0.53
90%	0.65
95%	0.73
99%	0.93

Several additional Monte Carlo analyses were performed to investigate the sensitivity of the resulting distribution to the approach used for the analysis. In the first alternative case, the explicit treatment of the correlation between the fractional fat volume and body weight was removed. In the second alternative case, only the seven parameters with the greatest sensitivities were varied, and all the rest of the parameters in the model were fixed at their preferred (mean) values. In the third alternative case, all of the parameter distributions were changed to lognormal instead of the mix of normal and lognormal shown in Table

I. In all three cases, the resulting mean, standard distribution, and 5th through 90th percentiles in the distribution of ingestion rates were within 1% of the values obtained in the primary analysis. Somewhat greater differences were observed in the 1st percentile as well as in the 95th and 99th percentiles of the distribution.

The results of the sensitivity analyses are shown in Table IV. The parameters identified as most significant for relating dietary ingestion to hair concentration by the analytical sensitivity analysis were the hair excretion rate constant (khi), hair/blood partition coefficient (PHB), the body weight (BW), gut tissue/blood partition coefficient (PG), fecal excretion rate constant (kfi), fractional fat volume (VFC) and fractional slowly perfused tissue volume (VSC). The same parameters were also identified as significant by correlation analysis, with the exception of VSC. The most sensitive parameters for predictions of half-life were similar to those for hair concentration, with the addition of the partition coefficient for slowly perfused tissue/blood.

Table IV

Parameter Sensitivity*

<u>Parameter</u>	<u>Analytical Sensitivity Coefficient</u>	<u>Pearson Correlation Coefficient</u>
BWF	0.24	0.19
kfi	-0.13	-0.23
khi	-0.77	-0.66
PG	-0.13	-0.32
PHB	0.22	0.42
VFC	0.08	0.15
VPC	0.02	-0.13
VRBCC	0.02	-0.13
VRemain	0.03	-0.13
VSC	0.09	0.01

* Parameters were only included in this table if they had an analytical sensitivity coefficient or Pearson correlation coefficient greater than 0.1 in absolute value

4. DISCUSSION

4.1 Variability versus Uncertainty

In performing a Monte Carlo analysis it is important to distinguish uncertainty from variability. As it relates to the impact of pharmacokinetics in risk assessment, uncertainty can be defined as the possible error in estimating

the “true” value of a parameter for a representative (“average”) person. Variability, on the other hand, should only be considered to represent true interindividual differences. Understood in these terms, uncertainty is a defect (lack of certainty) which can typically be reduced by experimentation, and variability is a fact of life which must be considered regardless of the risk assessment methodology used. Unfortunately, in practice it is often difficult to differentiate the contribution of variability and uncertainty to the observed variation in the reported measurements of a particular parameter.⁽⁵⁹⁾ The parameter distributions used in the Monte Carlo analysis described here were chosen to represent interindividual variability; however, where there was doubt regarding whether differences between studies represented experimental uncertainty or population variability the conservative position was taken that the differences should be assumed to reflect interindividual variability.

For example, it is likely that the study-to-study differences in the means of reported evaluations of the hair/blood partition coefficient for MeHg reflect experimental bias due to differences in the analytical methodologies used rather than to real differences in the populations. If the differences in reported means were indeed due to experimental bias, the study means could have been combined in an unweighted fashion, and a coefficient of variation could have been separately determined from one or more of the larger studies. A narrower distribution would have been obtained if the studies were combined in this way. However, the assumption of experimental bias cannot be supported by comparison data, so the hair/blood partition distribution was calculated assuming all of the reported measurements represented estimates of a single global distribution.

The large standard deviation produced by the Monte Carlo analysis for half-life probably reflects the fact that the distributions calculated for the input parameters do indeed reflect not only interindividual variability, but also uncertainty regarding the true value of the parameters. The performance of the PBPK model with respect to half-life is a reliable indicator of its ability to estimate the variability in the DCF, because the sensitivities of these two outputs (half-life and hair concentration) to the various input parameters were very similar. Thus the result for half-life provides some assurance that the variability of ingestion rates predicted by the Monte Carlo analysis would also provide a conservative (broad) estimate.

4.2 Comparison with Results from Empirical, One-Compartment Models

The variability in ingestion rates predicted by this PBPK analysis is comparable to the results of two previous compartmental analyses.^(1,6) In the PBPK analysis, the ratio of the ingestion rate at the 5th percentile in the distribution to the median ingestion rate is 0.66. The same ratio in the compartmental analyses is approximately 0.5. The agreement of these three analyses is encouraging since they not only reflect different choices for the data underlying the distributions of common parameters (hair/blood partition

coefficient, body weight), but also are to a large extent based on different types of parameters. For example, one of the important input parameters in the compartmental models is the apparent half-life for MeHg in the blood. In the case of the PBPK model, on the other hand, the half-life is actually one of the outputs of the model, and is predicted on the basis of the more fundamental physiological, partitioning, and kinetic parameters.

The somewhat greater variability predicted by the empirical one-compartment analyses probably results primarily from the inability of the empirical approach to represent the functional relationships between parameters. In the analysis performed by Stern⁽⁶⁾ only one relationship was described explicitly: blood volume with body weight, while in the USEPA⁽¹⁾ analysis three correlations between parameters were considered: blood volume with body weight, fraction of MeHg in blood with body weight, and hair/blood partition with elimination half-life. In the PBPK model each of these relationships, along with many others, is defined functionally within the structure of the model and interacts with the parameter selections during the Monte Carlo analysis. For example, the USEPA⁽¹⁾ estimated a correlation of -0.5 for hair/blood partition with half-life; the correlation observed in the PBPK Monte Carlo analysis was -0.66. In the case of the PBPK analysis, however, the variability in the half-life was not used as one of the inputs to determine the variability of DCFs for hair, rather it was an output predicted in parallel with the DCFs.

The use of a PBPK model in place of an empirical compartmental description in an analysis of variability provides several benefits. The principal benefit is the structural framework the model provides which defines the functional relationship between the physiological, chemical, and pharmacokinetic factors determining the uptake, disposition, and clearance of MeHg in an individual. In the empirical approach it is necessary to combine parameters which are primary determinants of kinetic behavior, such as body weight and hair/blood partition, with parameters which are empirical measures of the kinetics resulting, in part, from these primary determinants, such as the fraction of MeHg body burden in the blood and the half-life for its excretion. In this case, the latter two parameters reflect the results of complex underlying processes, and are functionally dependent on the former two parameters. However, in the compartmental description any functional relationship between the parameters must be determined empirically, an approach which is often hindered by the lack of adequate data.⁽¹⁾

While an empirical compartmental analysis provides a useful means for summarizing and generalizing kinetic information, its use in extrapolation or uncertainty/variability analysis must be carefully considered. An example of the potential shortcomings of an empirical modeling approach is the importance in the one-compartment models of the blood volume. In contrast, in the PBPK model there is very little sensitivity to the plasma and RBC volumes. Indeed, there is no biological reason to expect a significant dependence of MeHg pharmacokinetics on the volume of the blood. The appearance of blood volume

in the equation for the one-compartment description is an artifact of the simplified model structure. The basic description of the one-compartment model is:

$$\frac{VdC}{dt} = d * BW * A * f - b * V * C$$

where,

- or:
- C = concentration of MeHg in blood ($\mu\text{g/L}$)
 - C = concentration in hair divided by hair/blood partition (P_{HB})
 - d = daily dietary intake ($\mu\text{g MeHg/kg/day}$)
 - BW = body weight (kg)
 - A = absorption factor (unitless)
 - f = fraction of daily intake taken up by the blood (unitless)
 - b = elimination constant (days⁻¹)
 - V = volume of blood in the body (L)

At steady state, $dC/dt = 0$ and the equation used by USEPA and ATSDR can be derived. Note that, in this description, the role of the blood volume is to calculate an apparent extrinsic clearance ($b * V$). From a biological viewpoint, it is this clearance (fecal, hair, etc.) which varies between individuals, and the separation into half-life and blood volume components is an analytical convenience. While this simplification makes no difference in terms of capturing steady-state behavior, it unfortunately imputes an unwarranted influence to a physiological factor (blood volume) which in itself is not actually an important determinant of MeHg kinetics.

5. CONCLUSIONS

On the basis of the results of this analysis, the use of an uncertainty factor of 3 for pharmacokinetic variability in the USEPA RfD appears to be more than is necessary. The 1st and 5th percentiles of the distribution of DCFs calculated in this analysis were a factor of 1.8 and 1.5 below the median, respectively. The other factor of 3, for database limitations, constitutes a judgment which specifically addresses limitations in the Iraqi study, and is not effected by this analysis. In deriving the MRL, on the other hand, ATSDR did not apply any uncertainty factor. Indeed, it can reasonably be assumed that the impact of pharmacokinetic variability on the dose-response relationship within an exposed population is adequately reflected in the results of an epidemiological study on a large population such as the Seychelles study cohort. Moreover, many of the concerns regarding limitations in the Iraqi study are addressed by the multigenerational nature of the exposure in the Seychelles and the prospective study design. However, the impact of pharmacokinetic variability in the U.S. population of women of child-bearing age must still be considered, since in deriving the MRL it was necessary to use a pharmacokinetic model to calculate an ingestion rate associated with the hair concentration derived from the epidemiological cohort.

The results of the present analysis of pharmacokinetic variability can be used to provide a consistent adjustment for pharmacokinetic variability in these two guidelines, by selecting an ingestion rate which would be associated with a hair concentration equal to or lower than the desired level for 95% of the population, rather than for an average person. Specifically, if the ratio of the 5th and 50th percentiles (1.5) is used in both cases as a measure of the pharmacokinetic variability in an ingestion rate for U.S. women of child-bearing age associated with a hair mercury concentration derived from an epidemiological study, and if the USEPA's uncertainty factor of 3 for database inadequacies is retained in the case of the RfD, the result would be an RfD of 0.2 $\mu\text{g}/\text{kg}/\text{day}$ and an MRL of 0.3 $\mu\text{g}/\text{kg}/\text{day}$. Derived in this way, both guidelines would represent reasonable estimates of an acceptable daily MeHg ingestion rate in a population of U.S. women of child-bearing age.

The sensitivity analyses performed in this study suggest that the most important determinants of pharmacokinetic variability for MeHg are the hair/blood partition, body weight, and hair growth rate. The first two parameters have been the subject of much greater attention than the third. The hair growth rate used in the PBPK model is in units of liters per hour. Thus it represents a composite of linear hair growth rate, hair diameter, hair follicle density, and body surface area covered with hair. Of course, the values of these components vary not only between individuals but also between different areas of skin on the same individual. More data is needed on the potential for racial or ethnic differences in both hair parameters, since they could lead to significantly different pharmacokinetic susceptibility to the effects of MeHg across populations.

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Appendix Calculation of Global Distribution for Hair-to-Blood Ratios

A global mean and standard deviation for the MeHg hair to blood ratio were computed from nine independent studies.^(24,48-55) In one case,⁽⁵³⁾ the hair to blood ratio of MeHg was defined by the sample size n , the estimated mean \bar{x} , and the estimated standard deviation, s . However, in most of the studies only the linear coefficient, sample size and the standard error from a regression analysis of the blood concentrations of MeHg to the hair concentrations of MeHg were available. In a few cases, the correlation coefficient R or its square was given instead of the standard error. In the cases where the linear regression coefficient, sample size and either the standard error or the correlation coefficient were given, the mean and standard deviation of the hair to blood ratio were determined based on the formulas given below.

Given the following definition of the regression equation:

$$\text{HairConc} = h$$

$$\text{Bloodconc} = b$$

$$h = a \times b + e$$

then \hat{a} is the estimate of a , n is the sample size and $se(\hat{a})$ is the standard error. When R or R^2 was given instead of the standard error, $se(\hat{a})$ was determined as

$$se(\hat{a}) = \frac{\hat{a} \times \left(\frac{1}{R^2} - 1 \right)}{(n - 2)}$$

From this, we determined the following:

$$\hat{a} = \frac{\sum b_i \times h_i}{\sum b_i^2} \approx \text{mean of } \frac{h}{b} = \bar{x}$$

$$\text{Var } h = \sigma^2 = \text{Var } e^2$$

$$\text{Var } a = \frac{\sigma^2}{\sum b_i^2}$$

$$\text{Var} \left(\frac{h}{b} \right) = \frac{\sigma^2}{b^2}$$

Then the standard deviation of h/b , the hair to blood ratio, is computed from

$$\begin{aligned} \text{Var} \left(\frac{h}{b} \right) &= E \left(\text{Var} \left(\frac{h}{b} \right) \right) + \text{Var} \left(E \left(\frac{h}{b} \right) \right) \\ &= \sigma^2 \times E \left(\frac{1}{b^2} \right) + 0 \\ &= \sigma^2 \times E \left(\frac{1}{b^2} \right) \end{aligned}$$

If we use the approximation

$$E \left(\frac{1}{b^2} \right) \approx \frac{n}{\sum (b_i)^2}$$

then

$$\text{Var} \left(\frac{h}{b} \right) = n \times \text{Var } \hat{a}$$

$$s \text{ of } \left(\frac{h}{b} \right) = \sqrt{n} \times se(\hat{a})$$

Once the means and standard deviations were computed for all the studies, the means and standard deviations were combined into a global mean and standard deviation using the equations

$$\text{Var } a = \frac{\sigma^2}{\sum b_i^2}$$

and

$$\text{Var} \left(\frac{h}{b} \right) = \frac{\sigma^2}{b^2}$$

then the standard deviation of h/b, the hair to blood ratio, is computed from

$$\begin{aligned} \text{Var} \left(\frac{h}{b} \right) &= E \left(\text{Var} \left(\frac{h}{b} \right) \right) + \text{Var} \left(E \left(\frac{h}{b} \right) \right) \\ &= \sigma^2 \times E \left(\frac{1}{b^2} \right) + 0 \\ &= \sigma^2 \times E \left(\frac{1}{b^2} \right) \end{aligned}$$

If we use the approximation

$$E \left(\frac{1}{b^2} \right) \approx \frac{n}{\sum (b_i)^2}$$

then

$$\text{Var} \left(\frac{h}{b} \right) = n \times \text{Var } \hat{a}$$

and

$$s \text{ of } \left(\frac{h}{b} \right) = \sqrt{n} \times se(\hat{a})$$

Once the means and standard deviations were computed for all the studies, the means and standard deviations were combined into a global mean and standard deviation as follows.

Given n_i , \bar{x}_i , and s_i^2 for each study i

where

$$s_i^2 = \frac{1}{n_i - 1} \sum_j (x_{ij} - \bar{x}_i)^2$$

and

$$N = \sum_i n_i$$

then

$$\text{Global mean} = \bar{x} = \frac{1}{N} \sum_i \sum_j x_{ij} = \frac{1}{N} \sum_i (n_i \bar{x}_i)$$

$$\begin{aligned} \text{Global } s^2 &= \frac{1}{N - 1} \sum_i \sum_j (x_{ij} - \bar{x}_i + \bar{x}_i - \bar{x})^2 \\ &= \frac{1}{N - 1} \sum_i \left(\sum_j [(x_{ij} - \bar{x}_i)^2 + n_i (\bar{x}_i - \bar{x})^2] \right) \\ &= \frac{1}{N - 1} \left(\sum_i (n_i - 1) s_i^2 + \sum_i n_i (\bar{x}_i - \bar{x})^2 \right) \end{aligned}$$

Chapter 6

Review and Evaluation of the Potential Impact of Age- and Gender-Specific Pharmacokinetic Differences on Tissue Dosimetry

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Abstract:

In standard risk assessment methods for carcinogenic or noncarcinogenic chemicals, quantitative methods for evaluating interindividual variability are not explicitly considered. These differences are currently considered by the use of statistical confidence limits or default uncertainty factors. This investigation consisted of multiple tasks aimed at making quantitative predictions of interindividual differences in susceptibility by using physiologically based pharmacokinetic (PBPK) models. Initially, a systematic, comprehensive review of the literature was conducted to identify any quantitative information related to gender- or age-specific physiological and biochemical factors that could influence susceptibility to chemical exposure. These data were then organized from a pharmacokinetic perspective by process and by chemical class to identify key factors likely to have a significant impact on susceptibility as it relates to internal target tissue dose. Overall, a large number of age- and gender-specific quantitative differences in pharmacokinetic parameters were identified. The majority of these differences were identified between neonates/children and adults, with fewer differences identified between young adults and the elderly. The next phase of this work consists of using PBPK models to develop examples of approaches through the development of case studies. The goal of the case studies is to continue to develop a methodology that incorporates PBPK modeling to assess the likelihood that a chemical or class of chemicals may present an age- or gender-specific risk. The case studies should also demonstrate practical methods for quantitatively incorporating information on age- and gender-specific pharmacokinetic differences in risk assessments for chemicals.

1. INTRODUCTION**1.1 The Role of Tissue Dosimetry in Risk Assessment**

The biological basis of interindividual variability is not explicitly considered in standard carcinogen risk assessments. Rather, statistical confidence limits are typically applied to account for this and other uncertainties inherent in the use of animal bioassay data or high dose occupational data. Noncancer hazard assessments usually apply a default uncertainty factor of 10 to account for interindividual variability. While it may be possible to distinguish specific groups of individuals, such as infants or the elderly, who appear to be more susceptible to a chemical's potential toxicity, numerous factors contribute to this variability within a defined population (interindividual) and among subgroups within a larger population (intraindividual). These factors may be placed into two broad categories: those that influence the target tissue dose for a given external exposure (pharmacokinetic factors) and those that influence the target tissue response at a given target tissue dose (pharmacodynamic factors).

In recent years, there has been an increasing use of pharmacokinetic data for a chemical or class of chemicals in risk assessment. In particular, physiologically-based pharmacokinetic (PBPK) models have been applied in chemical risk assessments to help make key pharmacokinetic factors more

explicit, and provide a means for estimating the significance of these factors in the final risk estimates. Use of these data provides a stronger biological basis for extrapolation across species or dosing patterns (from high to low dose, across temporal dosing patterns or routes of exposure) and provides estimates of the relevant tissue dose for the target population. However, because of variability in populations and subgroups within populations, tissue doses may vary even at the same exposure level. PBPK modeling has the capability to quantitatively describe the potential impact of the pharmacokinetic aspects of this variability in individual susceptibility. Specifically, a PBPK model can provide a quantitative structure for determining the effect of various age- and gender-specific factors in the relationship between the external (environmental) exposure and the internal (biologically effective) target tissue exposure. For example, PBPK models can be used to determine the impact of differences in key metabolic enzymes not only due to normal variation in enzyme activities within the general population, but also due to differences in metabolism between males and females and across age groups. The focus of this investigation is to identify those pharmacokinetic factors that may be used quantitatively to assess intraindividual variability across lifestages (infant, child, adult, elderly) and between genders.

1.2 General Description of Age- and Gender-Specific Differences in Physiology and Metabolism with Potential to Impact Tissue Dosimetry

The many physiological and biochemical determinants of the concentration time course (pharmacokinetic) of a chemical can be broadly organized into four secondary processes, absorption, distribution, metabolism and elimination, collectively referred to as ADME. Each of these secondary processes results from one or more primary processes that correspond to actual physiological or biochemical elements. Elimination, for example, is a secondary process that is comprised of fecal excretion, metabolism, glomerular filtration, tubular secretion and pulmonary exhalation. These processes are dynamic, in some cases changing significantly during development, maturation, and aging in humans. General changes in body weight (Figures 1 and 2) and corresponding changes in organ volumes, particularly the fat composition, occur throughout the human lifespan. For example, glomerular filtration rates (GFRs) are relatively low in human newborns, as is hepatic metabolism by the cytochrome P450, but both rise rapidly after birth, reaching adult levels after several months. The capacities of some enzyme systems involved in xenobiotic metabolism decline during old age, as does renal elimination. Changes in lung morphology also occur during development and maturation. The dynamic, developmentally related state of these processes implies that there is a uniqueness to each lifestage with respect to its exposure-tissue dose relationship. This applies equally to gender differences, where such differences can also be significant.

While the pharmaceutical sciences have begun to develop chemical- and lifestage-specific data useful for characterizing and assigning significance to the effects of age and gender on pharmacokinetic processes, risk assessment for environmental xenobiotics has not.

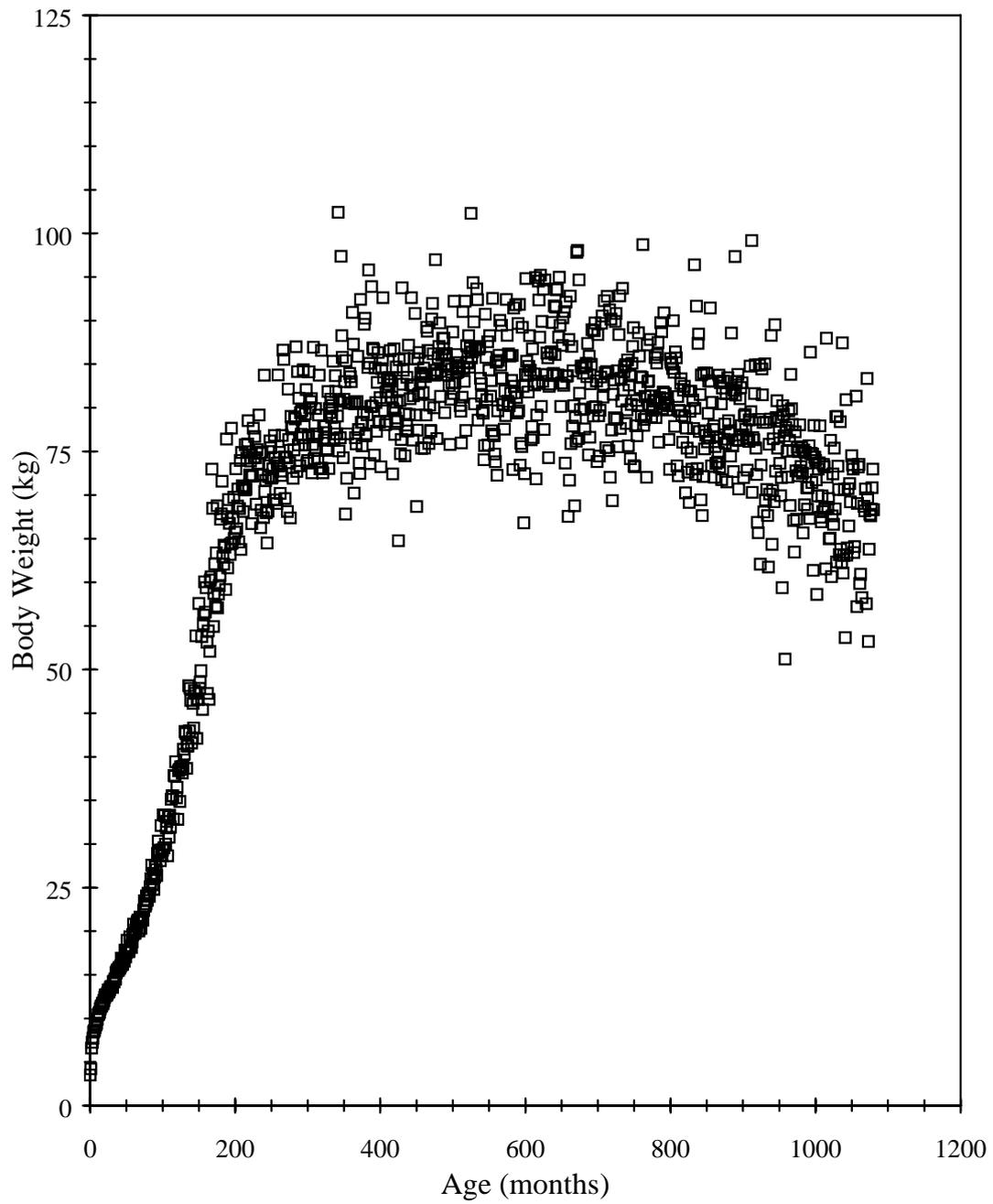


Figure 1. Male body weight data from NHANES data.

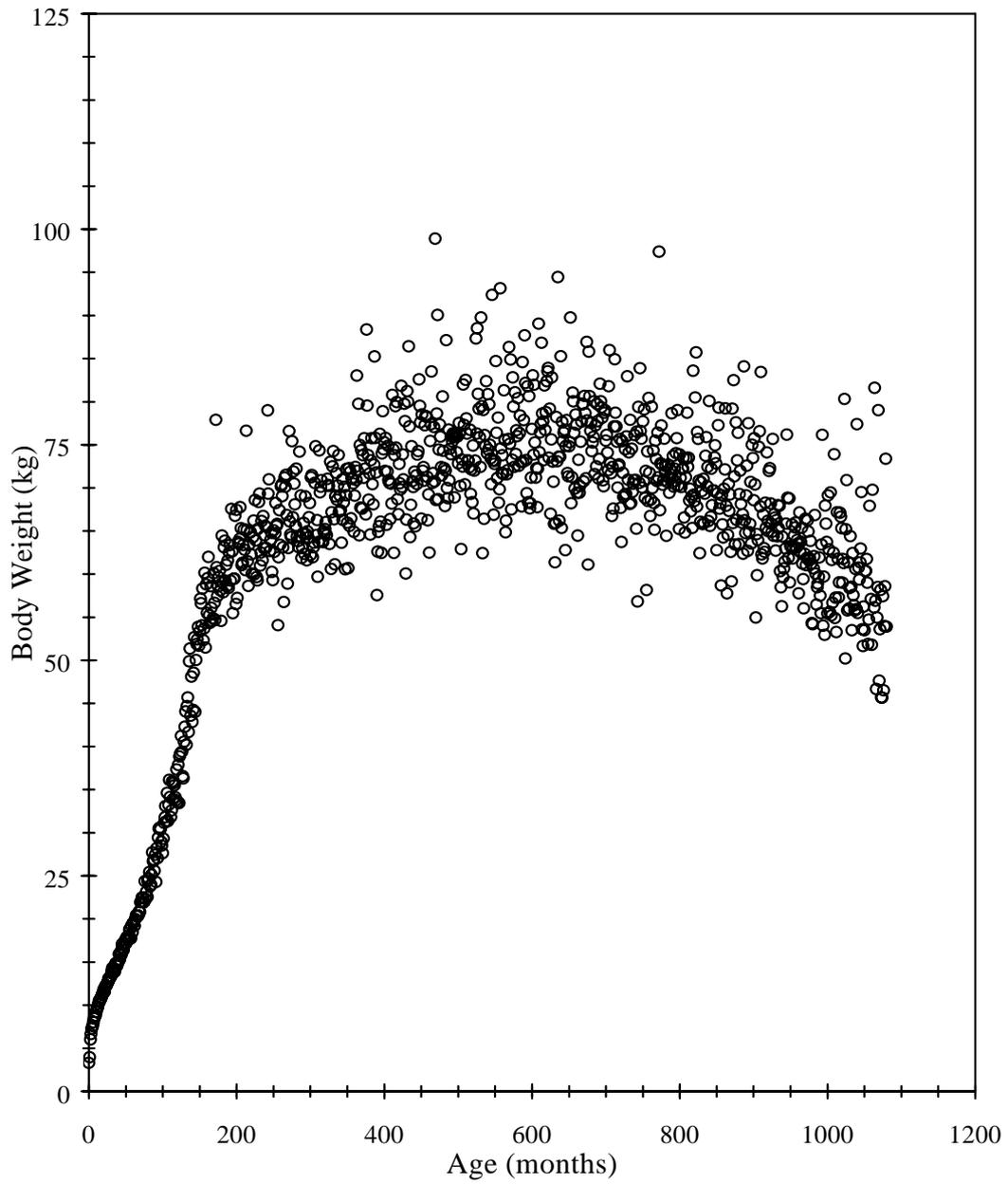


Figure 2. Female body weight data from NHANES data.

Since the development of PBPK models is strongly influenced by *a priori* knowledge regarding the significance of the various pharmacokinetic processes that determine the tissue concentrations of a chemical of interest, there is significant value in developing tools to broadly define, for chemical classes, the important processes at various lifestages.

1.3 Purpose and Objectives

The purpose of the analyses described in this report is to perform a comprehensive review of the literature to determine the information available in humans to support the use of PBPK modeling to address the impact of age- and gender-specific physiological, biochemical, and pharmacokinetic differences in individual risk. One objective is to organize, from a pharmacokinetic perspective, the key factors that are likely to have a significant impact on susceptibility, as it relates to estimates of target tissue concentrations. A second objective is to develop a predictive pharmacokinetic framework that can be used to characterize the effect of age and gender differences on tissue dosimetry for a chemical or class of chemicals. A major objective would be to use the available data in combination with a PBPK model to develop examples of approaches that could provide data-derived uncertainty factors to be used in place of default values in risk assessment.

1.4 Technical Approach

The data needs for this analysis were extensive, comprising two levels of organization: summary descriptions of age and gender differences and primary literature providing specific quantitative differences in relevant physiological parameters and processes. A systematic, comprehensive literature survey was conducted using available electronic databases for journals and reference books. On occasion, principle investigators were contacted directly.

The retrieved literature was reviewed to determine: 1) which pharmacokinetic or primary process or physiological parameters give rise to lifestage or gender-specific differences in tissue dosimetry; 2) chemical-specific examples of these effects; and 3) quantitative data sufficient to support additional analyses. The literature was grouped for review into one of two major categories, physiological parameters that can impact pharmacokinetics (i.e., body weight, ventilation rate) or data on pharmacokinetic processes. The pharmacokinetic processes category was divided into the four classic subcategories of absorption (A), distribution (D), metabolism (M), and elimination (E). These four subcategories were further divided by chemical class, chemical name, enzyme system or a specific process (e.g., glomerular filtration). The relevant quantitative and qualitative information on age- and gender-specific differences in pharmacokinetics, as well as the age- and gender-related information on physiological parameters, was then placed in a tabular matrix using the relevant key words for rapid organization. Table 1 presents the classifications used to characterize the physicochemical characteristics of the compounds that are a part of this analysis.

Table 1
Classification of Chemicals by Their Physicochemical Properties

Class	Number of Chemicals in Matrix	Typical Example
A. Volatile: v.p. > 1 mm-Hg 1. Reactive (lung only) 2. Water soluble: $K_{ow} < 1$ 3. Lipophilic: $K_{ow} > 1$	0 2 6	Formaldehyde Ethanol Chloroform
B. Nonvolatile: v.p. < 1 mm-Hg 1. Water soluble a. Hydrophilic: $K_{ow} < 1$ b. Acidic: $pK_a < 5$ c. Basic: $pK_a > 9$ 2. Lipophilic: $K_{ow} > 1$ a. Moderately: $K_{ow} < 4$ b. Highly: $K_{ow} > 4$	19 6 3 32 4	Methotrexate Benzoic acid Caffeine Phenobarbital Estradiol
C. Insoluble (lung only)	0	Carbon dust

These data were organized from a pharmacokinetic perspective and the key factors that are likely to have significant impact on susceptibility as it relates to estimates of dose metrics or target tissue concentrations were identified and evaluated. A predictive pharmacokinetic framework was then developed, based on the available data, which can be used to characterize the effect of age and gender differences on tissue dosimetry for a chemical or class of chemicals. In addition, this manner of organizing the data resulted in the identification of physicochemical or biochemical data that would be needed to place a chemical within the predictive framework. It also allowed for identification of chemicals for which adequate data were available to conduct further quantitative analysis of the impact of age- and gender-specific differences in pharmacokinetics on risk assessment.

As described in this report, the effect of age/gender on pharmacokinetics is relatively well characterized for pharmaceuticals, but less well characterized for xenobiotics. Pharmaceutical pharmacokinetics generally focus on aggregate properties, such as volume of distribution and whole body clearance, while PBPK modeling is typically more concerned with primary processes, such as tissue partitioning and metabolism. The results of the literature searches reflect this difference. Much of the data focus on secondary processes: clearance (elimination), bioavailability, and measures, such as area under the blood concentration time curve (AUC). There are fewer cases where data are available on primary processes, e.g., absorption, metabolism, and excretion. While it is possible to use aggregate properties in a PBPK model, it is preferable to relate these secondary processes to the physiological and biochemical differences that give rise to them within the quantitative framework developed here. The latter

approach is more informative, and makes it possible to use the predictive power of the PBPK modeling to extrapolate across chemicals. These considerations are reflected in the choice of data sets used throughout the manuscript.

2. SUMMARY AND DEVELOPMENT OF A PREDICTIVE PHARMACOKINETIC FRAMEWORK

Based on our review of the literature, a large number of age- and gender-specific quantitative differences in absorption, distribution, metabolism and elimination were identified. The quantitative differences are summarized in a matrix (Table 2) that can be used to predict age- and gender-related pharmacokinetic differences for a particular class of chemicals (lipophilic, water soluble, etc.). The majority of the age-related differences identified were between neonates/children and adults, with fewer differences reported between young adults and the elderly. With the exception of differences in distribution and P450 metabolism, no gender-specific differences were identified.

Table 2
Summary of Age- and Gender-Dependent Changes

Absorption			
Route of Exposure	Neonate/Child	Elderly	Gender
Oral Lipophilic	↑↓	↑↓	M=F
Water Soluble	↑↓	↑↓	M=F
Inhalation Lipophilic	↑	I	M=F ¹
Water Soluble	↑	I	I
Particulates	↑	I	M=F
Dermal Lipophilic	I	↓	I
Water Soluble	I	↓	I
Distribution			
Compound Characteristics	Neonate/Child	Elderly	Gender
Lipophilic	↑	↑	F↑
Water Soluble	↑	↓	F↓
Protein Binding	↓	↓	↑↓
Metabolism			
Metabolic Pathway	Neonate/Child	Elderly	Gender
Glutathione-S-Transferase	↓	I	I
Sulfotransferase	↑	I	I
Glucuronyl Transferase	↓	I	F≤M
P450	↓	I	M>F
Carboxylesterase	↓	I	I
Alcohol Dehydrogenase	↓	I	I

Elimination			
Compound Characteristics	Neonate/Child	Elderly	Gender
Lipophilic			
Protein binding	I	I	I
Glomerular filtration	↓	↓	M=F
Tubular secretion	↓	↓	I
Tubular re-absorption	I	I	I
Water Soluble			
Protein binding	I	I	I
Glomerular filtration	↓	↓	M=F
Tubular secretion	↓	↓	I
Tubular re-absorption	I	I	I

¹ Data for one compound, methylene chloride

↑ Higher than adult; ↓ lower than adult; ↑↓ increases and decreases demonstrated

I Insufficient data for conclusion

F Female; M=Male

2.1 Absorption

The observed differences in absorption are the net result of the interplay between multiple physiological processes and the physicochemical characteristics of the compound. The size, in particular the surface area, flow rates of blood, air and gastric contents, status of the tissue forming the barrier as well as the biochemistry, such as gastric pH and lung surfactant, can influence rates of absorption⁵⁻⁹. For instance, changes in absorption would be expected to parallel increases or decreases in surface area. In addition, the effect of each of these physiological properties on absorption depends on the physicochemical properties of the ingested compound⁸. Lipophilicity, water solubility, and reactivity of the exogenous chemical have a particularly large impact on absorption.

2.1.1 Oral Absorption

Sufficient data on the absorption of lipophilic and water soluble pharmaceuticals were collected to generalize absorption following oral exposure to these classes of compounds in the neonate and elderly populations. To the extent that T_{max} values reflect differences in rates of absorption, the rate effect of age on absorption was not predictable by chemical class. Compared to adults, increases and decreases in the rate of absorption were reported for both lipophilic and water soluble compounds in neonates and the elderly. While the data generally suggest a lower rate of absorption in elderly versus adult populations, the extent of absorption was the same. This was similarly true for the neonate. A recent review of gender-related differences in pharmacokinetics provides no convincing data that gender differences in gastrointestinal absorption rate constants unrelated to differences in first pass intestinal metabolism exist¹⁰.

2.1.2 Dermal Absorption

Data on the dermal absorption of xenobiotics in neonates and children were not available. The epidermis of the fetus is unkeratinized, becoming fully keratinized 3-5 days after birth. Because the keratinized epidermal layer represents a protective barrier, an increase in dermal absorption during this time frame would be expected¹¹. However, Cunico et al.¹² reported no differences in penetration for infants and adults. The hydration state of the epidermis is also elevated in neonates¹³, suggesting the potential for increased absorption of some compounds.

Data on the dermal absorption of xenobiotics in the elderly, which indicated lower, but not statistically different absorption of lipophilic compounds, conformed with expectations based on reports of lower hydration of the epidermis¹⁴. In general, the available data provide mostly qualitative suggestions of age-dependent differences, but are not sufficient to guide a more quantitative PBPK analysis.

2.1.3 Respiratory Tract Absorption and Deposition

The available literature provided excellent quantitative characterizations of age-dependent changes in pulmonary parameters affecting uptake of volatile compounds and deposition of particulates. Lung surface area, total lung capacity, vital capacity, functional residual capacity, and residual volume all increase in proportion to lung growth. The number of conducting airways is complete at birth, and increase only in size with age¹⁵. However, the size of the pulmonary airways increases from birth to adolescence, primarily by addition of new alveoli^{11, 16}. The resulting surface area for respiratory absorption also increases¹⁷. Age-related differences in ventilation parameters have been reported, with the greatest differences occurring between neonates and adults¹⁸. Morphometric studies have consistently found a decrease in surface area of airspace wall per unit of lung volume beginning in the third decade of life which continues throughout life¹⁹. Total lung capacity does not change significantly with age²⁰. These age-specific differences in characteristics of the respiratory tract lead to age-specific differences in uptake and deposition.

The characterization of age-dependent differences in the pulmonary uptake of anesthetics is indicative of expected behaviors for similar volatile organic xenobiotics. The uptake rate of volatile anesthetics is higher in both infants and children than in adults²¹. A larger alveolar ventilation rate, relative to FRC and body weight, as well as greater perfusion rates and lower fat content, are believed to be responsible for this age-specific difference in pulmonary absorption²¹. To the extent that these data reflect the behavior of water soluble and lipophilic volatile organics, it appears the uptake of both classes of compounds is higher in infants and children. Age-dependent changes in the physiological parameters affecting these lifestage-specific effects can be integrated into a predictive PBPK model to determine the effect of these

differences on uptake and on steady-state blood concentrations for water soluble and lipophilic compounds.

Children also receive a higher particle dose per unit surface area of the lung compared to adults. Direct measurement of vapor deposition has not been conducted to explore age-related effects. However, mathematical models that have been developed to quantitatively describe the effect of postnatal changes in the human lung on dosimetry predict a higher deposited fraction in children compared to adults, for both inhaled reactive vapors and particulates. Reports of differences in pulmonary uptake between elderly populations were not available. Only a single report of gender-related differences in the uptake of volatile organics was identified and does not provide sufficient data to form expectations for gender differences in pulmonary uptake.

The available quantitative data on respiratory tract anatomy and physiology can be integrated into a predictive model structure to provide estimates of age-related differences in the uptake of volatile organic compounds and the deposition of particulates. Detailed morphometric measurements of lung volume, surface area, minute ventilation, breathing frequency, and airway dimensions are available in humans for different age groups (from neonates to adults). Case studies that provide specific quantitative characterization of age-related effects of particulate deposition from the neonatal through adult periods would provide useful insights into expectations for differences in risk in these age groups. In addition, developing a similar quantitative approach for organic vapors would be useful for predicting differences in tissue doses for two important classes of chemicals and for dispelling some currently held beliefs about the relationship between increases in uptake or ventilation rates and steady-state blood concentrations.

2.2 Distribution

Physiological processes and constructs, as well as the physicochemical properties of a chemical, influence distribution of a chemical throughout the body. The extent to which a chemical distributes throughout the body is influenced by five main physiological properties, which include body composition (body water, fat, lean body mass), blood flow, composition and concentrations of plasma binding proteins, tissue-protein concentration, and fluid pH². In addition, plasma lipoprotein concentrations have an impact on the distribution of lipophilic compounds²²⁻²⁴. From the pharmaceutical literature, sufficient data were available to characterize age- and gender-specific effects on the distribution of both water soluble and lipophilic compounds.

The main determinant of differences in volume of distribution is the relative size of three body compartments, total body water, fat and lean mass. In general, the largest age-related differences in distribution occur in the first 10-12 months of life, after which distribution is similar to adults²⁴. Based on a database published by Hattis et al.²⁵, 84% of the compounds with differences in Vd in adults and children had volumes of distribution that were higher in the

neonate compared with the adult. For the majority of these compounds, the volume of distribution was 1.3- to 2.8-fold higher in the neonate. There was no apparent relationship to chemical class. The consistent effect for both lipophilic and hydrophilic compounds is likely attributable to a larger body water compartment and reduced binding to plasma proteins, including lipoproteins. Generally, reduced plasma protein binding relative to adult levels is observed in newborns²⁶. Consistent with the observed decreases in lean body mass in the elderly, changes in the volume of distribution correlated with lipophilicity, with the volumes of distribution of some lipophilic compounds increasing and that of the water soluble compounds decreasing with age. Two modestly lipophilic compounds did not fit this pattern.

Comparing men and women, the observed differences in the Vd of both lipophilic and hydrophilic compounds are consistent with gender-specific differences in lean body mass. The Vd of lipophilic compounds is higher, and that of water soluble compounds lower, in women compared with men. In fact, normalization of the Vd for lean body mass minimizes gender-specific differences in Vd for some chemicals¹⁰. The relationship between body composition, chemical class and Vd was clearest when men and women were compared and weakest when neonates and adults were compared. The relationship in newborns seems confounded by issues of plasma protein binding, but as a function of gender and in the elderly, the relationship is sufficiently clear to be suitable for use in a predictive PBPK framework.

While examples of reduced protein binding in neonates were documented, this effect is chemical- rather than chemical-class-specific and is not suitable for integration into a general predictive framework. Such an approach requires specific knowledge of binding characteristics, protein concentrations and the effect, if any, protein binding has on hepatic and renal clearance.

2.3 Metabolism

For Phase I reactions, the cytochrome P450 family of enzymes is the most abundant and the most important system with regard to xenobiotic metabolism. The overall activity of P450 has been reported to be approximately 50% higher in adults when compared to the fetus or neonate²⁴. In addition, there have been age-related differences in specific P450 isozymes reported. Specifically, the activity of the CYP3A family, the most abundant P450 isozyme in the human liver and a family of isozymes that are involved in the metabolism of numerous xenobiotics, has been reported to be approximately 25-50% lower in newborns^{27, 28}. In addition, the profile of the CYP3A family is different in the fetus and neonate, when compared with the adult, in that CYP3A7 is responsible for up to 85% of the total P450 activity in the fetal liver²⁹. However, within 3-12 months of age, CYP3A7 levels decline to adult levels and CYP3A4, which is not present in the fetal liver, becomes the major P450 isoenzyme in the newborn and adult liver²⁹⁻³¹.

There is conflicting information regarding the decline of CYP3A4 levels with age with one investigator reporting a decrease with age³². However, there is no decrease in the activity of CYP3A4 with age, but there is a significant decrease in liver mass (35%), liver blood flow (35%) and volume (24 to 44%) between adulthood and late old age that could account for the decline in total systemic clearance of CYP3A4 prototype compounds³³. Consequently, these age-related differences in the metabolic capabilities of the fetus/neonate and children/adults should be considered in a predictive framework for xenobiotics that are metabolized by the CYP3A isozymes. Moreover, the changes in liver mass, liver blood flow and volume that occur with advancing age between adulthood and late old age that could account for the decline in total systemic clearance should be considered in a general predictive framework.

There are quantitative age- and gender-specific differences in the activity of several different P450 isozymes and age-related differences in the capacity to form certain conjugates. An increase P450 metabolic activity does not infer a decrease in susceptibility to the toxicity of that chemical. Many chemicals are minimally toxic; however, when acted on by metabolic enzymes, such as the cytochrome p450 enzymes, these chemicals are activated, i.e., the result of metabolism is the formation of toxic metabolites. Consequently, the presence of increased P450 for that particular chemical would potentially make that particular age group or sex a sensitive subpopulation. Therefore, the toxicity of the chemical and/or metabolites should be considered.

Quantitative differences in the levels of esterases in the neonate and in adults have been reported³⁴. For example, the levels of plasma pseudocholinesterase have been reported to be 50-60% that of adults³⁴. This difference would be an important factor in sensitivity to chemicals that are detoxified by these enzymes, such as organophosphorous pesticides.

Gender-related differences in activity have also been reported for the P450 enzymes, CYP1A2^{10, 32, 35, 36}, CYP3A4³², CYP2D6³⁷ and CYP2E1^{28, 37}. CYP1A2 activity has been reported to be greater in males than in females^{10, 32, 35, 36}. One study reported an approximate 1.5-fold decrease in half-life for theophylline, a CYP1A2 substrate, in males when compared to females³⁷. CYP1A2 is involved in the metabolism of polycyclic hydrocarbons, heterocyclic amines and aromatic amines. Consequently, the half-lives of these types of chemicals would be expected to be longer in females than in males. The activities of CYP3A4, CYP2D6 and CYP2E1 have also been reported to be higher in males.

One the most important Phase II pathways, glucuronidation, was reported to be at most 20% of the adult levels in the fetus during the first 4.5 months of gestation³⁸. Thus, at least during early gestation, the ability of the fetus to detoxify xenobiotics by glucuronidation is limited. The fetal liver has been reported to up to 20-fold less glutathione activity than adult livers³⁹⁻⁴¹. Based on studies with acetaminophen, the ability to form glucuronide conjugates is also less in the neonate and young children, when compared with adults. In infants and children 12 years of age or less, approximately 50% of the administered

dose was eliminated as a sulfate conjugate⁴². In adults, approximately 50% of the dose was eliminated as glucuronide conjugates. Thus, the sulfation pathway was more important in children and the glucuronide pathway more prominent in adults. These quantitative differences should be considered when either of these pathways is important in the detoxification of chemicals. The data regarding gender differences in Phase II capacity are mixed with no evident differences in the ability to form conjugates by any of the Phase II pathways identified.

2.4 Elimination

The effects of development and aging on renal function and elimination have been well characterized for lipophilic and water soluble pharmaceuticals. The renal clearance rates of compounds that are GFR-dependent and tubular secretion-dependent are both reduced in the neonate. Renal clearance was lower for all chemical classes, lipophilic and water soluble, including organic ions. The magnitude of reduction was significant, being on the order of 30-50%. These reductions parallel the immaturity of glomerular filtration and tubular secretion systems in the neonate. Reductions in renal clearance that are similar in magnitude are also observed the elderly. In the elderly, reductions in renal clearance parallel age-dependent deficits in renal function. Very complete quantitative data are available that detail the postpartum maturation of GFR and tubular secretion systems, and the rate of decline after age 30. These data, which include rates, are both necessary and sufficient to develop quantitative, age-dependent predictions of the effect of clearance on steady-state blood concentrations across the entire lifespan. While gender effects in renal clearance generally suggest a lower renal clearance in women compared to men, the data on differences in GFR between men and women are not consistent. Quantitative data on gender differences in tubular secretion were not available. The case for supporting gender differences in renal clearance is not as strong as for age-dependent changes.

3. AGE AND GENDER DIFFERENCES IN ADSORPTION, DISTRIBUTION, METABOLISM AND ELIMINATION

3.1 Absorption

In the context of pharmacokinetics, absorption is the movement of external material across biological membranes at one of four principle portals of entry, the respiratory tract, skin, gastrointestinal tract or, in the case of fetal exposure, through the placenta¹¹ into the systemic circulation. Absorption is the net result of the interplay between multiple physiological processes and the physicochemical characteristics of the compound. Physiological characteristics, such as the size, surface area, blood flow rates, gastric contents, status of the tissue forming the barrier, as well as biochemical parameters, such as gastric pH and lung surfactant, can influence rates of absorption^{5-8, 43}. For instance, changes in absorption would be expected to parallel increases or decreases in surface area exposed. In addition, the effect of each of these physiological properties on absorption depends on the physicochemical properties of the

compound⁸. Lipophilicity, water solubility, and reactivity have a particularly large impact on absorption; therefore, these properties were the basis for the categories within which compounds were organized.

The following sections contain a description of information concerning age- and gender-specific differences in the physiological processes that influence absorption, and their impact on absorption of chemicals following oral, dermal, inhalation or fetal (placental) exposure. This information is presented at two levels of organization, grouped first by route of exposure and second by physiological construct or process (organ size, flow rates). Because the preponderance of data is on pharmaceuticals, compounds will be classified by physicochemical properties (i.e., lipophilicity) to highlight the relationship to less well studied, environmentally-relevant compounds with different physicochemical properties.

3.1.1 Oral Absorption

The anatomical structures comprising the gastrointestinal tract—stomach, small and large intestines—are optimized to support its primary function, absorption of nutrients and water. The wall of the stomach is covered by folds or rugae, while the walls of the small intestine are lined with villi, both which serve to maximize surface area. The external surface of each villus is covered by microvilli. Lipophilic compounds are primarily absorbed by passive diffusion, although water soluble compounds may also be absorbed to a limited extent by passive diffusion⁴⁴. However, the absorption of most water soluble compounds occurs via active transport systems that are also involved in the absorption of nutrients⁴⁴. For example, several divalent ions, including lead, may be absorbed via the calcium transport system⁴⁵. Active transport systems have been identified for several nutrients, including sugars, amino acids, gamma globulins (newborns), pyrimidines, triglycerides, fatty acids, bile salts, vitamins, electrolytes (Na^+ , Cl^-) and iron. For xenobiotics to be absorbed via these systems they must be structurally similar to the nutrients allowing them to be transported by these specialized transport systems. For this portal of entry, net absorption may be influenced by the available surface area in the upper and lower gastrointestinal tract, blood flow rates, gut transit times and pH, as well as the status of the intestinal mucosa (villi and microvilli)^{5-8, 43}.

No quantitative data regarding changes to surface area of the gastrointestinal tract were found, but clearly as the surface area increases with intestinal length, surface area increases with body weight⁴ (Figure 3). The length, and presumably surface area of the small intestine, increases as a function of body weight at a higher rate than the large intestine, consistent with the role of the small intestine in nutrient uptake. However, atrophy of gastric mucosa and intestinal microvilli with a decrease in mucosal surface area has been reported in the elderly⁴⁶.

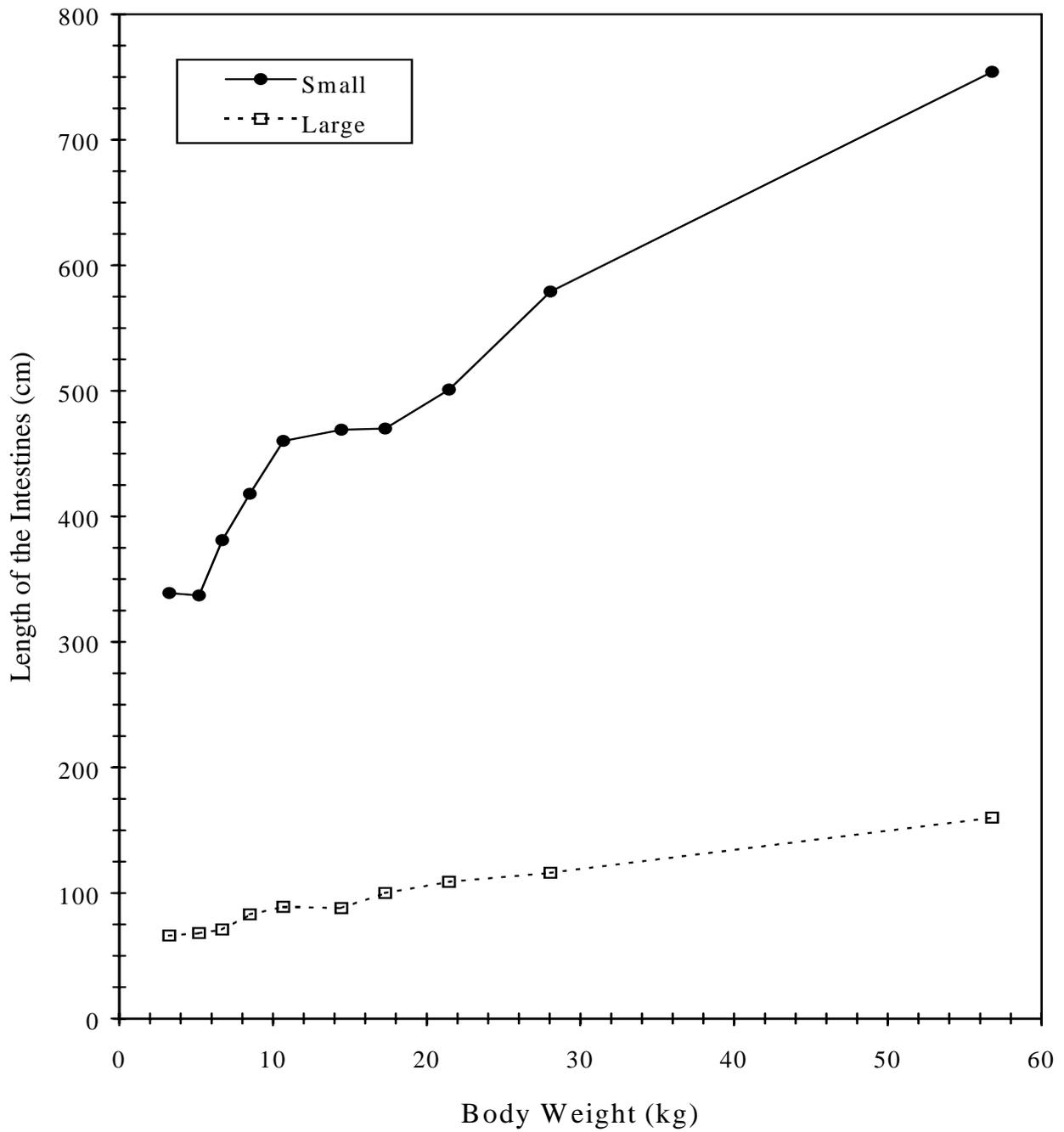


Figure 3. The relationship between intestinal length and body weight. Adapted from: ICRP¹ and Ogiu *et al.*⁴.

In response to the nutrient demands of early development, the absorptive capacity of the intestine in children for some nutrients is higher when compared with adults. For example, calcium absorption from intraluminal contents is higher in children when compared with adults¹¹. This could have important consequences for the absorption of other divalent metals, such as Pb²⁺. Pancreatic enzyme function and bile acid secretion are also lower in neonates¹³. Consequently, absorption from the gastrointestinal tract of elderly individuals may be decreased, while increased for some compounds in the neonate.

Changes in blood flow patterns with age may also impact the absorption of chemicals from the gastrointestinal tract. For example, splanchnic blood flow decreases with age at a greater rate than cardiac output, leading some investigators to hypothesize a reduced capacity for absorption from the gastrointestinal tract in the elderly⁷. It has been reported that splanchnic blood flow decreases approximately 40% by age 70⁸.

Gastric pH varies significantly with age; gender differences have also been reported¹⁰. Gastric pH is elevated in newborns relative to adult levels¹¹. Adult values are typically 1 to 2.5⁸, while newborns have a gastric pH near neutrality²⁶. Full acidification of the stomach does not occur until several months of age¹¹. Women secrete less gastric acid, and have a slower gastric emptying time, which is affected by increases in sex hormones, particularly in pregnancy and following the use of exogenous hormones. A decrease in gastric acid production causes an increase in gastric pH in the elderly⁴⁶. The elevated pH in neonates or the elderly can influence the ionization state of ingested weak acids and bases, increasing or decreasing the neutral fraction, which is the species available for absorption by diffusion.

There is an increased incidence of intestinal stasis in the elderly compared with adults⁴⁶, slowing the transit time of gut contents. In contrast, the gastric emptying time may increase with the increase in gastric pH in the elderly^{8, 46}. Two studies reported an increase in gastric emptying time, and two reported no change². The impact of increases in gastric residence times would have a variable effect on absorption. If the stomach is the primary site of absorption, as might be expected for weak acids, absorption might be higher⁸. The contrary would be true for weak bases, where the site of absorption is the intestine. Any delay in movement from the stomach to the intestine would delay the absorption of these compounds².

3.1.2 Dermal Absorption.

A primary function of the skin is to form a selectively permeable barrier to chemical substances. Materials passing through the skin into the systemic blood flow must first cross the nonperfused keratinized epidermal layer into the dermis, where they may be cleared by blood flow. Absorption through the epidermis occurs by passive diffusion via different pathways for nonpolar and polar compounds. Nonpolar compounds diffuse through the lipid matrix in the stratum corneum, while polar compounds may diffuse through the outer surface of

protein filaments within the stratum corneum⁴³. The status of the stratum corneum, thickness and hydration state, has a large impact on absorption⁴³. The permeability of skin to hydrophilic compounds increases, and that of lipophilic compounds decreases with increasing hydration state. In general, the mass of a compound absorbed by this route is a function of surface area (exposed), the hydration state of the epidermis, blood flow to the dermal layer and the physicochemical characteristics of the compound.

In adults, the keratinized epidermal layer (stratum corneum) of the skin is a protective barrier against transdermal absorption, particularly for hydrophilic compounds. The epidermis of the fetus is unkeratinized, becoming fully keratinized 3-5 days after birth¹¹. This developmental change, unique to the fetus/newborn, could potentially result in an increase in dermal absorption during the first few days of life¹¹. However, Cunico et al.¹² reported no differences in two parameters, indicative of skin penetration rates when 22 infants 1-3 days of age were compared with 30 adults. Based on these findings, Cunico et al.¹² concluded that penetration rates in adults and infants should be similar for polar compounds.

Aging affects changes in the physiology of skin in the elderly as well. Reduced hydration state of the stratum corneum and atrophy of the capillary network as well as a reduction in skin surface lipids have been reported¹⁴. Depending on chemical class, these changes in the elderly would be expected to reduce absorption, but predicting outcomes are difficult because one change (blood flow) is not chemical-specific, while the others (reduced hydration, decrease in lipids) would have opposing effects on the absorption of a class of chemicals.

The skin surface area of newborns is 3-fold larger per kilogram body weight than in adults^{11, 47}, suggesting the potential for higher per kilogram doses for equivalent surface area exposures. Based on higher surface area to body weight ratios for children, exposure to a chemical that is capable of penetrating the skin is expected to result in a dose which is 40-50% higher in children on a body weight basis, compared with adults⁶.

3.1.3 Respiratory Tract Absorption

The respiratory tract consists of the nose, nasal cavity, trachea, bronchi, and bronchioles, with the bronchioles terminating in alveolar sacs that contain the alveoli, the primary region where gas exchange occurs. Chemicals may be absorbed across any of these structures. Respiratory tract absorption of volatile compounds is a function of the flow rate of mass through the lung, the available surface area, pulmonary blood flow, thickness of the airway epithelium. Absorption is also a function of tissue permeability, which is determined by physicochemical parameters, such as molecular weight, lipid solubility, water solubility, and partition coefficients. The respiratory tract absorption of substances deposited in the airways additionally depends on airway clearance

kinetics at the site of deposition. Airway clearance parameters do not show any significant variation with gender or age⁴⁸.

The relative impact of mass flows and tissue blood flows depends on the physicochemical characteristics of the compound, as well as the nature of the absorptive surface. For instance, pulmonary absorption of highly water soluble, volatile organic compounds is lower than expected based on simple blood partitioning⁴⁹. Drawing conclusions regarding the relationship between systemic tissue doses and ventilation rates without consideration of all the processes involved can be misleading. For highly water soluble compounds such as alcohols, the steady state arterial blood concentration is not, as commonly believed, dependent on ventilation rate, or fraction (as a percent of pulmonary ventilation) of inhaled material absorbed^{50, 51}. Differences in ventilation rates, however, may impact upper respiratory or pulmonary tissue doses of some compounds, such as particulates and reactive chemicals.

The human lung can be broadly divided into three regions based on structure and function: the nasal cavity, the conducting airways, and the gas-exchange region or the pulmonary airways. The nasal cavity has a complex and convoluted structure and forms the first line of defense against inhaled toxicants. Its main function is to condition the inhaled air before entering the gas exchange region and filter the air of inhaled dust particles. The respiratory and olfactory tissue that line the nasal cavity are also metabolically active and well perfused by blood, resulting in an efficient scrubbing of inhaled vapors in this region. Certain metabolized vapors, such as vinyl acetate, are extracted in the nasal cavity by up to 90%, thus limiting the inhaled dose available for systemic exchange. Quantitative information on the human nasal cavity was characterized only available for adult males;^{52, 53} no information was available on age and gender related variations.

Lung surface area, total lung capacity, vital capacity, functional residual capacity, and residual volume all increase in proportion to lung growth. In infancy and childhood, the overall growth of the lung parallels that of the body as a whole and these changes are best correlated with body length or stature⁵⁴. The rate of development is maximal in the neonatal period and then declines until the start of the adolescent growth spurt at about age 11⁵⁵. Thereafter, the rate of growth increases to a peak. In girls, this occurs at about age 13-14 years and growth ceases a few years later. In boys, the peak is delayed by about 2 years, and growth continues into adulthood⁵⁶. The growth and maturation of specific regions of the lung, however, are not always parallel to changes in body weight.

The number of conducting airways is complete at birth, and they increase only in size with age¹⁵. Hence, the conducting airways of the infant may be regarded as a miniature version of those in the adult and the growth of the conducting airways in both length and diameter takes place in a symmetrical manner with age, with constant relation to the rest of the lung⁵⁷. However, the size of the pulmonary airways increases from birth to adolescence, primarily by

the addition of new alveoli^{11, 16}. The resulting surface area for respiratory absorption also increases¹⁷. At birth, the alveolar surface area is approximately 3 m²; however, in adults, the alveolar surface area is 75 m², an approximate 25-fold increase¹⁸. This increase in surface area for absorption is in rough proportion to body weight, resulting in similar body weight adjusted values for alveolar surface area. Estimates of alveoli present at birth vary widely from 17 to 71 million, with a mean of about 55 million. This value in the adult lung varies from 200 to 600 million¹⁶. The maximal number of alveoli is reached at about 10 years of age and, thereafter, maturation of the respiratory system accelerates until maximal function is reached, at approximately the age of 20 years for females and 25 years for males⁵⁸. In this respect, the growth pattern of the conducting airways is different from the alveoli, since the latter increases in both number as well as size. Alveolar surface area increases rapidly until 8 years and then more slowly until the adult area of 70-80 m² is attained by 20 years⁵⁹. Similarly, lung volume increases rapidly in infants and then shows a moderate increase to an average value of 4000 cm³ at about 20 years⁵⁹. The lung surface area and volume in males increase more rapidly than those in females do during adolescence and adulthood⁶⁰. Females reach their maximum value at 14-20 years, while males continue to increase until 18-25 years⁵⁸.

Age-related differences in ventilation parameters have been reported, with the greatest differences occurring between neonates and adults¹⁸. The respiratory ventilation rate in infants is significantly larger in relationship to lung surface area (133 ml/min/kg BW/m²), when compared with adults (2 ml/min/kg BW/m²). Therefore, in infants, there is potentially a greater exposure of the lung surface to airborne compounds on a body weight basis¹⁸. Differences in minute ventilation normalized to body weight between neonates, infants (1 year) and children up to 10 years of age are small, but the rates are approximately 40-50% higher than those of adults⁶. Activity adjusted values for total intake (L/kg/day) by inhalation are highest for children and lowest for newborns⁶. In comparison, a 25% reduction in the vital capacity and an increase in the residual volume of 50% have been reported in the elderly⁴⁶. The different characteristics of ventilation between infants/children and adults can also impact the pulmonary uptake of volatile compounds. In infants and children, the alveolar ventilation rate (V_{alv}), normalized to functional reserve capacity (FRC) (V_{alv}/FRC), is much larger than in adults²¹. More rapid turnover of alveolar air allows more rapid equilibrium with blood and potentially higher uptake in infants and children²¹.

Changes in lung parameters are also observed in the elderly. Morphological changes in the senescent adult lung result in a loss of the alveolar surface area. Alveolar ducts in humans increase in diameter and alveoli become wider and more shallow with age. Morphometric studies have consistently found an increase in the average distance between airspace walls and a decrease in surface area of airspace wall per unit of lung volume beginning in the third decade of life. The decrease in airspace wall surface area per unit lung volume is approximately linear and continues throughout life¹⁹. Furthermore, the flattening of the internal surface of the alveoli is associated with a reduction in alveolar

surface (75 m^2 at age 30 years and 60 m^2 at age 70 years, a reduction of $0.27 \text{ m}^2/\text{year}$)⁶¹.

Total lung capacity does not change significantly with age²⁰. This constancy in the total lung capacity is maintained due to the complementary change to its constituent volumes: the vital capacity (mobile volume) and the residual capacity (fixed volume). The vital capacity decreases progressively in adults, even after adjusting for height and weight⁶², due to a decrease in the lung elasticity. The progressive narrowing and closing of small lung airways increase the residual volume with age until the mean value at 60 years is approximately 118% of the value at 20 years⁶³. Gas exchange efficiency is also reduced progressively with age in adults. Ventilation-perfusion imbalances and alveolar hypo-ventilation may be factors in the decline of gas-exchange efficiency⁶⁴, but other factors, such as the reduction in the surface area, increase in membrane thickness, reduced membrane permeability⁶⁵, and a reduced capillary blood volume⁶⁶, also contribute significantly.

The most important physiological changes associated with aging are a decrease in the static elastic recoil of the lung, a decrease in compliance of the chest wall, and a decrease in the strength of respiratory muscles. Most of the age-related functional changes can be related to these three phenomena. After the age of 50 years, a proportion of the elastic fibers in the region of the respiratory bronchiole and alveolus degenerate and appear ruptured and coiled⁶⁷. These changes are most marked around the alveolar ducts. Consequently, dilatation of the alveolar ducts occurs and this is followed by enlargement of the air spaces⁶⁸. A consequence of the reduction in supporting tissues around the airways is a tendency for the small airways (<2 mm) to collapse. Premature closure of the airways, therefore, may occur during tidal breathing.

Quantitative relationships between lung surface area, volume and age have been developed and reported in the literature. Based on morphometric measurements, Thurlbeck⁶⁰ developed regression equations for lung surface area (in m^2) as a function of age. The equations for boys and girls, respectively, were

$$21.9+10.7*\ln(\text{age}) \text{ and } 18.5+7.7*\ln(\text{age})$$

Similarly, the regression equations for lung volume (in liters) in boys and girls, respectively, were

$$0.973+\ln(\text{age}) \text{ and } 0.794+\ln(\text{age}).$$

Petrini et al.⁶⁹ measured pulmonary capillary blood flow (in L/min) in 176 normal subjects (94 males, 82 females), ranging from 17 to 70 years of age, and expressed blood flow rate as a function of age (in years) in males and females, respectively, using

$$Q = \exp(1.25-0.0089*(\text{age})) \text{ and } Q = \exp(1.25-0.0089*(\text{age})).$$

Changes in the total lung surface area and volume alone are insufficient to predict regional dosimetry of inhaled pollutants as a function of age. Anatomical information on airway dimensions on a generation-to-generation basis is required to compute regional lung dose metrics. Age-dependent lung models have been developed⁷⁰ to characterize the dimensions of a maturing lung and these model have been used to predict particulate and vapor dosimetry in the lungs as a function of age^{71, 72}. Based on airway dimensions in the Weibel model, Hofmann⁷⁰ derived a series of empirical relations to determine the length and diameter of the trachea, bronchial airways, and alveoli diameter and total number of alveoli in the pulmonary airways as a function of age (Table 3).

Table 3
Empirical Relations for Airway Length, Diameter, and Function for an Age-Dependent Lung

Trachea Diameter (cm)	$D_0(t) = \frac{D_0(t)}{1.67} [1.271(1 - e^{-0.07t}) + 0.55]$
Trachea Length (cm)	$L_0(t) = \frac{D_0(t)}{12.29} [8.72(1 - e^{-1t}) + 4.0]$
Bronchial Diameter (cm)	$D_i(t) = \frac{D_i(t)}{1.26} [0.863(1 - e^{-12t}) + 0.42]$
Bronchial Length (cm)	$L_i(t) = \frac{D_i(t)}{4.285} [2.931(1 - e^{-1t}) + 1.5]$
Alveoli Diameter (μm)	$D(t) = [172.76(1 - e^{-2t}) + 100]$
Alveoli Number	$N(t) = 10^6 [286.21(1 - e^{-4t}) + 37.6]$
Tidal Volume (resting - mL)	$TV(t) = 21.7 + 35.13 t - 0.64 t^2$
Respiratory Frequency (resting - min^{-1})	$RF(t) = 11.75 + 15.17 / (0.25 t + 0.5)$

Source:⁷⁰

The ventilation conditions of humans are also age-related. Although the reported values are highly variable, Hofmann⁷⁰ estimated that the resting tidal volume increases from about 22 cm^3 at birth to about 500 cm^3 in adulthood, whereas the resting respiratory frequency decreases from about 42 min^{-1} to about 14 min^{-1} . The empirical formulas derived by Hofmann⁷⁰ for the resting tidal volume and respiratory frequency as a function of age t (in years) are summarized in Table 3.

Respiratory tract clearance is accomplished by various regionally distinct processes. In the nasal cavity and the conducting airways, clearance occurs via the mucociliary transport and in the pulmonary region clearance is primarily by macrophages⁷³. Nasal mucus flow rate in healthy adults is about 5 mm/min ⁷⁴,

similar to the tracheal mucus transport rate⁷⁵. There appears to be no clear evidence for any age- or gender-related differences in either mucociliary clearance or macrophage clearance.

3.1.4 Observed Differences in Absorption

Neonates, Infants and Children. There is limited experimental evidence of age-specific differences in gastrointestinal tract absorption of xenobiotics in children. The example of lead, a water soluble hydrophilic compound, is well known. Neonates through 2 years of age absorb 4-5 times more lead (42-53%) than adults (10%)⁴⁷. From ages 2 through 6, the amount of absorption drops slightly to 30-40% and then drops again between the ages of 6 and 7 years⁶. The mechanism responsible for these differences is unknown, but it has been speculated that the gastrointestinal tract develops more selective intestinal absorption processes during maturation⁶. Pulmonary absorption of lead is also greater in children (42%) than in adults (15-30%)⁷⁶.

Compared with xenobiotics, neonatal pharmacokinetics of pharmaceutical compounds have been widely studied, providing classical pharmacokinetic characterization of water soluble and moderately lipophilic compounds. Expectations for environmental chemicals of similar characteristics can be built on these characterizations. However, measures of absorption, such as time to peak concentration (T_{max}), which reflects outcomes influenced by other factors (e.g., gastric emptying time) rather than simple absorption alone, are typically reported. The T_{max} values for anticonvulsants, several classes of antibiotics and digoxin for neonates and adults were reviewed by Morselli et al.²⁶. To the extent that T_{max} reflects differences in the rate of absorption, the rate was variable and ranged from higher (phenobarbital, theophylline) or equivalent (digoxin) to lower (rifampicin) for neonates compared to adults²⁶. These changes are not predictable by chemical class; phenobarbital, digoxin and rifampicin are moderately lipophilic and acetaminophen and theophylline are water soluble.

In contrast to rates of absorption, the extent of absorption may be equivalent. Delayed absorption of both moderately lipophilic (phenobarbital) and water soluble (acetaminophen) compounds in neonates has also been documented¹³. The effect appears to be related to more than reduced gastric emptying time; the enteral absorption rate constant increases with age¹³. These data only support an age-related difference in the extent of gastrointestinal tract absorption. An exception is lead, where it appears that absorption is greater.

Minimal experimental data are available regarding differences in the rate or extent of dermal absorption between adults, children and infants. This likely reflects the limited use of the dermal route for administration of pharmaceuticals and the inappropriateness of experimental work in humans.

The characterization of age-dependent differences in the pulmonary uptake of anesthetics is likely an indication of expected behaviors for similar volatile organic xenobiotics. The initial uptake rate of volatile anesthetics, both lipophilic

(e.g., halothane, and cyclopropane) and water soluble (e.g., nitrous oxide), is higher in infants and children than adults²¹. A larger alveolar ventilation rate relative to FRC and body weight, as well as greater perfusion rates and lower fat content, are believed to be responsible for this age-specific difference in pulmonary absorption²¹. To the extent that these data reflect the behavior of water soluble and lipophilic volatile organics, it appears that the initial rate of uptake of both classes of compounds would be expected to be higher in infants and children.

Inhaled particles deposit in any one of the three regions of the lung depending on the aerosol size, breathing characteristics, and airway morphometry. Fractional deposition of particles in the developing lungs has been studied by direct *in vivo* measurement, and has been inferred using mathematical models that quantitatively describe the postnatal changes in the human lung⁷⁰. These models provide a basis to evaluate both the age-dependence on inhaled particle dosimetry, as well as investigate aerosolized drug dosage extrapolation to children based on formulations in adults.

Swift⁷⁷ constructed replica nasal casts of an infant and adult and studied deposition using particles from 1 to 10 μm . Swift observed comparable nasal cavity deposition efficiency in adults and children for equivalent states of activity. Oldham et al.⁷⁸ examined the deposition of monodispersed particles, having diameters of 1, 5, 10, and 15 μm , in hollow airway models designed to represent the trachea and the first few bronchial airway generations of an adult, a 7-year-old child and a 4-year-old child. In most cases, the total deposition efficiency was greater in the child-size models than in the adult model⁷⁸. Bennett and co-workers compared the deposition of 4.5 μm , poorly soluble iron oxide particles in children and adults with mild cystic fibrosis and found that children had higher deposition in the extrathoracic airways⁷⁹. However, no significant difference was observed in total respiratory tract deposition between the children and adults. In a subsequent study, Bennett et al.⁸⁰ compared the total fractional deposition of 2 μm wax particles in children (7-14 years), adolescents (14-18 years) and young adults (19-35 years). Although no significant age-related difference in fractional deposition, based on smaller lung size and higher minute ventilation in children, was observed, they concluded that children would receive a higher dose of particles per lung surface area compared to adults.

Hofmann⁷⁰ developed a theoretical age-dependent lung morphology by scaling airway anatomical parameters and airflow rates and examined their effects on particle deposition. They showed that particle deposition is strongly dependent upon age, with children receiving a significantly higher dose than adults. Xu and Yu⁸¹ also modeled particle deposition as a function of age and found higher total deposition in children than adults in the tracheobronchial and alveolar region. Using morphometric measurements taken from 21 replica airway casts of children and adolescents, Phalen et al.⁸² constructed mathematical models to estimate tracheobronchial particle deposition efficiency for infants, children, and adolescents. Their computed particle deposition efficiencies indicated that under most levels of physical activity and for most

particle sizes (0.01-100 μm), children exhibited a higher tracheobronchial deposition than adults. More recently, a theoretical lung model incorporating changes in morphology and breathing pattern with growth was used by Musante and Martonen¹⁷ to predict regional particle deposition in children. Based on model derived estimates they concluded that under both resting and active breathing conditions, tracheobronchial deposition decreased with age. In particular, if ventilation rate and cumulative surface area are considered, children may receive a localized dose that is three times higher than adults. They also concluded that the pulmonary deposition was highest in the 4- to 6-year-old for all particle sizes examined¹⁷. Hence, both *in vivo* experimental measurements and mathematical models predict a higher fractional deposition of inhaled particulates in children compared to adults.

The impact of physiological differences specific to the neonatal and childhood periods on absorption is route-specific. The extent of gastrointestinal tract absorption appears to be equivalent for the lipophilic and water soluble compounds studied, while the rate of absorption varies by chemical but is independent of physicochemical property. Absorption of compounds which enter through the gastrointestinal tract through specialized transport processes, for example, lead, may be higher in neonates, reflecting higher absorptive capacity of the neonatal intestine for some nutrients during this lifestage. Dermal absorption data are lacking, but one study reported no differences in penetration between children and adults¹². Pulmonary absorption appears to be higher in infants and children for water soluble and lipophilic volatile organics as does deposition of particulates. Increased uptake and deposition result from age-specific differences in airway geometry, increases in ventilation rates relative to body weight and differences in body composition.

Elderly. The physiological and biochemical changes that accompany aging have been shown to affect gastrointestinal absorption². Reduced splanchnic blood flow, gastric emptying times and increased gastric pH have been reported in the elderly as have reductions in pharmacokinetic parameters correlated with absorption (C_{max} , T_{max}), but attributing decreases in absorption specifically to changes in one or more of these physiological processes is difficult. In addition, based solely on the data presented here, differences in the rate and extent of absorption are not classifiable by physicochemical characteristics.

Decreases in splanchnic blood flow in the elderly increase the apparent absorption of compounds undergoing high hepatic extraction such as nifedipine⁸. The absolute bioavailability of nifedipine is 30% higher in the elderly when compared with young adults⁸. As indicated by a longer T_{max} , the rate of absorption of some moderately lipophilic (quinidine, chlordiazepoxide, nalidixic acid) and hydrophilic (ethambutol, practolol) pharmaceuticals has been shown to be lower in the elderly⁴⁶. The absorption rate constant for practolol is 1.13 L/hr in adults and 0.71 L/hr in the elderly⁴⁶. The rate constant for absorption of nalidixic acid is similarly lower in the elderly (0.52/h vs. 0.29/hr)⁴⁶. On the other hand, for a group of water soluble drugs, clomethiazole, cimetidine and propranolol, the extent of absorption is higher in the elderly⁴⁶.

A recent review reports that there are no data that support an effect of gastric pH on gastrointestinal absorption in the elderly². In addition, evidence suggests that the extent of gastrointestinal absorption of antibiotics in the elderly is comparable to that in younger patients, even when differences in the rate of absorption are apparent⁸³.

In summary, the relevant data suggest that in general, the extent of gastrointestinal absorption in the elderly is unaltered but the rate of absorption is lower for some compounds², with the physicochemical characteristics of the compound playing little role in producing these differences. Differences in gastrointestinal absorption in the elderly result in delayed peak concentrations, but similar AUCs⁸³.

Roskos et al.¹⁴ compared the dermal absorption of compounds with aqueous solubilities of ~ 0 (modestly lipophilic, testosterone) to 21.7 g/L (water soluble, caffeine) in young adults 22-40 years of age and older adults ages greater than 65 years. The extent of absorption in older adults was lower, but not statistically different for the modestly lipophilic compounds, and 36-52% lower for water soluble compounds¹⁴. The tested compounds included acetylsalicylic acid, caffeine, benzoic acid, hydrocortisone, testosterone, and estradiol. This pattern conforms with expectations based on reports of lower hydration of the epidermis¹⁴, which should have a greater effect on the absorption of hydrophilic compounds. Generally, dermal absorption of water soluble compounds can be expected to be lower in the elderly, compared with younger adults.

Gender. A recent review of gender-related differences in pharmacokinetics provides no convincing data that gender differences in gastrointestinal absorption rate constants unrelated to differences in first pass intestinal metabolism exist¹⁰. The authors concluded that gender differences in absorption and bioavailability are rare, and are likely without clinical significance.

No data characterizing gender differences in dermal uptake were available. One study used a PBPK model of dermal absorption from bath water of various temperatures to estimate chloroform skin permeability rates⁸⁴, and reported a sex difference in the rate for one of two tested water temperatures. Because of uncertainties associated with model assumptions (i.e., equivalent skin blood flow, skin thickness and subcutaneous fat between male and females), the difference in rates could not be solely attributed to a difference in skin permeability between males and females⁸⁴.

Experimental work evaluating gender differences in pulmonary uptake of xenobiotics in humans is similarly lacking. The paucity of available literature evaluating gender difference in pulmonary uptake limits the characterization of any such differences. One study reported no differences in the pulmonary uptake of methylene chloride between males and females⁸⁵.

Unlike vapors, gender-related differences in particle deposition in the respiratory airways have been explored. In general, studies on gender-specific effects on particulate dosimetry are equivocal and do not show any significant gender-related changes to particulate dosimetry in the adult lung. Using 2.5 and 7.5 μm particles, Pritchard et al.⁸⁶ concluded that females receive a higher extrathoracic and tracheobronchial deposition and a smaller pulmonary deposition compared to males for the same particles and at similar inspiratory flow rates. More recently, Bennett et al.⁸⁰ measured total respiratory tract deposition in adult males and females aged 18-80 years using 2 μm particles and showed comparable deposition efficiency under normal breathing conditions. Dosimetry studies by Kim and Hu⁸⁷ and Jaques and Kim⁸⁸ assessed regional deposition efficiency in healthy adult males and females using a bolus delivery technique with 1, 3, and 5 μm . Deposition was comparable in males and females for 1 μm particles, but a higher deposition (15%) was noted in females compared to males for the 3 and 5 μm particles^{87, 88}.

3.2 Distribution

Distribution of a chemical throughout the body is influenced by physiological processes and constructs, as well as the physicochemical properties of a chemical. Distribution of chemicals is typically described as the volume of distribution, V_d , or volume of distribution at steady state $V_{d_{ss}}$. As a human ages, the extent to which a chemical distributes throughout the body is influenced by five main physiological properties, which include body composition (body water, fat, lean body mass), blood flow, composition and concentrations of plasma binding proteins, tissue-protein concentration, and fluid pH². In addition, plasma lipoprotein concentrations have an impact on the distribution of lipophilic compounds²²⁻²⁴. Changes in these physiological properties during development, maturation and aging have demonstrable influence on distribution^{2, 24, 46, 89}. Gender differences, predominantly in lean body mass, and percent body fat can also result in differences in volumes of distribution^{8, 10}.

Changes in each of these parameters with age or gender have an individual impact on the volume of distribution. However, changes in overall chemical distribution with age or gender may be the result of a combination of changes in these parameters, depending on the physicochemical properties of a chemical. The following sections provide information on the age- and gender-related differences in each of these physiological processes and their potential impact on distribution, followed by a discussion of measured overall differences in distribution of specific chemicals by age or gender.

3.2.1 Body Composition

While the absolute volumes of some tissues increase from birth to stable adult levels (Figure 4) in rough proportion to height⁹⁰ or body weight⁴ (Figure 5), the relative volumes of total body water, extracellular water, body fat and lean body mass do not increase in proportion to height^{24, 89, 90} (Figure 6 and Table 4).

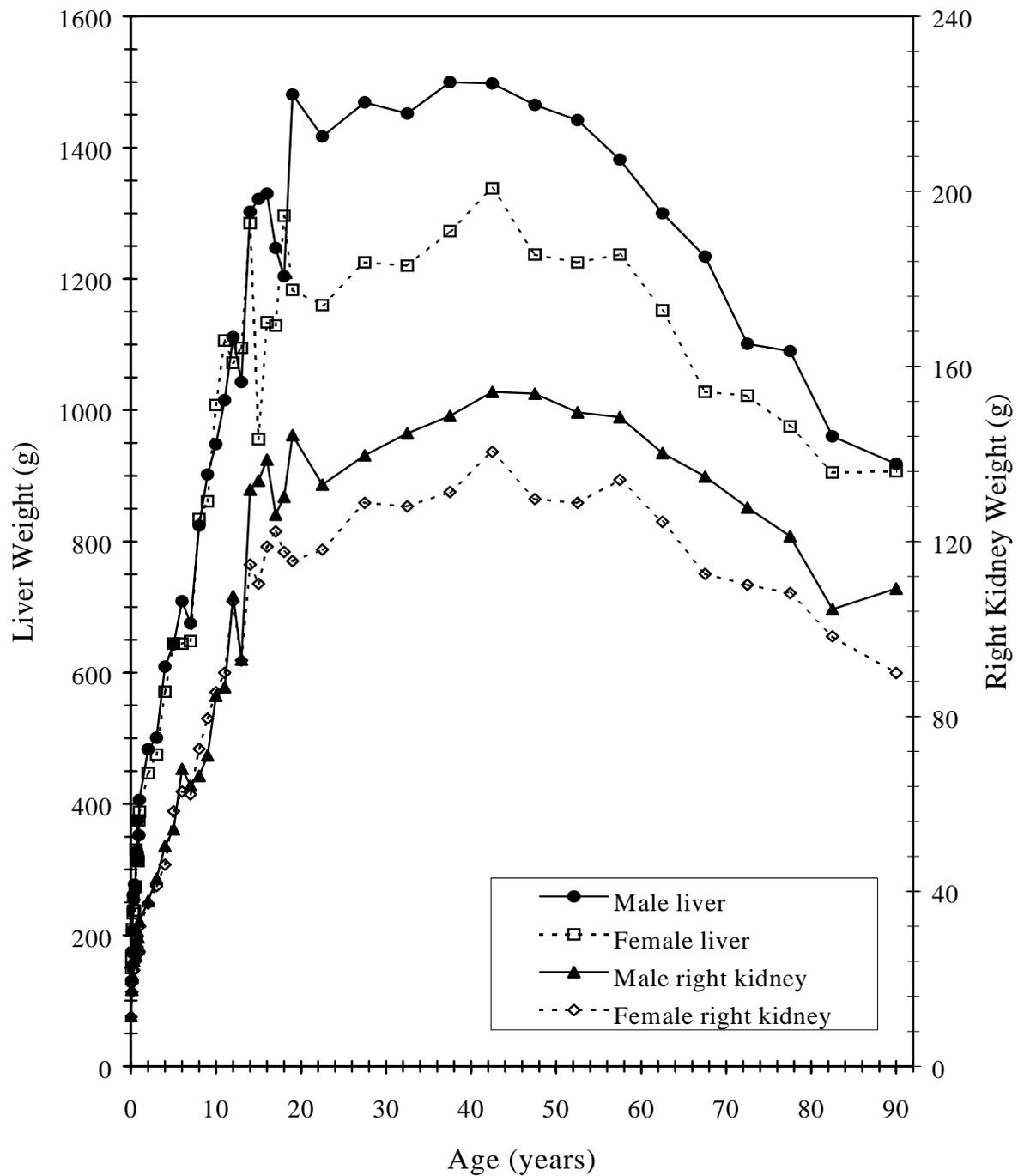


Figure 4. The relationship between right kidney or liver weight and age in Japanese from birth to old age. Adapted from: Ogiu *et al.*⁴.

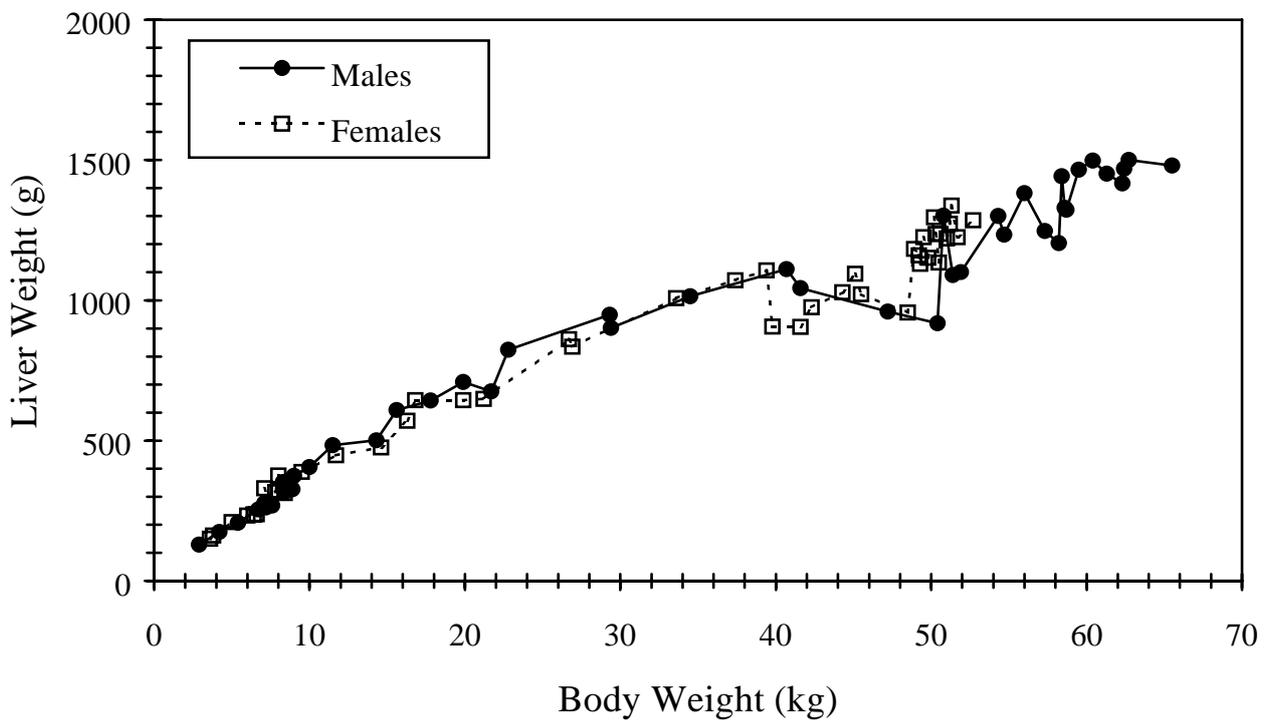
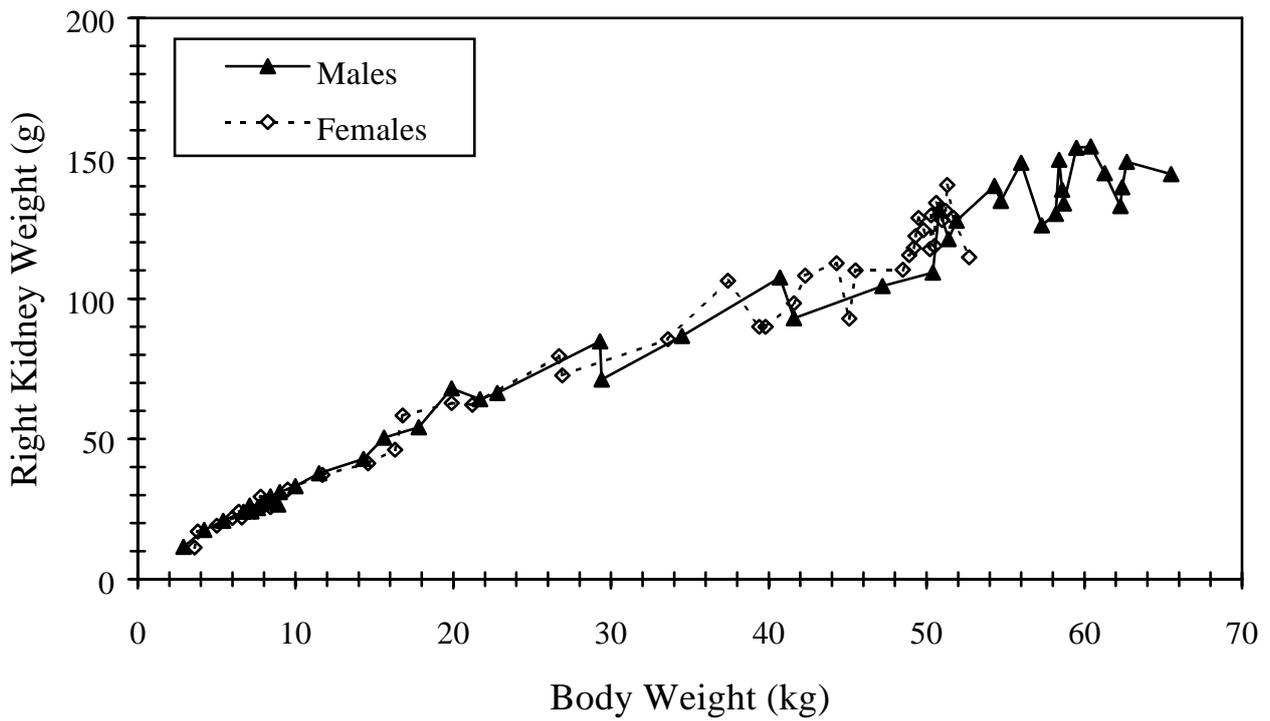


Figure 5. The relationship between right kidney or liver weight and body weight in Japanese from birth to old age. Adapted from: Ogiu *et al.*⁴.

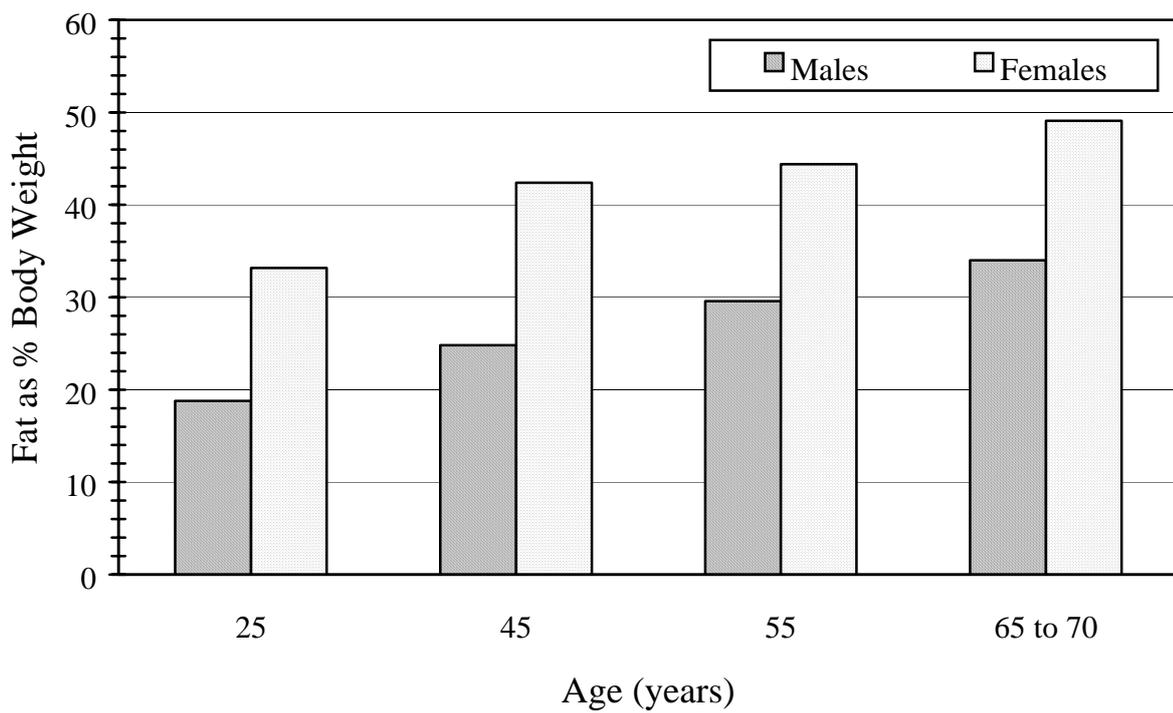
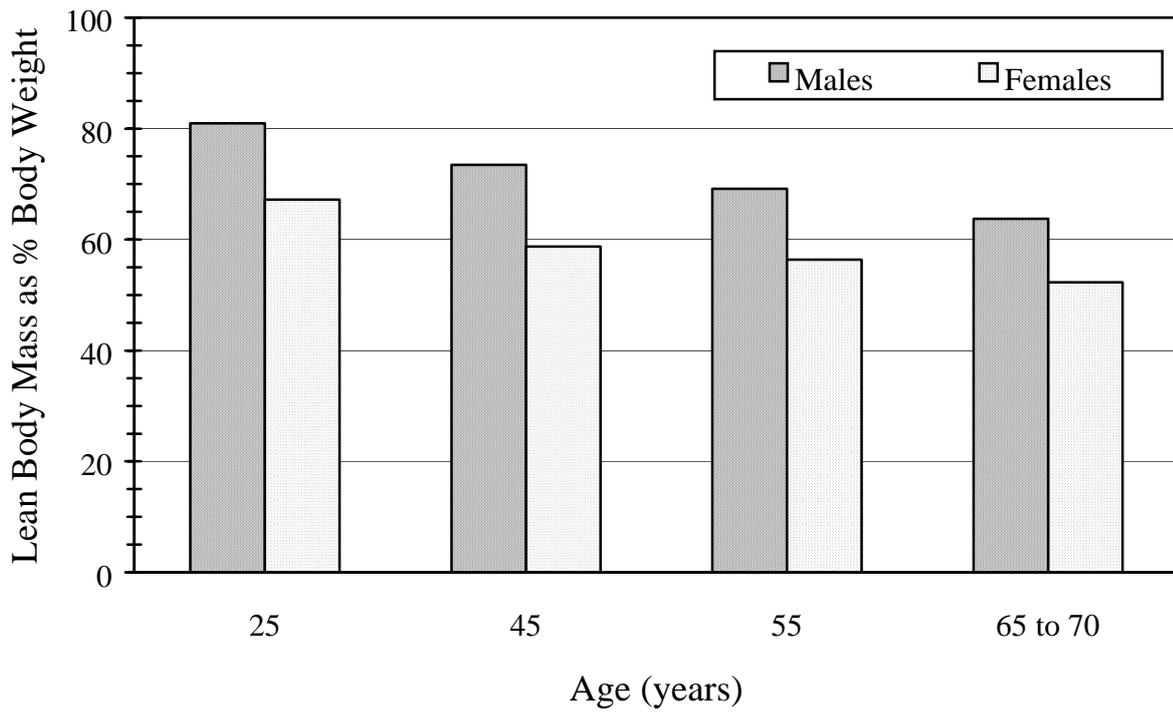


Figure 6. The relationship between lean body mass or body fat as percent of body weight and age. Adapted from: Mayersohn².

Table 4
Fractional Organ Weight as a Function of Age^a

Organ Weight as a % of Body Weight		
Organ	Neonate	Adult
Skeletal Muscle	25.0	40.0
Skin	4.0	6.0
Heart	0.5	0.4
Liver	5.0	2.0
Kidneys	1.0	0.5
Brain	12.0	2.0

^aAdapted from¹⁴⁴.

The relative volume of lean body mass and fat undergo additional changes during aging, and are notably different in the elderly² (Figure 6). In women, lean body mass decreases from 76% to 52% and percent body fat increases from 33% to 49% between ages of 25 and 65-70. In men, lean body mass decreases from 80% to 64% and percent body fat increases from 19% to 34% during the same periods (Figure 6).

Body water constitutes approximately 77% of body weight in full-term infants, 73% at 3 months, and decreases to adult levels of 55% by age 12⁸⁹. Similarly, extracellular water is 45% of body weight in full-term neonates, dropping to 33%, 28% and 20% after 3 months or 1 year, respectively, before reaching the adult level of 20²⁴.

Body fat increases in a gender-dependent fashion during development and maturation. Fat content increases between the ages of 5 and 10 in boys, dropping afterward until age 17. In women, fat content increases rapidly during puberty, after which women maintain body fat levels approximately 2-fold higher than men⁸⁹. Body fat increases in women from an average of approximately 33% at age 20 to approximately 48% on average in women over 70 years of age. The corresponding age-dependent increase in men is from an average of 18 to 36%⁹¹. In neonates, the average percent body fat is lower than that observed in adults, representing 15% of body weight²⁶.

3.2.2 Serum Binding Proteins

The extent of binding to serum proteins influences the volume of distribution by changing the storage capacity of the blood compartment for a chemical. Both the composition and concentrations of plasma proteins have the capability to influence the volume of distribution of a compound²⁴.

In humans, there are four serum-binding proteins which are known to impact the bound/free concentrations of pharmaceutical agents or xenobiotics, and hence the volume of distribution of these compounds^{22, 23, 92-94}. These

include the steroid hormone binding protein (SHBG)⁹²⁻⁹⁴, albumin, α_1 -acid glycoprotein (A1G)^{24, 46, 95}, and the serum lipoprotein family (SLPF), which includes chylomicrons, high density (HDL), and low density (LDL) lipoproteins^{22, 23}. Other serum-binding proteins that impact the free fraction of endogenous hormones are also present in human blood^{96, 97}, but are not addressed here because characterization of their influence on drug and chemical binding and distribution is very limited.

Total plasma protein concentration does not seem to change significantly with age, but changes in the concentrations of specific binding proteins do occur⁴⁶. Changes in the concentrations of binding proteins are developmental stage-specific, typically showing the largest change between birth and full maturation. Total protein (7.1 vs. 5.5 g/100 ml), albumin (4.5 vs 3.7 g/100ml) and globulins (2.58 vs 1.6 g/100 ml) are higher in adult plasma, compared with newborn cord plasma⁹⁸. Bilirubin concentrations are higher in the newborn (2.0 vs 0.2) compared with adults. Plasma protein concentrations do not change significantly in healthy children between the ages of 2 and 18⁸⁹. Differences in serum binding protein concentrations, particularly in SHBG, which binds estrogen, show gender differences as well.

Albumin. Albumin is the major drug binding protein in the serum². Plasma albumin is reduced in the elderly, compared with younger adults⁴⁶. The decrease is modest⁹⁵. The free fraction and free serum concentrations of benzodiazapines and most neuroleptics depend primarily on the serum albumin levels⁹⁵. Hepatic extraction of the benzodiazapines is limited by protein binding. Age-related reductions in serum albumin concentrations can increase hepatic extraction of these compounds^{95, 99}. Changes in the free fraction could cause changes in the volume of distribution as well. Albumin concentrations in the neonate are equivalent or modestly lower than adult values^{24, 98}. At birth and to 3 months of age, the range of normal albumin concentrations is 3.2-4.8 g/dL, increasing only slightly to 3.7-5.7 g/dL at 1 year of age¹⁰⁰. Neonatal albumin has different physiochemical properties (amino acid sequence) than the adult protein, and shows a lower affinity for drugs¹⁰¹.

Steroid Hormone Binding Globulin. One-year-old boys have a blood SHBG concentration of ~ 110 nM. The concentration declines steadily from age one, through puberty, until reaching adult levels of ~ 30 nM¹⁰². Similarly, SHBG levels are higher in young female children compared with adults¹⁰³. SHBG levels are ~ 70 nM in young girls between ~ 3.7 and 6.5 years of age, and drop during puberty (~ 13.4 years) to adult levels of 40 nM¹⁰³. Levels of SHBG are higher in adult women (40 nM) compared with men (20 nM), and rise significantly during pregnancy to 400 nM^{93, 94}. SHBG concentrations are reported to be higher in the elderly compared with young adults. Mean SHBG levels are ~ 53 nM and 70 nM in elderly men and women of approximately 70 years of age, respectively¹⁰⁴.

Glycoproteins and Lipoproteins. A1G levels are decreased in the neonate and do not reach adult levels until about 1 year of age¹³. There appears to be a moderate increase in A1G with age⁹⁵, although concentrations of this acute

phase protein can also rise in response to disease states. However, another study reported no change in A1G with age². The tricyclic antidepressants bind both albumin and A1G, but free concentrations were determined primarily by the serum concentrations of A1G^{95, 99}.

The concentration of globulins is lower in newborns. The concentrations of α_1 -globulin remains constant, while α_2 and β globulins increase with development. At birth, α_1 -globulin concentrations are 0.2-0.03 g/dL, and increase to 0.5-1.1 g/dL by 1 year of age. Similarly, β -globulins increase from 0.3-0.6 g/dL at birth to 0.4-1.0 g/dL at 1 year of age¹⁰⁰. In addition, the binding properties of the globulin fraction in newborn cord blood are different from those of the adult globulins¹⁰⁵. Lipoprotein concentrations are lower in newborns⁹⁸.

3.2.3 Tissue Binding

Digoxin binding to erythrocytes shows age dependence. The erythrocyte/plasma digoxin ratio is reported to be 3.6 in infants on long-term treatment and 1.3 in adults²⁴. This is consistent with findings that neonatal erythrocytes have 2.5 times the digoxin binding sites found in adult erythrocytes²⁴. Because measurement of tissue binding of drugs is not typically made directly, but rather inferred from compartmental analysis of plasma-concentration time course data, it is difficult to conclude with confidence that increases or decreases implied by the model are attributable to actual changes in tissue binding².

3.2.4 Observed Differences in Distribution

Neonates, Infants and Children. In general, age-related differences in distribution occur in the first 10-12 months of life, after which distribution is similar to adults²⁴. Hattis and co-workers have compiled a database containing volumes of distribution for compounds in neonates, children and adults²⁵. The volume of distribution was higher in neonates or children compared with adults for 10 of the 12 compounds with apparent differences in the volume of distribution (Table 5). The ratio of the perinatal Vd/adult Vd was calculated for these compounds (Table 5). The majority of the compounds had a ratio between 1.3 and 2.8. This range may be reasonable bounds for the increase in Vd for water soluble and moderately lipophilic compounds in neonates and children. Only two compounds, one of that is lipophilic, presented a Vd that was lower in the perinatal period. The volume of body water in children (<12 years) results in larger volumes of distribution (35% of body weight) for the water soluble aminoglycoside antibiotics when compared with adults (20-30% of body weight)⁸⁹. Volumes of distribution in neonates and children are also higher for modestly lipophilic compounds. The volume of distribution of the diuretic furosemide is increased in newborns (0.25-1.13 L/kg) compared with adults (0.07-0.18 L/kg)²⁶. The volume of distribution of the anticonvulsant phenytoin is higher in newborns (0.8-2.0 L/kg) compared with infants (0.3-1.0) and adults (number not given). The volume of distribution of two local anesthetics, lidocaine and mepivacain, is larger in the neonate than the adult²⁶. In light of

the larger volume of body water in the neonate, the higher volumes of distribution for lipophilic compounds are at first surprising. The increase may be attributable to lower serum protein content, including albumin, which has a lower affinity for drugs than the adult form, lower lipoprotein concentrations, and higher bilirubin levels. These factors would reduce the carrying capacity of blood, and increase the volume of distribution of lipophilic or other compounds transported in the blood bound to these proteins or lipoproteins.

Table 5
Ratio of Perinatal to Adult Volume of Distribution of Selected Pharmaceuticals

Compound	$Vd_{\text{perinata}} / Vd_{\text{Adult}}$	Physicochemical Characteristic	Age of Perinatal Group
Fentanyl	1.3	Highly Lipophilic	Neonate < 1 mo
Fentanyl	1.9	Highly Lipophilic	Child 1-5 yr
Fentanyl	2.8	Highly Lipophilic	Infant 1-12 mo
Lorazepam	0.7	Moderately Lipophilic	Full term neonates
Ticarcillin	1.5	Moderately Lipophilic	1 mo - 2 yr
Ketamine	1.6	Moderately Lipophilic	3-12 mo
Metoclopramide	2.0	Moderately Lipophilic	Premature neonates, 1-7 weeks post-natal
Furosemide	2.1	Moderately Lipophilic	Premature neonates
Lignocaine	2.5	Moderately Lipophilic	Premature neonates, 9-42 days
Ketamine	4.6	Moderately Lipophilic	< 3 mo
Furosemide	7.3	Moderately Lipophilic	Neonates
Vancomycin	0.7	Not Classified	Premature, 4-17 days postnatal
Theophylline	1.4	Water Soluble	Neonates, 3-36 d
Theophylline	1.4	Water Soluble	Infants
Caffeine	1.6	Water Soluble	Premature neonates
Theophylline	1.6	Water Soluble	Neonates, 1-26 d
Clavulanic Acid	1.6	Water Soluble	1 mo - 2 yr
Tobramycin	2.3	Water Soluble	10-14 yr
Theophylline	2.3	Water Soluble	Neonates

Generally, reduced plasma protein binding relative to adult levels is observed in newborns²⁶. Morselli et al. attributed reduced binding to several factors including reduced total plasma proteins concentration, presence of fetal albumin, which has lower affinity for drugs, and lower concentrations of gamma globulins and lipoproteins. Gamma globulins are lower in newborns as well as infants²⁶. Decreased binding to plasma proteins in neonates is responsible for a parallel increase in the volume of distribution of two modestly lipophilic compounds, phenobarbital and phenylbutazone¹⁰¹. Similarly, reduced levels of serum protein binding of salicylate in newborns have been reported²⁴. The volume of distribution of the hydrophilic compound, theophylline (adult 0.44-

0.55, neonate 0.2-2.8), is ~3-fold higher in the neonate, attributable to a 30% lower level of protein binding in the neonate¹³. A lower fraction of protein-bound phenytoin (modestly lipophilic) in the neonate similarly leads to a 2-fold increase in the volume of distribution¹³. The volume of distribution of gentamicin, a hydrophilic, weakly basic antibiotic with similar free fractions (or amount bound) in the neonate and adult, is ~2.5-fold higher in the neonate than the adult¹³. Amikacin, also hydrophilic, also has a Vd which is ~2 times higher in infants and children compared with adults²⁶. Other water soluble compounds, the cephalosporins and penicillins, also have a higher volume of distribution in infants and children compared with adults. The increases are attributed to increases in body water, and in the case of penicillins, additional reductions in protein binding²⁶.

Kurz et al.⁹⁸ measured the adult and newborn cord plasma binding of 20 drugs composed of several drug classes (antibiotics, barbiturates, antihistamines, opiates, sulfonamides, benzodiazepines), which included lipophilic and hydrophilic compounds, to determine if the measured differences in blood constituents affected the free fraction. Differences were small, ranging from adult/newborn ratio of fraction bound of 1 to 2.4⁹⁸. Differences in the extent of binding were not due to differences in protein concentrations, or the presence of ultrafiltrable plasma constituents, but may have been the result of higher bilirubin levels, which can compete effectively for drug binding sites on albumin. Kurz et al. extended this analysis by measuring differences in drug binding to the albumin and globulin fractions of adult and newborn plasma in the presence and absence of bilirubin. Of the six tested compounds, only promethazine, a highly lipophilic compound, had a difference in binding to the albumin fraction; binding was higher to cord blood albumin. Bilirubin had no influence on the binding of three lipophilic compounds, promethazine, thiopental or desipramine, nor did it influence binding of water soluble salicylic acid to albumin. Nevertheless, decreased binding of two hydrophilic compounds, nitrofurantoin and sulfamethoxydiazine, was observed. The effect was greatest in cord albumin from newborns. Binding of the lipophilic compounds (promethazine, thiopental and desipramine) to newborn globulins was lower than to adult globulins. These three drugs have the highest octanol water partition coefficients (~14-600) of the test drugs. Reduced binding of these drugs to globulins in the newborn can be attributed to lower concentrations of lipoproteins in the globulin fraction. The water soluble compounds (nitrofurantoin and meticillin) were not bound to adult or newborn globulins, reflecting their low octanol water partition coefficients (0.01-2). Collectively, these binding data demonstrate the quantitative differences in binding to plasma proteins that can lead to differences in measured volumes of distribution and clearance. Such differences are influenced by the physiochemical characteristics of the compound and the composition and concentration of plasma constituents, including albumin, globulins and bilirubin. In addition, it has been suggested that competition between bilirubin and other toxicants may result in release of these compounds from albumin binding sites, unexpectedly high serum free concentrations and associated toxicity²⁶. The volume of distribution of digoxin is 5-12 times lower in newborns than adults²⁶. This difference has been attributed

to several factors, including lower plasma protein binding, higher tissue binding and larger extracellular fluid volume²⁶.

Elderly. Searching a large database of pharmacokinetic parameters of drugs in the young and elderly developed by Ritchel et al.⁴⁶ revealed several drugs with significant differences in volumes of distribution. Consistent with the observed decrease in lean body mass in the elderly, changes in the volume of distribution correlated with lipophilicity. Lipophilic compounds, such as the benzodiazepines chlordiazepoxide, clobazam, and diazepam, as well as the aminoglycoside antibiotic, amikacin, had higher volumes of distribution in the elderly. Water soluble drugs, such as propicillin, had a lower volume of distribution in the elderly reflecting a reduction in the relative size of the body water compartment. Amantadine, a tricycloamine of modest lipophilicity, had a lower volume of distribution in the elderly¹⁰⁶, in contrast to expectations.

Age-associated reductions in serum albumin concentrations increase the serum free fraction of the lipophilic benzodiazepines, chlordiazepoxide, diazepam, lorazepam, and alprazolam⁹⁵. Proportionate increases in their hepatic clearance are observed, resulting in minimal changes in free concentrations at steady state⁹⁵. For compounds whose hepatic clearance is restricted by protein binding, increases in free fractions resulting from reductions in binding proteins may not affect a change in serum free concentrations at steady state because with the increases in free fraction comes a proportionate increase in clearance^{46, 95}. Chemicals that bind A1G or albumin, such as the lipophilic tricyclic antidepressants, have the capacity to free other bound compounds, increasing pharmacologically active free concentrations⁹⁵. This has been observed for both water soluble (salicylate, and sulfadiazine) and lipophilic drugs (phenylbutazone) in elderly patients undergoing multi-drug treatments⁴⁶.

Increases in A1G may increase binding of propranolol, which is significantly bound by A1G in the elderly², although several studies found no difference in the amount of serum A1G, or the free or total concentrations of propranolol between healthy, young adults (25-33 years) and elderly adults (62-79 years)^{99, 107-109}. Disease states common in the elderly induce increases in A1G and cause reductions in free fraction of propranolol¹¹⁰. Increases in the free fraction of propranolol can be attributed to disease-induced changes in A1G, not to age-dependent changes in A1G. The volume of distribution of the hydrophilic antibiotic gentamicin is between 0.2 and 0.37 L/kg in both the young and elderly¹¹¹. This is one of many water soluble or modestly lipophilic pharmaceuticals (e.g., aspirin, digoxin, lidocain, theophylline) that present no difference in the Vd between the young and elderly¹⁰⁶, demonstrating the influence of physiological changes in the elderly on distribution is chemical-specific.

Gender. Gender differences in lean body mass, body fat, and to a lesser extent, plasma protein concentrations, are expected to lead to parallel differences in the disposition of drugs between males and females. Wilson's summary of gender differences in drug disposition indicates that for two lipophilic

compounds, chlordiazepoxide and diazepam, the Vd is 27-28% higher in women^{11,2}. In contrast, the Vd of more water soluble, less lipophilic paracetamol is 36% lower in women compared with men. These changes are statistically significant. While the direction of the difference depends on the properties of the chemical, the magnitude of the difference appears to be reasonably similar. Gender differences for vancomycin, fluoroquinolones and theophylline have also been documented¹⁰. The Vd of vancomycin (solubility not reported) is 11% higher in women¹⁰. The Vd of the hydrophilic fluoroquinolones, fleroxacin, levofloxacin and ofloxacin, are each lower in the female, levofloxacin being 15% lower. Similarly, theophylline, which is hydrophilic, has a Vd that is 10% or 22% smaller in young and elderly women, respectively, when compared with corresponding male groups¹⁰. The observed differences in the Vd for these compounds, both lipophilic and hydrophilic, are consistent with gender-specific differences in lean body mass. In fact, normalization of the Vd for lean body mass minimizes gender-specific differences in Vd for some chemicals¹⁰.

3.3 Metabolism

The terms metabolism and biotransformation are often used interchangeably to refer to the enzymatic transformation of xenobiotics to metabolites. Metabolism may also refer to the total fate of a chemical in the body, including absorption, distribution, biotransformation, and excretion. However, in this section, metabolism will be used to refer to the process of enzymatic transformation of xenobiotics. There are two types of metabolic reactions catalyzed by enzymes: Phase I and Phase II²⁸. Phase I reactions involve hydrolysis, reduction, or oxidation of the xenobiotic to expose or introduce a functional group. Phase II reactions involve glucuronidation, sulfation, acetylation, methylation, and conjugation with glutathione or amino acids. The Phase I and Phase II reactions and the enzymes involved in each are listed in Table 6. Some xenobiotics undergo both Phase I and Phase II reactions; however, a Phase II reaction does not have to be preceded by a Phase I reaction²⁸. Both types of reactions may be influenced by variations in age and gender.

Metabolic enzymes are present throughout the body in several subcellular compartments²⁸. In humans, the liver is the primary source of metabolic enzymes. However, metabolic enzymes can also be found at the major routes of entry for environmental contaminants, including the skin, lung, nasal mucosa, eye, and gastrointestinal tract²⁸. In addition, enzymatic activity can also be seen in the kidney, adrenal glands, pancreas, spleen, heart, brain, testis, ovary, placenta, plasma, erythrocytes, platelets, lymphocytes, and aorta²⁸. Chemicals may undergo metabolic transformation in these tissues, although the metabolic capacity of these tissues is limited when compared with the liver.

Table 6
Phase I and Phase II Metabolic Reactions and Associated Enzymes

TYPE OF REACTION	ENZYME
Phase I	
Hydrolysis	Carboxylesterase
	Peptidase
	Epoxide hydrolase
Reduction	Axo- and nitro-reduction
	Carbonyl reduction
	Disulfide reduction
	Sulfoxide reduction
	Quinone reduction
	Reductive dehalogenation
Oxidation	Alcohol dehydrogenase
	Aldehyde dehydrogenase
	Aldehyde oxidase
	Xanthine oxidase
	Monoamine oxidase
	Diamine oxidase
	Prostaglandin H synthase
	Flavin Monooxygenase
	Cytochrome P450
Phase II	
	Glucoronide conjugation
	Sulfate conjugation
	Glutathione conjugation
	Amino acid conjugation
	Acylation
	Methylation

Both Phase I and Phase II reactions undergo maturation during the time between infancy and early childhood^{24, 89}. The process of aging, however, does not uniformly affect the hepatic metabolic capacity⁸. The Phase I and II metabolic pathways develop at different rates, vary among individuals, and can be induced *in utero*, making age-related changes in biotransformation complex and difficult to predict²⁴. Total enzyme and esterase activity is decreased during the neonatal and post-natal periods¹³. Phase I activity is present at birth and gradually increases to adult levels by about 6 months of age. Phase II conjugation reactions are reduced at birth but activity does vary. For example, glucuronic acid is decreased at birth; however, sulfate conjugation is well expressed. Glycine conjugation in neonates is reported to be comparable to that of adults. Enzymes responsible for theophylline oxidation and caffeine methylation are found in premature infants; however, enzymes responsible for

oxidative demethylation do not develop until several months after birth. In general, drugs and chemicals that undergo Phase I-type reactions tend to have a slower clearance in the elderly, while Phase II-type reactions are not affected by the aging process⁸.

A review of the scientific literature indicated that data regarding age and gender differences in metabolism have been collected for several pharmaceutical agents, with considerably less data available for environmental chemicals. However, the data for the pharmaceutical agents can be used to demonstrate any age- and gender-specific differences in metabolism. For an enzyme that acts on a particular chemical class or substrate (e.g., an enzyme that acts by adding hydroxyl groups to benzene rings), any quantitative age- and gender-specific difference in metabolism applicable to these pharmaceutical agents would also be relevant for structurally similar environmental chemicals.

3.3.1 Physiological and Biochemical Determinants of Metabolism

While the majority of tissues have the capacity to metabolize some xenobiotics, the enzyme systems responsible for metabolism of foreign compounds are primarily present in the liver. While extrahepatic metabolism can contribute significantly to local metabolism and toxicity, this section focuses on hepatic metabolism because the liver has the highest metabolic capacity for xenobiotics, it is the central organ for systemic metabolism, and it is the most completely understood.

Two sources of blood deliver xenobiotics to the liver, arterial blood through the hepatic artery and venous flow through the portal vein. Xenobiotics absorbed through the intestinal tract may be delivered directly to the liver through the portal vein, or transported within the triglyceride pool in chylomicrons to the vena cava via the thoracic duct. Delivery of compounds through the portal vein to the liver before entering the general venous blood compartment results in a first pass effect for this route of exposure. Compounds delivered to the liver enter hepatocytes primarily by passive diffusion, where they are subject to metabolism. The Phase II reactions typically increase transfer of the conjugated product across hepatic, intestinal and renal membranes and as a result, elimination. Phase I enzymes include the P450 family of proteins, esterases, epoxide hydrolase and alcohol and aldehyde dehydrogenases among others. Phase II enzymes include glucuronosyltransferases, sulfotransferases and glutathione-S-transferases among others.

The extent of metabolism depends on the concentration, composition and metabolic constants (V_{max} , K_m) of the hepatic enzymes, as well as sub-cellular localization and the rate of delivery (equal to the perfusion rate * concentration). The maximum capacity (V_{max}) and Michaelis Constant (K_m) are intrinsic properties of the enzyme and are not expected to change with age. The expression and concentration of Phase I and II enzymes in the liver are regulated by various factors, hormone status for instance, that may change with age,

increasing or decreasing the total metabolic capacity of the liver. The size of the liver also changes with age, leading to changes in total metabolic capacity, which in the absence of disease is proportional to body weight raised to the $3/4$ power. Changes in hepatic blood flow can affect changes in the rate of delivery, and under some conditions (perfusion limited metabolism), changes in metabolism.

During aging and development changes in the size of the liver, the composition and concentration (collectively "expression") of the Phase I and Phase II enzymes, as well as the hepatic blood flow, have the potential to influence the extent of metabolism. Age-dependent changes in these processes do occur and are described in the following sections, organized by enzyme system.

3.3.2 Influence of Age on Phase I Enzyme Systems

Phase I reactions are catalyzed by the mixed function oxidase (MFO) system, including cytochrome P450, cytochrome b5, and NADPH cytochrome c reductase, which are present primarily in the adult, fetal and neonatal liver²⁴. In the fetus, premature, infant and full-term neonate, cytochrome P450 is approximately 50-70% of the measured levels in adults²⁴. NADPH cytochrome c reductase activity is lower in premature infants than in full-term infants, and both are lower than adult values²⁴. Milsap and Jusko¹³ reported that esterase activity in premature infants does not reach normal infant levels until 10-12 months of age.

Cytochrome P450. The cytochrome P450 family of enzymes is the primary enzyme system involved in Phase I metabolic reactions²⁸. The P450 family consists of several different isoenzymes with varying degrees of substrate selectivity. Total cytochrome P450 activity in humans appears to remain stable from fetal development (both premature and full-term) through the first year of life^{24, 30}. One study reported similar cytochrome P450 activity between fetal and adult human liver¹¹³. However, other studies have indicated that total cytochrome P450 activity varies significantly between children and adults^{113, 114}. Hepatic cytochrome P450 activity levels in the fetus and neonate are reported to be approximately 50-70% of measured values in the adult²⁴.

The influence of age on the activity of independent isoenzymes has been studied. Isoenzymes of the CYP4A and CYP3A families are the major isoenzymes detected readily in the fetal liver¹¹⁵. The relative concentration of CYP4A1 in the fetal liver was 40% or more of the adult concentration during the first week after birth¹¹⁵. The CYP3A family of isoenzymes, consisting of CYP3A4, CYP3A5, and CYP3A7, is the most abundant P450 enzyme in human liver microsomes³⁰, and is age-dependent, with newborns having 25-50% less activity than adults^{27, 28}. CYP3A5 isoenzymes are present in embryonic liver tissue, and to a lesser extent, fetal liver tissue. Reports of one study showed 50% of infant liver expressed CYP3A5 activity, while only 29% of adult livers expressed CYP3A5 activity³⁰. While variations have been reported in the concentrations of the isoenzymes in

the CYP3A family, total CYP3A concentrations appear to remain constant from early gestation to adulthood^{115,116}.

CYP3A7 has been detected in the fetal liver at 17-32 weeks of gestation and accounts for 36-85% of the total P450 present in the fetal liver²⁹. The activity of CYP3A7 is positively correlated with testosterone 6 β -hydroxylase and dehydroepiandrosterone 16 α -hydroxylase activities²⁹. Levels of CYP3A7 decline to adult levels at 3-12 months of age and CYP3A4, which is not present in the fetal liver, becomes the major P450 isoenzyme in the newborn and adult liver²⁹⁻³¹. Levels of CYP3A4 begin to rise in the newborn around day 7 and peak at about 1 year of age³⁰. LaCroix et al.¹¹⁶ reported that CYP3A4 levels are very low at birth and reach 30-40% of adult levels by 1 month of age.

There is evidence that CYP3A4 levels are lowest in newborns and peak during adulthood³². Decreases in the levels of CYP3A4 have been noted in adults from the ages of 20 to 80 years³². However, other investigations concluded that there was no decrease in the activity of CYP3A4 with age in the human liver³³. There is, however, a significant decrease in liver mass (35%), liver blood flow (35%) and volume (24-44%) between adulthood and late old age that could account for the decline in total systemic clearance of CYP3A4 prototype compounds³³. Hepatic regional blood flow decreases by 0.3-1.5% per year after age 25⁴⁶.

In addition to its presence in the fetal liver, CYP3A7 mRNA and protein have been detected in the endometrium and placenta³⁰. The levels of CYP3A7 are higher during pregnancy and increase significantly from the first to the second trimester³⁰. Compared to CYP3A7 levels in the fetal liver, the amount of CYP3A7 in the placenta and the endometrium per gram of tissue ranged from 0.6% to 5.5%³⁰.

The CYP3A family of isoenzymes is involved in the metabolism of a large group of xenobiotics and steroids²⁸. In order to understand the effects related to the age-dependent variation of the CYP3A isoenzymes, one must be aware of the magnitude and variety of substances and compounds that are metabolized by the CYP3A isoenzyme family (Table 7).

Most of the interactions between CYP3A isoenzymes and its substrates are the result of either induction or inhibition of the CYP3A enzyme. Induction increases the content of the isoenzyme and enhances drug clearance. Substrates known to induce CYP3A4 activity *in vivo* and *in vitro* include corticosteroids, anticonvulsants, and several antimicrobials³⁰. Compounds that are known to inhibit CYP3A4 both *in vivo* and *in vitro* include imidazole derivatives, erythromycin, clarithromycin, troleandomycin, gestodene, ritonavir, fluvoxamine, and grapefruit juice. Dexamethasone, rifampicin, and phenobarbital are known to induce CYP3A4 but do not appear to induce CYP3A5 activity. However, the CYP3A5 gene does contain the sequence information needed to corticosteroid regulation of transcription³⁰. Triazolam, gestodone, ketoconazole and fluconazole have been shown to inhibit CYP3A5 activity, as well

as CYP3A4 activity. Rifampicin induces CYP3A7 expression in adult hepatocytes and gestodene inhibits its activity. Dose-dependent increases have been noted following pretreatment of HepG2 cells expressing CYP3A7 with dexamethasone, rifampicin, troleandomycin, erythromycin, phenobarbital, or lovastatin³⁰.

Table 7
Important Substrates of CYP3A Isoenzymes

Pharmaceuticals in the following categories:

Antihistamines
Antireflux
Anti-emetic
Anticonvulsants
Anti-HIV
Antimicrobials
Antifungals
Immunosuppressants
Chemotherapeutics
Benzodiazepines
Anaesthesia-analgesics
Antihypertensives
Anti-arrhythmics
Antidepressants

Xenobiotics:

Aflatoxin B1
Benzphetamine
Benzopyrene
Heterocyclic amines
Sterigmatocystin

Endogenous substrates:

Androstenedione (6 β -hydroxylation)
Cortisol (6 β -hydroxylation)
Dehydroepiandrosterone
Dehydroepiandrosterone sulfate
Estradiol
17 β -Ethinylestradiol
Progesterone (6 β -hydroxylation)
Testosterone (2 β -hydroxylation, 6 β -hydroxylation, and 15 β -hydroxylation)

Source: ³⁰.

When one considers the age-dependent variation of the CYP3A family and the magnitude of substrates related to this family of isoenzymes, it is obvious that age-dependent effects are important. This is especially true when treating infants and children with pharmaceutical substrates of the CYP3A family. Nifedipine, a CYP3A substrate and antihypertensive drug, has a shorter half-life

in children age 5-68 months when compared to adults. Cyclosporin and tacrolimus, both CYP3A substrates, are immunosuppressants used in pediatric transplant patients to prevent rejection. Both drugs have a higher plasma clearance rate in children, requiring higher doses per kilogram body weight in younger children to maintain plasma concentrations equal to adults. Some of the variation in plasma clearance could be due to changes in body weight; however, it is also suggestive of increased hepatic and intestinal CYP3A activity in younger children³⁰.

In studies performed by Jacqz-Aigrain and Cresteil¹¹⁷, results indicate that in adults, CYP3A4 is the major enzyme involved in the N-demethylation of dextromethorphan; and O- and N-demethylations of dextromethorphan are carried out by isoenzymes in the CYP2D and CYP3A subfamilies, respectively. Using dextromethorphan as an indicator of the levels of CYP3A and CYP2D6 present, concentrations in the fetal and adult liver were compared. O-demethylation activity was present in the fetal liver but it did not exceed 5-10% of the adult values. During the first week after birth, O-demethylation rates increased to 25% of the adult levels. However, all of the fetal and neonatal liver preparations were active in the N-demethylation, reaching 30% of the adult values. Immunoinhibition studies using anti-CYP3A IgG showed that the anti-CYP3A IgG had only a small inhibitory effect on methoxymorphinan formation in fetuses. These results indicate the fetal liver contains a specific form of CYP3A with different catalytic properties for dextromethorphan than those in the adult liver.

CYP2D6 protein levels in fetuses and neonates of less than 24 hours of age were reported to be less than 5% of adult values^{117, 118}. The levels of CYP2D6 begin to rise steadily during days 1 through 28 following birth. The increase observed in CYP2D6 was independent of gestational age at birth and reaches 2/3 of the adult value in infants aged 1 month to 5 years. A similar pattern was reported for CYP2C (Figure 7). Little or no protein content was reported in infants less than 24 hours of age. However, CYP2C levels steadily increased during the first week of birth and reached 1/3 of the adult rate by the end of the first month¹¹⁹.

Other studies have shown variations in the CYP1A, CYP2C, and CYP2E isoenzyme family. Sonnier and Cresteil¹²⁰ examined the ontogenesis of CYP1A proteins in human fetal, neonatal, and adult liver samples. Results indicated that there was no CYP1A2 protein present in the fetal and early neonatal liver samples. In the first month of life, CYP1A2 was about 3% of the adult values. The levels began to increase during the next 3 months and were approximately 50% of adult values at the end of 1 year (Figure 8).

The developmental expression of CYP2E1 in the fetal and newborn liver was studied by Vieira et al.¹²¹. Results showed that the CYP2E1 isoenzyme was not present in the fetal liver; however, a surge in the isoenzyme occurred during the first few hours after birth regardless of gestational age. At 1 year of age, the CYP2E1 levels reached approximately 40% of the adult levels (Figure 9).

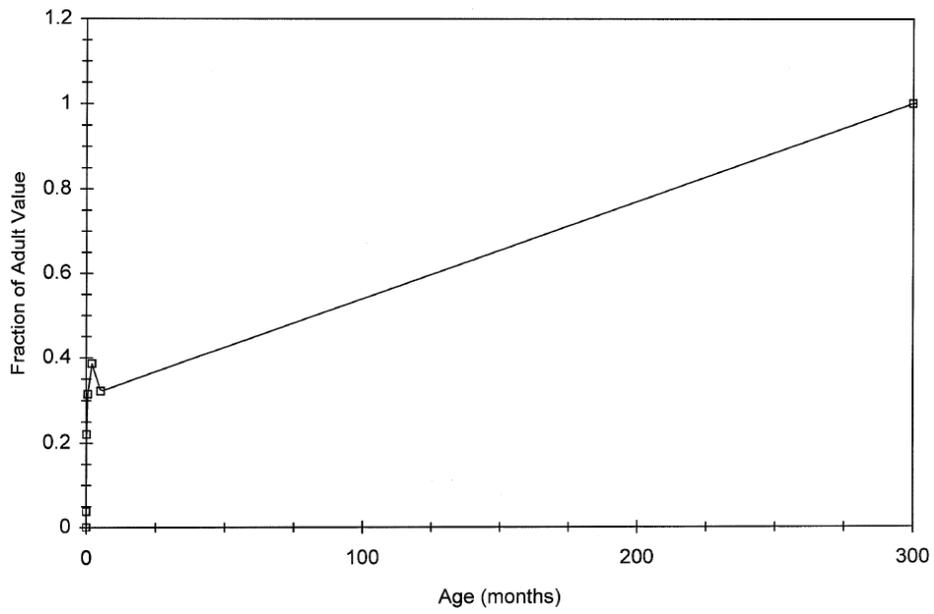


Figure 7. Age-dependent activity of CYP2C.

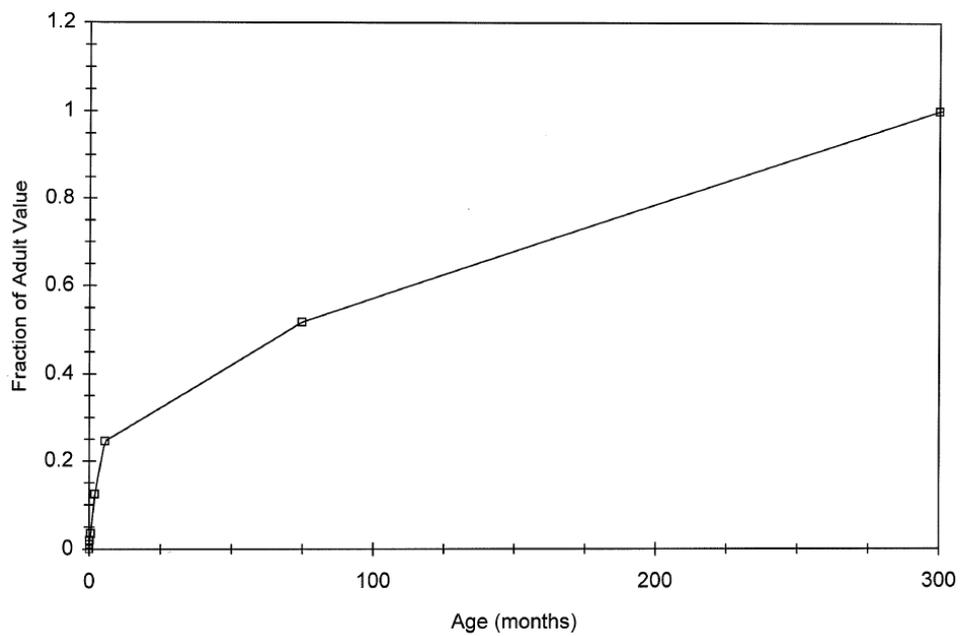


Figure 8. Age-dependent activity of CYP1A2.

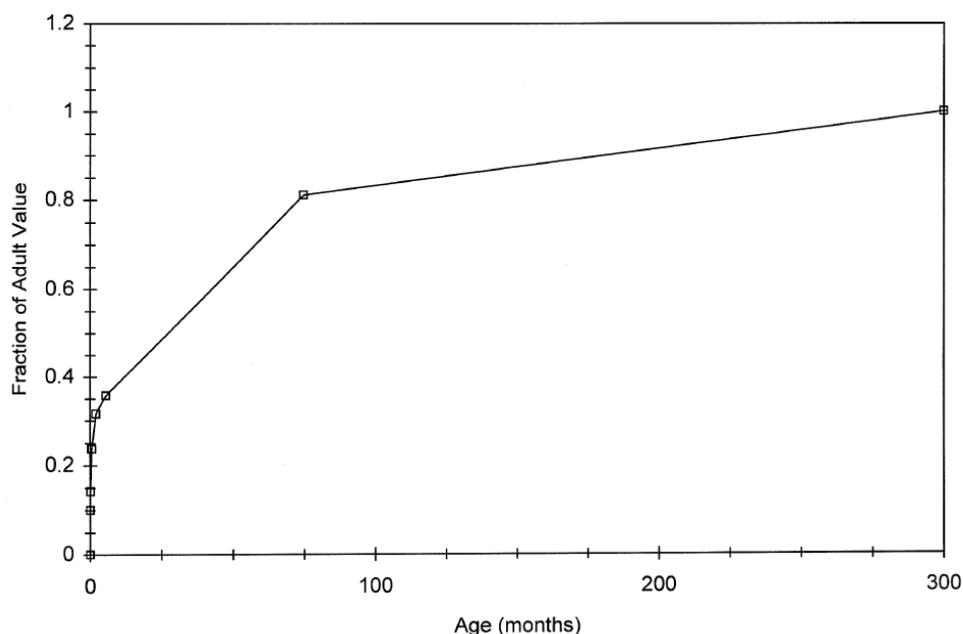


Figure 9. Age-dependent activity of CYP2E1.

In contrast to the abrupt increase in CYP2E1 protein content, the CYP2E1 RNA moderately increased throughout early postnatal development. The concentration of CYP2E1 RNA was 15 times lower in early neonates when compared with adults with major increases occurring in newborns 1 to 3 months of age. At 3 to 12 months of age the infants reached 50% of the adult levels of CYP2E1 RNA.

In epilepsy patients ranging from 3 months to 29 years old, there is a significant correlation between age and the dose ratio of carbamazepine, an anticonvulsant drug. The metabolism of carbamazepine to carbamazepine-10, 11-epoxide is catalyzed by CYP3A4. In addition, there is some evidence that CYP3A7 also catalyzes this reaction based on the presence of the epoxide metabolite in stillborn fetuses of mothers receiving carbamazepine during pregnancy. An inverse relationship between the epoxide metabolite and carbamazepine also exists in children from 2 weeks to 15 years old being treated with carbamazepine³⁰. Cisapride is a prokinetic drug metabolized primarily by CYP3A4. When given with other CYP3A4 inhibitors, cisapride has been shown to cause prolonged QTc intervals and serious ventricular arrhythmias, suggesting that low levels of CYP3A4 in infants predisposes them to concentration-related effects of the drug.

Although the compounds used in this example are pharmaceutical agents, a similar scenario could also be observed with environmental chemicals that are metabolized by CYP3A. Consequently, depending on the chemical and if the parent or metabolite is the toxic moiety, the fetus and neonate may be more or less sensitive to a toxic chemical that is metabolized by CYP3A.

Influence of Age on Other Phase I Reactions. Hydrolysis is a major biotransformation reaction for certain substrates, such as procaine, pethidine,

meprobamate, paroxone, and acetylsalicylic acid¹¹³. Hydrolytic activity develops after birth in animals, in all organs, and in humans.

Esterases, which are involved in Phase I hydrolysis reactions, catalyze hydrolytic cleavage of ester bonds to produce carboxyl groups and an alcohol. The carboxyl groups and alcohols can then undergo Phase II conjugations. There are four main groups of esterases¹²²:

- Arylesterases, which hydrolyze aromatic esters,
- Carboxylesterases, which hydrolyze aliphatic esters,
- Acetylersterases, in which the acid group of the ester is acetic acid,
- Cholinesterases, which hydrolyze esters where the alcohol group is choline.

Many drugs and exogenous chemicals undergo esterase-catalyzed hydrolysis, including analgesics, steroids, local anesthetics, organophosphorous ester insecticides, and phthalates³⁴. Studies have been performed to determine the age-related effects and perinatal development of red cell acetylcholinesterase (ACHE), plasma proteins, plasma pseudocholinesterase (ppACHE), and arylesterases (ArE)³⁴. Results showed an increase in ACHE plasma protein concentration between 28 and 40 weeks of gestation and a slower rate of increase until one year of age. Rapid increases in ppACHE and ArE occurred between 28 weeks of age to one year with no changes occurring after one year. Red cell ACHE levels in infants were approximately 55% of adult values and ppACHE levels were 50-60% of adult values. At 34 weeks gestation ppACHE levels were comparable to infant values; however, at 28-31 weeks of gestation ppACHE levels were about 41% and 65% of the adult levels, respectively.

Plasma pseudocholinesterase is responsible for the hydrolysis of local anesthetics including procaine. In an experiment to compare ppACHE activity with the rate of procaine hydrolysis, results indicated low hydrolysis activity at 28 weeks of gestation. These results correspond to the low levels of ppACHE at 28-31 weeks of gestation reported in the earlier study³⁴.

Lower esterase activity has also been reported in premature infants, when compared to full-term infants. The decreased blood esterase levels and corresponding low hydrolysis rate could lead to an increase in the volume of distribution. This could account for the prolonged effect and cardiorespiratory depression in preterm infants administered drugs containing ester bonds²⁶.

Dehydrogenation is another Phase I reaction important in the metabolism of certain alcohols. Alcohol dehydrogenase (ADH) is detectable in the human fetal liver in the second month of gestation at approximately 3% to 4% of the adult levels¹¹³. However, adult levels of ADH were not detected until 5 years of age (Table 8). Studies have shown that one, two, and four isoenzymes have been detected in the fetal, newborn, and adult liver, respectively.

Table 8.
Development of Alcohol Dehydrogenase Activity
in Fetal and Postnatal Human Liver

	Age	ADH Activity	
		mU/g liver wet weight	mU/100 mg soluble protein
Fetal	2 - 3 months	111	97
	3 - 4 months	145	135
	3 - 4 months	155	147
	4 months	163	155
	4 months	211	201
	4 - 5 months	246	236
	4 - 5 months	239	228
	4 - 5 months	411	318
	5 - 6 months	328	321
Postnatal	0.3 months	495	550
	2 months	444	555
	7 months	797	1025
	2 years	620	1030
	5 years	3170	2830
	10 years	945	2360
	15 years	1940	3880
Adult	20 years	1625	2030
	50 years	2040	2550
	50 years	6530	5430

Source:¹⁴⁵.

3.3.3 Influence of Gender on Phase I Enzyme Systems

Cytochrome P450. The cytochrome P450 system includes a subfamily of approximately 30 isoenzymes. Gender differences have been noted in some of these isoenzymes, including CYP1A2, CYP2A1, CYP2C19, CYP2D1, CYP2D6, CYP2E1, CYP3A4, CYP2C7, CYP2C12, CYP2C13, and CYP2C11. It has been suggested that the activity of most of these isoenzymes is greater in males when compared to females^{10, 32, 37, 123}. However, conflicting information has been reported for CYP3A4 and CYP2C19 activity in males versus females³². Other studies have shown that some isoenzymes are gender-specific¹²⁴. Several different factors have been investigated to determine the reason(s) for these gender differences. Some factors suggested to contribute to the gender differences include sex hormones, body weight, body water content, and diet.

One isoenzyme that has been shown to have gender-specific differences in activity is CYP1A. The CYP1A subfamily plays a primary role in the oxidative metabolism of some critical environmental contaminants, including polycyclic

hydrocarbons, heterocyclic amines and aromatic amines. The CYP1A2 isoenzyme is expressed in human tissues, with the liver being the main site of CYP1A2 expression¹²³. Human CYP1A2 is an activator for a series of aromatic amines and heterocyclic amines during the metabolic activation of various procarcinogens and plays a major role in the activation of cigarette smoke condensate³⁵. Human CYP1A2 has been shown to catalyze phenacetin O-deethylation, 4-aminobiphenyl (4-ABP) N-oxidation, ethoxyresorufin O-deethylation, and caffeine 3-demethylation³⁵.

At least two mechanisms are suggested to regulate CYP1A2 activity, one that regulates constitutive levels of expression and one that regulates inducibility¹²³. Both aryl hydrocarbon receptor (AhR)-dependent and AhR-independent pathways may be involved in CYP1A2 induction. It has been suggested that individual susceptibility to arylamine-induced cancers in humans is highly dependent upon levels of hepatic CYP1A2³⁵. Therefore, for metabolic activation of carcinogenic arylamines and arylamides, CYP1A2 isoenzyme is thought to be the primary P450 contributor³⁵.

Studies have shown increased CYP1A2 activity in males, when compared to females^{10, 32, 35, 36}. Some believe this gender difference is due to the involvement of female hormones as demonstrated by decreased CYP1A2 activity in females using oral contraceptives versus females not using them¹²³. A lower CYP1A2 activity level has also been observed in females with caffeine toxicity; however, this decreased activity was not observed in males¹²³. The half-life of theophylline, which is metabolized in part by CYP1A2, was approximately 1.5 times less in male subjects compared to females³⁷.

Another theory noted for gender differentiation in CYP1A2 activity is diet¹²³. Studies have shown decreased CYP1A2 activity in females consuming their normal diet, when compared to males. However, following consumption of a controlled diet in males and females for one week, no gender difference in CYP1A2 activity levels was noted between males and females¹²³.

The results of drug metabolism studies have indicated greater CYP1A2 activity in males when compared to females¹⁰. In one study, decreased CYP1A2 activity was suggested, based on 2-fold higher plasma levels of fluvoxamine in females who received daily oral doses of 100 or 200 mg, when compared to plasma levels in males who received the same doses for the same time period¹⁰. In a second study, the same results were noted in the 100 mg/day group, but not in the 200 mg/day group. Because fluvoxamine is both metabolized in part by CYP1A2 and is a potent inhibitor of the CYP1A2 isoenzyme, autoinhibition of fluvoxamine metabolism could occur with increasing dose. Concentrations of clozapine and norclozapine were 35% higher in females undergoing steady-state therapeutic treatment for schizophrenia, when compared to males involved in the same treatment regime. Again, these results suggest decreased CYP1A2 activity in females, since clozapine is mainly metabolized by the CYP1A2 isoenzyme¹⁰.

The CYP3A4 isoenzyme is also involved in the Phase I metabolism of numerous pharmaceutical agents. Over 50% of therapeutic drugs, including midazolam, triazolam, carbamazepine, lidocaine, erythromycin and cyclosporin, are metabolized by CYP3A4^{10, 37}. The CYP3A4 isoenzyme is considered one of the most important isoenzymes in the P450 subfamily because it is found in critical tissues, including the gastrointestinal tract and liver³⁷. The CYP3A4 isoenzyme accounts for 60% of the cytochrome enzymes located in the liver and 70% of those in enterocytes¹⁰. Conflicting gender differences in CYP3A4 activity have been reported. CYP3A4 activity in young females has been reported as approximately 1.4 times that of males, with this difference attributed to steroid hormones. Results of *in vitro* studies have shown that progesterone induces CYP3A4 activity, while estrogen and progesterone can be competitive inhibitors of CYP3A4 activity, suggesting that the higher levels of CYP3A4 in women may be due to higher levels of progesterone in the females¹⁰. However, the results of other *in vitro* and clinical studies have also shown decreased CYP3A4 activity in males, when compared to females³². Following multiple oral doses of 12 milligrams of sertindole, an antipsychotic drug primarily metabolized by CYP3A4 and CYP2D6, plasma sertindole concentrations were 20% higher in young females and 31% higher in elderly females compared to males, suggesting increased CYP3A4 and CYP2D6 activity in males¹⁰.

The CYP2D6 isoenzyme is another member of the P450 family that plays a role in Phase I metabolism of several compounds, such as debrisoquine, sparteine, imipramine, propranolol, and codeine³⁷. The CYP2D6 isoenzyme displays a polymorphic pattern in the metabolism of these compounds. There is limited information on the influence of gender for the CYP2D6. Some studies have suggested that males have higher CYP2D6 activity than females. Males were reported to have a higher rate of clomipramine metabolism, which is mediated by CYP2D6 and CYP2C19, when compared to females³⁷. Racemic propranolol, metabolized through ring oxidation by CYP2D6 and side chain oxidation by CYP2C and CYP1A and glucuronidation, was metabolized faster in males compared to females. In addition, the oral clearance of propranolol was 63% higher in males compared to females and CYP-mediated side-chain oxidation clearance was 137% higher in males compared to females. The mechanisms for these differences are not clear; however, they suggest that men have higher levels of CYP2D6 activity than women.

The isoenzyme CYP2C19 also demonstrates a polymorphic pattern of metabolism and greater activity has been reported in males versus females^{10, 37}. CYP2C19 is responsible for the aromatic ring hydroxylation of S-mephenytoin, an anticonvulsant. CYP2C19 is stereoselective for the S-enantiomer¹²⁵. Chemicals may also undergo *N*-demethylation by CYP2C19, including diazepam, citalopram, clomipramine, amitriptyline, imipramine, S-mephenytoin, methylphenobarbital and propranolol³⁷. An increased rate of mephobarbital metabolism was reported in males who received a single oral dose (400 mg) of racemic mephobarbital, when compared to females. Males were also reported with greater CYP2C19 activity in piroxicam metabolism, since females exhibited higher plasma concentrations of piroxicam, when compared to males. Also, in a phenotypic

study involving 166 individuals, males had lower 5-mephenytoin 4'-hydroxylation than females, following a single oral dose of mephenytoin (100 mg), therefore, suggesting greater CYP2C19 activity in males. However, some reports have shown an increased CYP2C19 activity in females, when compared to males³².

The isoenzyme CYP2E1 is responsible for the metabolism of ethanol and a large number of other halogenated alkanes²⁸. Increased activity of the CYP2E1 isoenzyme has been reported in males, when compared to the activity levels determined in females³⁷. The metabolism of chlorzoxazone was reported to be one-third greater in males than females, indicating a higher rate of CYP2E1 activity in males; however, this difference was minimized when normalized according to body weight³⁷.

Influence of Gender on Other Phase I Reactions. It has been suggested that aldoketoreductase activity is higher in males, when compared to females¹⁰. This suggestion was based on an approximate 2-fold greater clearance of doxorubicin in males (59 L/h/m²), when compared to females (27 L/h/m²), with gender accounting for half of the variability as denoted by a multivariate analysis of the data. This was also confirmed by the higher proportion of doxorubicinol, the major metabolite of doxorubicin, detected in males, when compared to females.

Higher ADH activity has also been suggested to occur in males, when compared to females¹⁰. Following ethanol dosing, females had greater blood alcohol levels, when compared to males. Since ADH oxidizes simple alcohols, such as ethanol, a greater ADH activity is suggested to occur in males, when compared to females. However, other factors that may contribute to the differences observed in blood alcohol levels include body weight, body water content, rate of metabolism, and sex hormones.

Recent investigations have suggested that females have a greater rate of methylation of drugs than males¹²⁶. In a population exposed to inorganic arsenic in the drinking water, females had approximately 3% more dimethylarsinic acid (DMA) in the urine, when compared to males. This suggests that females have a higher rate of methylation of arsenic, when compared to males.

The results of the pharmacokinetic studies conducted with various pharmaceutical agents have demonstrated age- and gender-specific differences in the activity of several different P450 isozymes. Although the examples provided above are for drugs, it is likely that these observed differences would also apply to environmental chemicals. Consequently, when exposed to an environmental chemical metabolized by one of these isozymes, differences in sensitivity to the toxicity of that chemical could result. For example, the data suggest that CYP1A2 levels are higher in males than females. Therefore, males could metabolize chemicals that are CYP1A2 substrates faster than females. Depending on the chemical this could serve to detoxify the parent or activate a metabolite.

3.3.4 Influence of Age and Gender on Phase II Enzyme Systems

Glutathione Transferase. The tripeptide, glutathione, is involved in the conjugation of an enormous array of electrophilic xenobiotics and xenobiotics that can be biotransformed to electrophiles²⁸. Glutathione transferase catalyzes the conjugation of xenobiotics with glutathione by converting glutathione to the glutathione thiolate anion through deprotonation. Substrates for glutathione transferase are hydrophobic, contain an electrophilic atom, and react nonenzymatically with glutathione²⁸.

Rane and Pacifici⁴¹ studied the metabolism of styrene oxide in human fetal livers and different extrahepatic human fetal tissues obtained from legal abortions. In liver preparations, microsomal hydrazase and glutathione-S-epoxide transferase activity were detected in 7-10 aborted fetuses, with activity levels in fetal livers ranging from 1.5 to 7.8 nmoles/min/mg protein. The microsomal styrene oxide hydrazase activity in the lungs, kidneys, gut and the placenta was less than 10% of the hepatic activity. However, glutathione-S-epoxide transferase activity levels were similar in all tissues examined.

Glutathione-S-transferase activity towards styrene oxide in adults has been reported by several authors, and is higher than in fetal/neonatal tissue^{39, 40}. Adult liver hepatic glutathione-S-transferase activity towards styrene oxide was reported by Mendrala et al. to be from 3 to 40 nmol/min/mg protein, and by Pacifici et al. to be 25 nmol/min/mg protein^{39, 40}.

Sulfotransferase. Sulfation is catalyzed by sulfotransferases, which are soluble enzymes found in the liver, kidney, intestine, lung, platelets and brain²⁸. The process of sulfation transfers the SO₃ group from 3'-phosphoadenosine-5'-phosphosulfate to the xenobiotic producing a highly water soluble sulfuric acid ester²⁸. Sulfate conjugation primarily involves the biotransformation of phenols, alcohols, and primary amines, which are often products of Phase I reactions^{28, 34}. However, some compounds are sulfated without prior Phase I transformation and include compounds such as primary alcohols, secondary alcohols, catechols, nitrogen oxide, aliphatic amines, aromatic amines, aromatic hydroxylamine, and aromatic hydroxyamide. It is not uncommon for sulfate conjugation to precede or occur simultaneously with glucuronidation, another type of Phase II biotransformation²⁸.

No data were located noting gender differences in the sulfation process. However, age-related differences in sulfation rates have been noted in children and adults and are probably best illustrated in the metabolism of acetaminophen. Acetaminophen undergoes both glucuronidation and sulfation during biotransformation³⁴. The overall elimination of acetaminophen does not appear to change from infancy to adulthood; however, the dominant metabolic pathway does appear to change⁴². Acetaminophen appears to be primarily metabolized via the sulfation pathway until approximately age 9, and the glucuronide pathway becomes the primary metabolic route at around age 12³⁴. Studies show that older children (>12 years old) and adults eliminate about 50% of the

acetaminophen dose as glucuronide conjugates and about 30% as sulfate conjugates. In infants and children (<12 years old), about 45-55% of the acetaminophen dose is eliminated as a sulfate conjugate and 18-30% as glucuronide conjugates⁴². This suggests that in neonates and young children, the glucuronidation rate is lower and the sulfation rate is higher compared to adults.

Glucuronyl Transferase. Glucuronidation is one of the most important Phase II conjugation reactions, both quantitatively and qualitatively²⁸. This is due to the vast number of substrates that are able to participate in this reaction and the diversity of the acceptor groups. During this process glucuronyl transferases transform endogenous and exogenous compounds to polar, water soluble compounds which are then eliminated in the urine or bile²⁸.

Glucuronidation is decreased in children, when compared to adults^{27, 38, 127}; however, adult values are reached by the third or fourth year of life²⁴. In the human fetus, the liver has been reported as the most active site of glucuronidation.

Low levels of glucuronidation activity are seen in fetal and newborn livers. It has been reported that levels of UDP-glucuronyltransferase, a catalyst for conjugation with glucuronic acid, are undetectable or 20% less than that of adult levels in fetal tissues during the first half of gestation³⁸. Prior to birth, UDP-glucuronyltransferase activity was shown to be higher than that of adults for substrates such as p-nitrophenol, aminophenol, o-aminobenzoate, and 1-naphthol. This activity then decreased to adult levels following birth. However, for substrates such as bilirubin, morphine, and a number of steroids, UDP-glucuronyltransferase activity was shown to develop after birth and did not exceed adult levels.

In three premature (25-32 weeks of gestation) infants who died 5 minutes to 98 hours after birth, UDPG-T was virtually absent in the liver³⁸. Hepatic bilirubin UDPG-T activity was reduced in living newborns 2-42 days old, with congenital gastrointestinal obstruction. It was also reported that human fetuses aged 8-22 weeks had markedly decreased hepatic activities of both bilirubin UDPG-T and UDPG-D, when compared to normal adults.

Calabrese³⁴ reported that adult levels of glucuronidation activity are reached by 3-4 months of age; however, other studies have reported that adult levels of glucuronic acid are not reached until 3-4 years of age^{24, 26}. Klinger¹¹³ reported that at 4 months gestation, glucuronyltransferase activity is detectable and glucuronidation capacity is fully developed in the human fetal liver. The low glucuronidation activity in fetuses and newborns decreases bilirubin excretion, which could lead to hyperbilirubinemia in infants, especially premature infants³⁴. The effects of low levels of glucuronic acid in the fetus and newborn can also be seen following exposure to trichloroethylene (TCE), a widely used halogenated hydrocarbon¹²⁸. The metabolic pathway of TCE involves the CYP2E1 isoenzyme, ADH, aldehyde dehydrogenase (ALDH) and glucuronidation. The presence and

activity of CYP2E1 in the young are still unclear and study results are contradictory. However, it is known that glucuronidation and ADH activity are reduced in newborns. Decreases in glucuronidation in infants exposed to TCE could increase the formation of the toxic metabolites, trichloroacetic acid and dichloroacetic acid¹²⁸. In addition, the decrease in ADH activity effects the clearance of chloral hydrate. The reported half-life of chloral hydrate was 39.8 hours in the preterm fetus, 27.8 hours in the neonate, and 9.7 hours in children ages 1-13 years¹²⁸.

In the case of chloramphenicol, decreased glucuronidation resulted in the accumulation of the parent compound in children and led to toxicity^{24, 27, 34}. Chloramphenicol has been known to produce death in premature infants being treated for infections with the antibiotic³⁴. In the case of morphine, which is also metabolized by glucuronidation, the metabolite has greater toxicity. Therefore, children show lower plasma levels of the toxic metabolite than adults exposed to morphine²⁷.

Gender differences are more easily detected in drugs that are directly metabolized by a Phase II reaction²⁸. Reports have suggested that glucuronyl transferase activity is higher in males, when compared to females¹⁰. However, this is only true for some isoenzymes and, therefore, only for certain drugs.

Glycine Conjugation. Glycine conjugation involves the conjugation of xenobiotics containing a carboxylic acid group with the amino group of glycine²⁸. Klinger¹¹³ reported that by 8 weeks of age, normal children reach adult activity levels of glycine conjugation. Conjugation between aromatic carboxyl groups and an α -amino group of amino acids (mainly glycine) has been observed in neonates, with adult values reached by 6 months of age²⁴. Following aspirin administration, concentrations of salicylic acid were greater in females, when compared to males, therefore, suggesting a higher metabolic activity for glycine conjugation in males¹⁰.

Acetylation. Acetylation involves the biotransformation of xenobiotics containing an aromatic amine or hydrazine group²⁸. The aromatic amine group is transformed to an aromatic amide and the hydrazine group is transformed to a hydrazide. N-acetylation is catalyzed by N-acetyl transferase and requires the cofactor acetyl-coenzyme A. The biotransformation occurs in two steps. First, the acetyl group from the coenzyme is transferred to an active site cysteine residue within an N-acetyl transferase with release of coenzyme A. Then the acetyl group is transferred from the acetylated enzyme to the amino group of the substrate with regeneration of the enzyme²⁸. Premature and mature newborns have been reported with a low acetylation rate for sulfonamides¹¹³. However, this hepatic acetylation rate increases postnatally.

3.4 Elimination

Elimination of chemicals can occur through the kidney (urine), liver (bile), lungs (air), oral cavity (saliva), mammary glands (breast milk), hair and skin. For drugs and other xenobiotics, routes other than the kidney are generally of minor importance^{43, 46}. Fecal elimination, which is dominated by biliary excretion, although typically less important than renal elimination, can be important for some classes of compounds⁴³. The kidney is the main route of excretion of water soluble compounds and the water soluble metabolites of lipophilic compounds^{46, 129}. Lipophilic compounds, presumably the nonbound serum fraction, can also be excreted by the renal system³. However, since clearance is typically reported as total clearance rather than Cl_{ren} , attributing changes in total clearance (Cl_{tot}) to changes in Cl_{ren} should be done only when Cl_{ren} dominates Cl_{tot} ⁴⁶. Lactational elimination occurs by passive diffusion of non-ionized or lipophilic compounds. Lipophilic compounds diffuse with dietary fats into milk, where the excreted compound becomes a source of exposure for the neonate. While some patterns of biliary excretion have been described, for instance, higher probability of biliary excretion for compounds with molecular weights greater than 325 grams/mole compared with lower molecular weight compounds, predicting biliary excretion by class of compound is not possible. The chemical properties that determine whether a compound is excreted into the bile are poorly understood⁴³. In addition, biliary excretion does not equate directly with elimination because re-uptake of the compound from the intestine, particularly those that are sufficiently lipophilic, is possible. This section will focus on renal and lactational elimination, reflecting the importance of the renal system for elimination of xenobiotics and the importance of lactational elimination for perinatal exposure. Other routes of elimination are not addressed because of limited information, and in the case of biliary excretion, the difficulty in predicting biliary excretion by physicochemical characteristics and with equating excretion by this route with elimination.

3.4.1 Physiological Determinants of Renal Clearance

Renal clearance is a function of three major processes, glomerular filtration, tubular secretion, and tubular re-uptake. Two processes, glomerular filtration and tubular secretion, move compounds to the proximal tubule and eventually to the urine, while tubular re-uptake moves compounds from the lumen of the proximal tubule back to the tubular cell. This latter process occurs by passive diffusion and is limited to non-ionic compounds³. Binding of compounds, the best studied of which are pharmaceuticals, to serum proteins diminishes renal elimination³. The net renal clearance is the difference between the rates of glomerular filtration, tubular secretion and tubular re-uptake.

Glomerular filtration is the transport of material from the renal blood flow, through the pores of the glomerulus into the proximal tubule. Renal blood flow, size of the kidney (number of nephrons), and maturity of the glomerulus all influence the effective GFR. This process accounts for a maximum of 20% of the total excretion capacity of the renal system under "conditions of maximum load"³.

Tubular secretion is an energy-dependent, active-transport process with unique systems for weak organic acids and weak organic bases³. Tubular secretion is activated when blood/plasma concentrations of a compound exceed a certain level. The process has a maximum transport value, T_m , which is characterized as being very high but limited. For chemicals that are 80% eliminated by tubular secretion rather than simple glomerular filtration, saturation of the tubular secretion process can easily be reached³. The capacity of tubular secretion is dependent on the mass and length of tubular cells, blood flow to the peritubular area, and the functioning of the necessary energy producing process³. Tubular reabsorption is a passive process of diffusion that is selective for non-ionic compounds, and is, therefore, dependent on urine pH³. Renal blood flow, kidney size, GFR, active tubular secretion and reabsorption decrease with age, with the attenuation beginning between the second and fourth¹³⁰. Renal clearance of drugs that are cleared predominantly by the kidney system is expected to parallel the maturation¹³ and decline of the renal system.

3.4.2 Influence of Age and Gender on Processes That Influence Renal Elimination

The processes governing renal function, GFR, tubular secretion and tubular absorption, which are themselves a function of kidney size, the number and size of nephrons, and renal blood flow, among others, are immature at birth. The absolute weight of the kidneys in newborns is 21.5 g (female) to 24.1 g (male). The ratio of kidney weight to body mass in newborns is 2-fold that of adults²⁶. Kidney size rises rapidly after birth, increasing approximately 3-fold by 9-12 months of age, and then enters a long phase of slower growth in proportion to body weight, which slows between the ages of 20 and 40 years¹ (Figure 4). From birth through adolescence, kidney weight as a fraction of body weight drops (Figure 10). At birth, kidney weight is 1% of body weight. The comparable adult level is 0.5%²⁴. The drop in fractional kidney weight is expected; kidney weight scales with body weight to the $3/4$ power (Figure 11), and so growth occurs in proportion to body weight to the $3/4$ power.

Renal blood flow is very low, and vascular resistance is high at birth²⁶. Increases in cardiac output and reductions in vascular resistance result in increases in renal blood flow²⁶. After reaching adult levels, renal blood flow decreases 1.1-1.9% per year after age 25⁴⁶ (Figure 12).

The reduced GFR in newborns is the result of the relatively small size and number of glomeruli, immature conditions of the nephrons, low renal blood flow and the reduced rate of functioning glomeruli³. GFR increases rapidly after birth in response to increases in renal blood flow²⁶ and is more advanced than tubular absorption at birth (Figure 13)^{13, 26}. This difference in maturation between the two processes disappears after 6 months. GFR at birth is between 2-4 ml/min, rising up to 4-fold in the first 72 hours of life¹³ to 8-20 ml/min⁶. When normalized to kidney mass (ml/min/kg cell mass), GFR reaches adult levels between 3 and 10 days of age³.

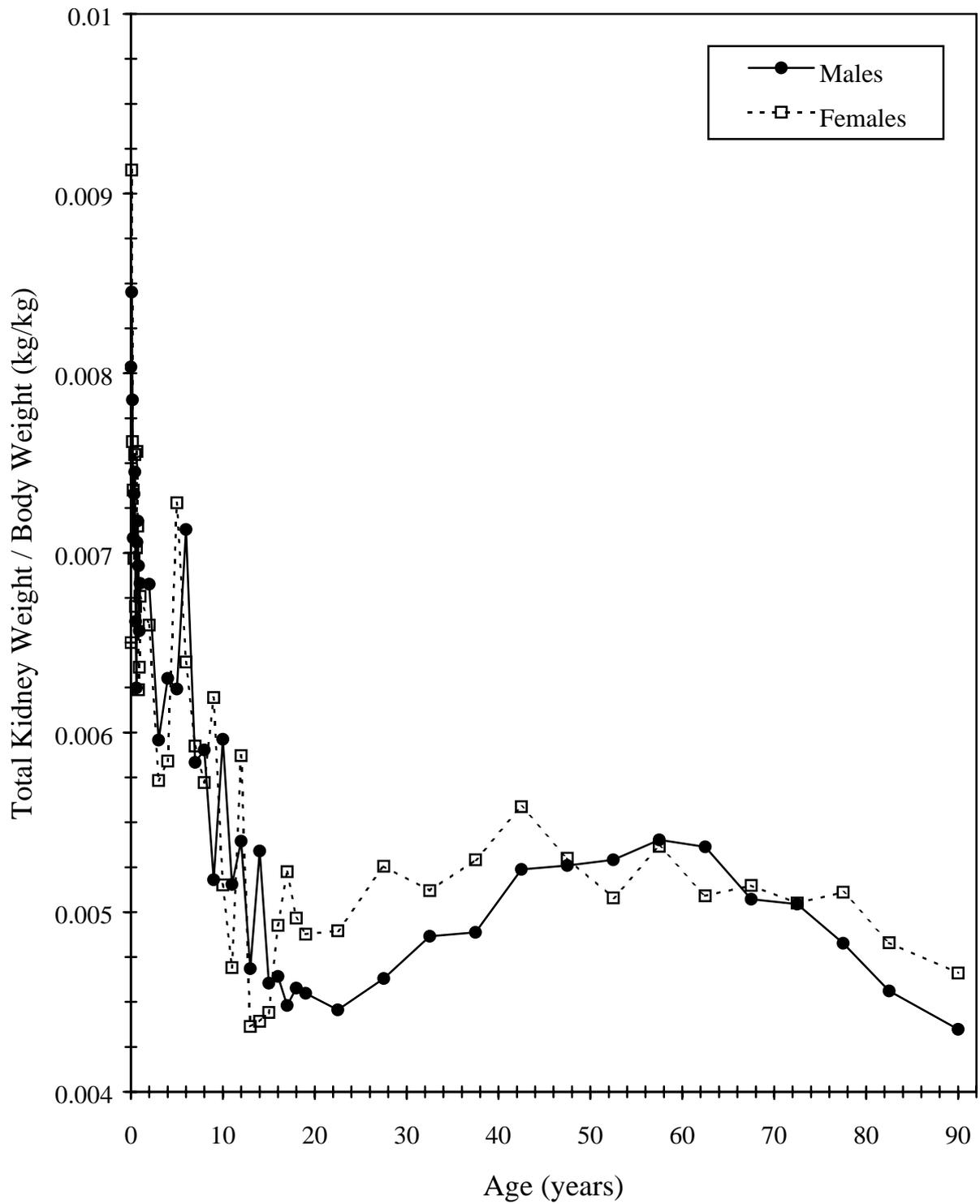


Figure 10. The relationship between relative total kidney weight and age in Japanese from birth to old age. Adapted from: Ogiu *et al.*⁴

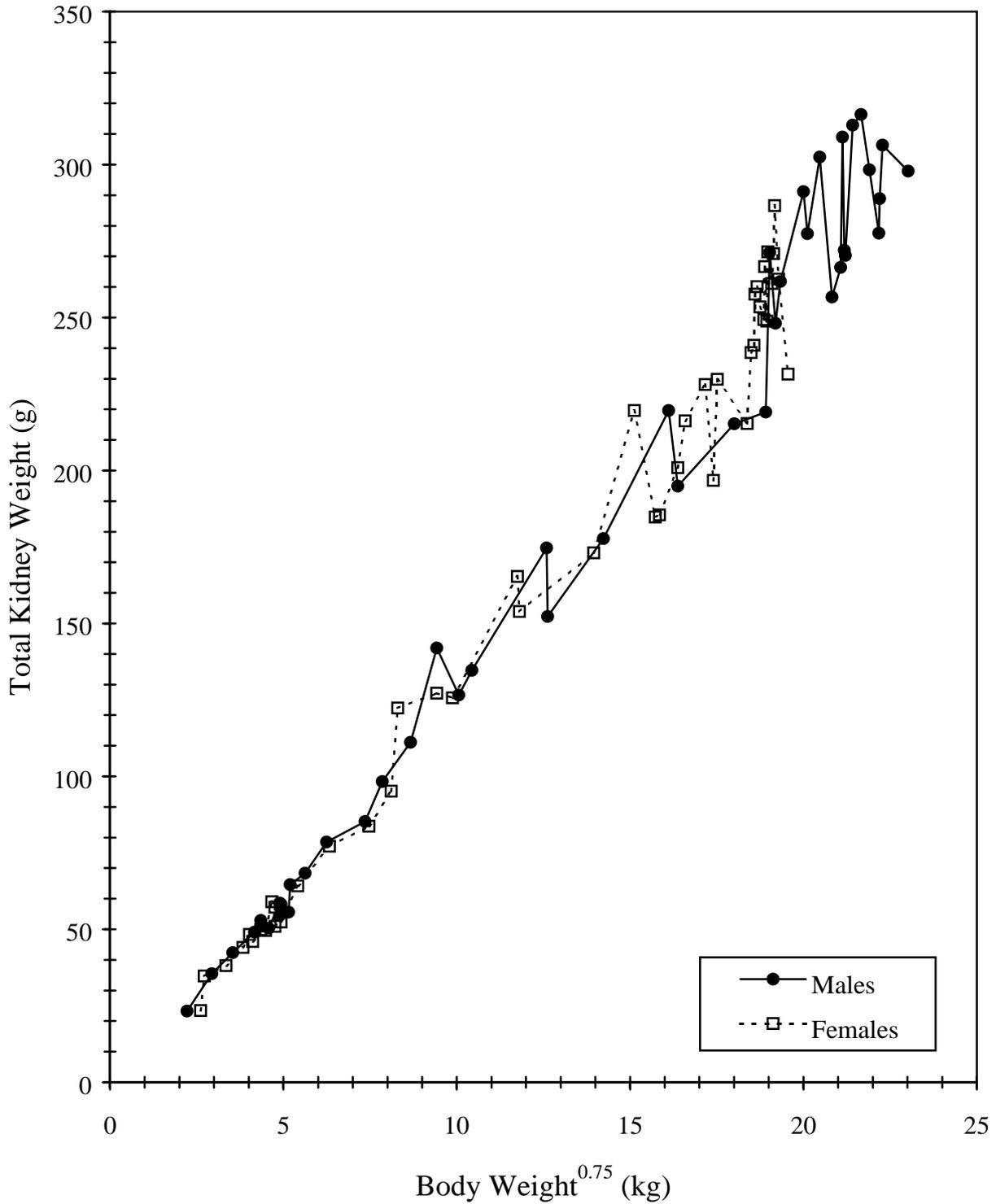


Figure 11. Total kidney weight scales with body weight to the 0.75 power ($BW^{0.75}$) from birth to old age. Adapted from: Ogiu *et al.*⁴.

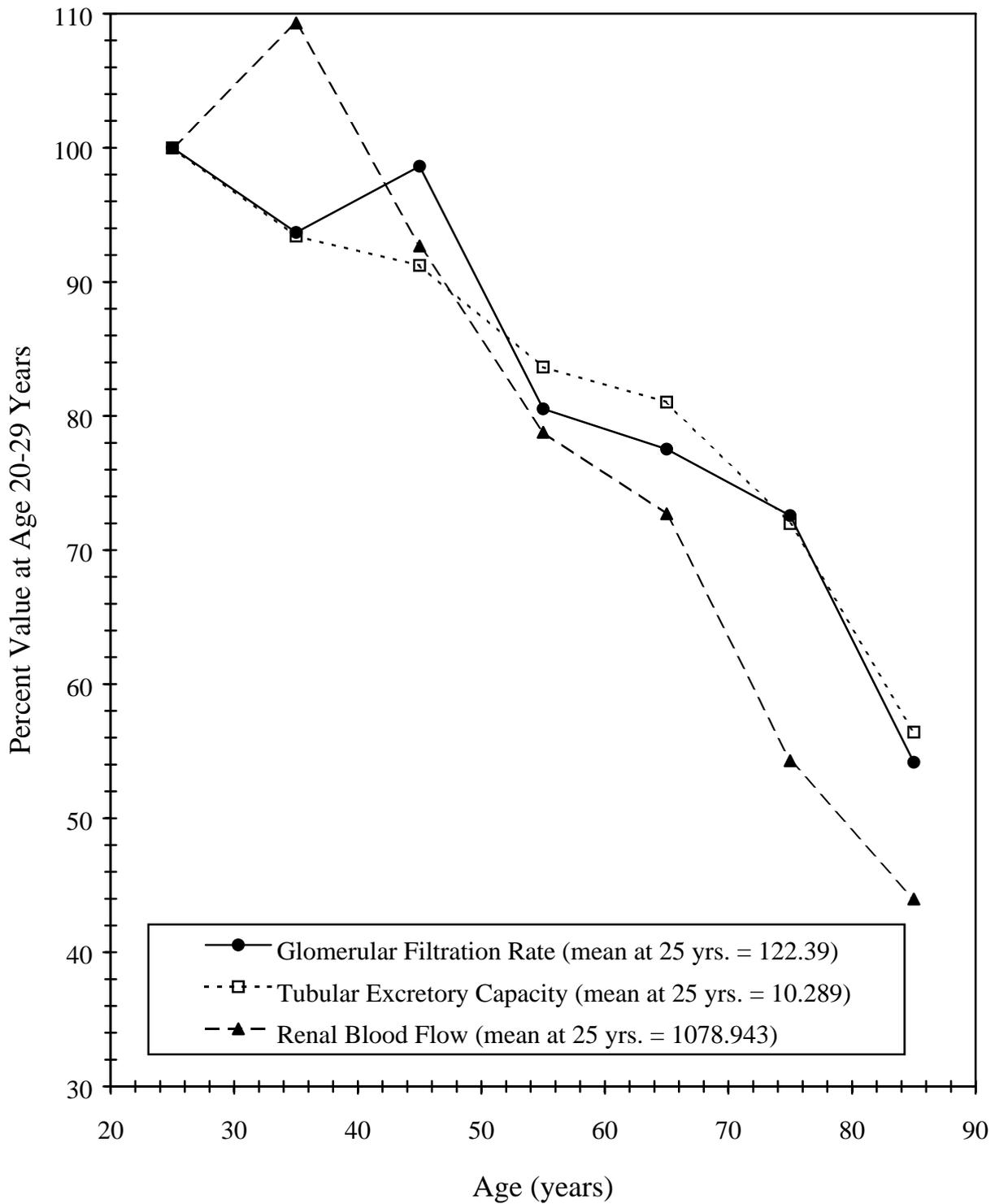


Figure 12. Age-related decrease in renal function parameters. Numbers are reported as fraction of the value at maturity (20-29 years). Adapted from: Mayersohn².

Glomerular filtration decreases approximately 0.66% per year after age 30, as measured by inulin clearance, almost identical in magnitude with the reduction of the maximum tubular secretion capacity—0.62%—supporting the hypothesis that the nephron loses its activity as a unit (Figure 12). Several equations relate age, body weight and creatinine serum concentrations to creatinine clearance (a surrogate for GFR):

$$Cl_{Creat} = \frac{(140 - Age) * BW}{72 * S_{Creat}} * GenderFactor \quad (1)$$

where Cl_{Creat} is the creatinine clearance, age is in years, body weight (BW) is in kg, gender factor is 1 for males and 0.85 for females¹³¹ and S_{Creat} is the serum creatinine concentration (mg/dL)⁴⁶. Recently, alternative measures of GFR using serum cystatin C concentrations, an endogenous compound that provides a measure of GFR, which is not confounded by differences in lean body mass, have been used¹³². Gender-related differences in GFR are not observed when serum cystatin C concentrations are used¹³².

Some authors report a modestly reduced GFR in women, compared with men, when normalized by surface area¹⁰. Beierle et al. noted that attributing these differences solely to gender and not other factors, such as body weight, is not feasible. These other factors may also contribute to differences in GFR. Glomerular filtration may rise 50% during pregnancy¹³³, and is reported to vary with menstrual cycle stage in one of two studies¹³³.

Tubular secretion of common blood solutes—glucose, phosphate and bicarbonate—as well as PAH (para amino hippuric acid) is reduced in neonates, but reaches childhood levels at 30-40 weeks of age²⁶ (Figure 13). Factors contributing to reduced tubular secretion include low blood flow to the peritubular region, immaturity of the active transport system, small mass of tubular working cells and smaller size of the proximal tubules²⁶. Passive reabsorption may also be reduced in the newborn²⁶. Maximum tubular secretion capacity decreases approximately 0.62% per year after age 30 (Figure 12).

Urine flow rate can impact excretion of chemicals by the renal system. The urine flow rate affects the urine concentration, increasing or decreasing the concentration gradient for excretion processes must overcome, and may affect the diffusion time for compounds diffusing out of the urine. This has been demonstrated for several compounds including chroamphenicol, ephedrine, phenobarbital, and theophylline¹²⁹.

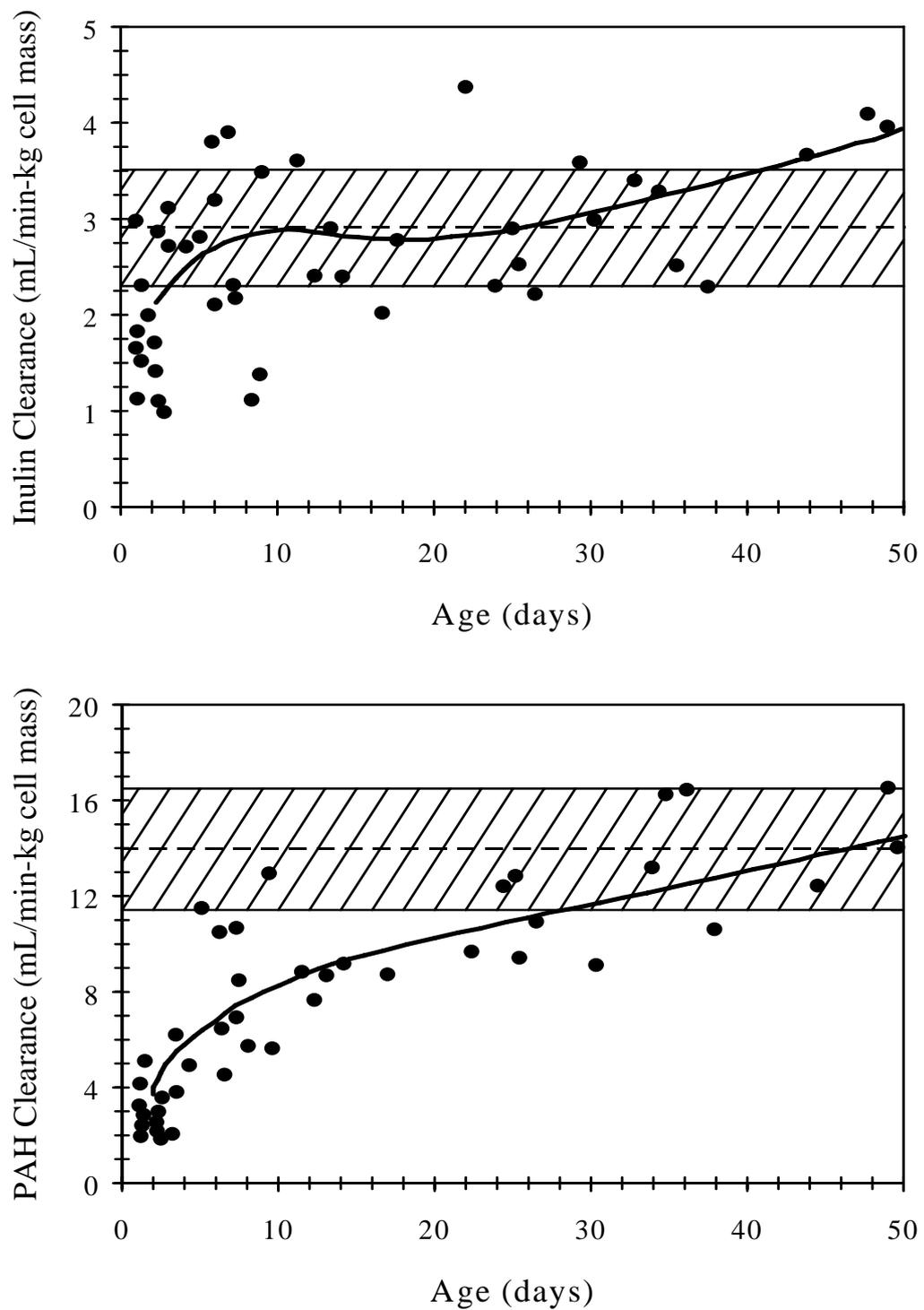


Figure 13. Increase in renal function during the early postnatal period: a) glomerular filtration rate, and b) tubular secretion. Hatched area represents adult values. Adapted directly from: Braunlich³.

3.4.3 Physiological Determinants of Lactational Clearance

Lactational elimination occurs by passive diffusion of non-ionized or lipophilic compounds. Elimination of ionizable compounds will be a function of Pk_a and milk pH, which is acidic (pH=6.5)⁴⁴. The dependence of pH results implies that concentrations of acidic compounds are likely to be lower in milk than plasma, and concentrations of basic compounds are likely higher in milk than plasma. The high fat content, particularly immediately following childbirth, makes milk a good storage depot for lipophilic compounds. Lactational excretion of lipophilic compounds, such as DDT, polychlorinated and polybrominated biphenyls, dibenzo-p-dioxins and furans, have been demonstrated, and may be a major source of elimination. Lipophilic compounds diffuse with dietary fats into milk, where the excreted compound becomes a source of exposure for the neonate⁴⁴.

3.4.4 Observed Differences in Renal or Lactational Clearance

Neonates, Infants and Children. The renal clearance rates of compounds that are GFR-dependent and those that are tubular secretion-dependent are both reduced in the neonate. Digoxin, which is modestly lipophilic, is cleared primarily by the renal system. Clearance is 35-75% lower in neonates, increasing from 32.9 ml/min/1.73 m² in infants less than 1 week old, to 88.9 ml/min/1.73 m² at 3 months and 144.4 ml/min/1.73 m² at 18 months of age¹²⁹. The clearance of pipercuronium, an aminosteroid neuromuscular blocking drug, which is cleared predominantly (43-56%) by the renal system, is 42% lower in infants than adults¹³⁴. The hydrophilic antibiotic, isepamicin, is cleared almost exclusively (97%) by the renal system, and also shows significant age-related differences in Cl_{ren} . Cl_{ren} in L/h/kg is 0.066 1-7 days after birth, rising to 0.128-0.157 between 2 weeks and ~14 years, after which it declines steadily to 0.055 by ages 71-80 years¹³⁵. Similarly, clearance of hydrophilic aminoglycoside antibiotics (gentamicin, amikacin, tobramycin, kanamycin), netilmicin, and indomethacin occurs through glomerular filtration and is reduced in the neonate. The serum half-lives of these compounds correlate with GFR and neonatal age¹²⁹. Clearance (total) of amikacin is lower in newborns (36 ml/min/1.73m²) than adult (100 ml/min/1.73 m²). Clearance of kanamycin is lower in newborns (7-9 ml/min/1.73m²) than adults (95-99 ml/min/1.73m²). Renal clearance of vancomycin is lower in premature neonates than adults, with rates that are 50% and 23% (premature neonates) lower than adults values, respectively²⁵.

The newborn kidney has a reduced rate of transport (tubular secretion) of organic anions, such as furosemide, chlorothiazide, ethacrynic acid, acetazolamide and aldactone, as well as weak organic acids, such as penicillins, sulfonamides, cephalosporins and phenolsulfonphthalein¹²⁹. This group of compounds comprises both water soluble and modestly lipophilic characteristics; the important characteristic appears to be a functional group targeting them for active tubular secretion. Renal clearance of furosemide is lower in neonates than adults, with neonatal rates that are 50% of adult levels²⁵. Renal clearance of

penicillins and sulfonamides is tubular secretion-dependent, and is similarly reduced in the neonate, in some cases. Ampicillin clearance is much lower in newborns (41.5-63 ml/min/1.73m²) than adults (260-420 ml/min/1.73m²)²⁶. Penicillin is cleared primarily by the kidney, by both GFR and tubular secretion. Clearance is reduced in the newborn compared to older children and adults. Renal excretion of semisynthetic penicillins is also lower in the newborn, increasing markedly by 3 weeks of age. Resulting serum half-lives are similar to those in adults¹²⁹.

The extent of binding of compounds to serum proteins (see Section 2.2) can influence renal elimination, and the resulting free serum concentrations³. Renal clearance of free ceftriaxone is reduced in the infant, but is partially compensated by a parallel ~2-fold decrease in plasma protein binding¹²⁹. The resulting fraction of the dose excreted unchanged in the urine is lower in adults (46%) than newborn infants (70%).

Differences are not observed for nafcillin, which is cleared primarily (90%) by biliary excretion. The renal clearance of digoxin is 35-75% lower in neonates (32-56 ml/mn/1.7 m²), when compared with adults (130-150 mg/min/1.7m²)²⁶.

Elderly. Lithium, a hydrophilic metal ion, is almost exclusively cleared by the kidney by glomerular filtration, which is limited by the high—80%—reabsorption in the proximal tubule¹³⁶. Consistent with the decline in GFR with age, unadjusted lithium clearance values are lower in the elderly (0.83-94 L/h) compared with adults (2.49 L/h). The hydrophilic antibiotics, gentamicin and isepamicin, are cleared almost exclusively (90-97%) by the renal system, and show significant age-related differences in Cl_{ren}. Isepamicin Cl_{ren} in L/h/kg is 0.066, 1-7 days after birth, rising to 0.128-0.157 between 2 weeks and ~14 years, after which it declines steadily to 0.055 by ages 71-80 years¹³⁵. Cl_{ren} of gentamicin declines with age, particularly between 60 and 70 years.

Gender. The renal clearance of two modestly lipophilic compounds, digoxin and amantadine, as well as azimilide and pramipexole, is lower in women than men. The renal clearance of digoxin is 12-14% less in women than men¹⁰. Renal clearance (Cl_{ren}) of amantadine, when corrected for body weight, mass index, and surface area, is 1.5-fold greater in males than females¹⁰. Cl_{ren} of azimilide is 19% higher in women when compared with men¹⁰. Pramipexole, a dopamine agonist, excreted predominantly by the kidney (80%), has a Cl_{ren}, which is 24-29% lower in women compared to men, similar to the calculated difference in creatinine clearance (GFR) (27.8%), but lower than other measures of GFR in women, which indicate no difference¹³².

Physiological Determinants of Lactational Clearance. Numerous chemicals have been found in human breast milk^{137, 138} and represent a potential source of exposure to the nursing infant. Human breast milk is a complex mixture of milk proteins, lactose, and triglycerides synthesized by mammary tissue and vitamins, fatty acids, and minerals added from the blood supply to the mammary tissue¹³⁸. Exogenous chemicals may also be transferred from the blood supply, across

mammary epithelial cells into the mammary duct lumen. The most important determinants for this transfer are 1) degree of ionization; 2) lipid solubility; and 3) molecular weight¹³⁸. The concentration of chemical in milk has been estimated for some volatile organic chemicals using a PBPK model that considers blood flows, tissue volumes, and chemical partitioning from blood to milk in the mammary gland^{139, 140}. In such models, more highly lipophilic compounds will preferentially partition from maternal blood into milk, and increases in blood flow will increase the rate at which such equilibration into milk occurs.

The transfer and accumulation of chemicals in breast milk is complex. It is not only a function of the physicochemical properties of the exogenous chemical and blood flow to the mammary tissue but also a function of the protein and fat composition of the milk. Changes in protein levels may result in increases in mammary excretion of protein-bound chemicals, such as heavy metals (lead, cadmium, mercury)¹³⁸. A 10-fold difference in protein concentrations have been noted between milk released immediately after birth (up to one week, post-partum) and mature milk¹³⁸. Consequently, exposure in the first week of life from compounds that tend to be protein bound may be higher than at a few weeks of age.

Milk fat is excreted as a fat globule with a lipoprotein membrane¹³⁸. Highly lipophilic chemicals, such as DDT, polychlorinated and polybrominated biphenyls, and dibenzo-p-dioxins/furans, may partition into milk and bind to or partition into the lipid core of the fat globule¹³⁸. Fat content can change over the time course of nursing with higher fat content, and, consequently, higher chemical excretion, the longer the infant nurses. Moreover, fat content varies among species; milk from mice contains three times more fat than human milk¹⁴¹.

The concentration of an exogenous chemical in milk is only one determinant of exposure to the nursing infant. The other is the amount of milk ingested daily and the month/years over which nursing occurs. According to Byczkowski et al.¹³⁸, milk intake increases from approximately 100 ml/day on post-partum day 2, rising to 500 ml/day by the second week and up to 700-800 ml/day in subsequent months with variations reported between 600-1000 ml/day at that time. Body burden will then change both with the increased milk intake but also with increased body weight as the infant grows.

Physiological Determinants of Placental Transfer. The placenta is a complex, multi-compartmented membrane that functions in maternal-fetal exchange of nutrients (from the maternal circulation) and excretory products (from the fetal circulation). The movement of endogenous and exogenous chemicals is a function of 1) molecular weight, 2) lipid solubility, 3) degree of ionization, 4) binding to tissue or plasma components, 5) degree of biotransformation by placental enzyme systems, and 6) the rate of placental clearance¹⁴².

Placental transfer occurs at the interface of maternal sinuses, into which blood flows from maternal arteries, and the placental villi, into which fetal capillaries, both arteries and veins, grow¹⁴³. The villi carrying fetal blood are surrounded by sinuses that contain maternal blood, both of which, villi and sinus, are contained in the area between the placental stratum spongiosum and the chorion-amnionic membrane¹⁴³.

Transfer from maternal blood to fetal blood is in general inversely proportional to molecular weight¹⁴². With the exception of gamma globulins, compounds with molecular weights greater than 1000 (units not given) are poorly transmitted, if at all. In general, as with other membranes and tissues, more highly lipid soluble compounds and/or unionized compounds will be more readily transported across the villi to fetal circulation. However, not all lipid soluble compounds are transported across the placenta at the same rate as across other tissues¹⁴².

Binding to tissue or plasma proteins and placental biotransformation significantly influence placental transfer¹⁴². Differential affinities of proteins in maternal and fetal plasma and tissues will alter placental transfer and fetal distribution. Preferential binding to maternal plasma relative to binding to fetal plasma will likely favor decreased distribution to the fetus. In contrast, preferential binding in fetal tissues, such as the liver, muscle, may create a fetal "sink" and enhance placental transfer.

The placenta is often thought of as an inert switching station in which nutrients and waste products are exchanged. However, the placenta is metabolically active. The placenta contains aryl hydrocarbon hydroxylase (AHH), aromatases, other MFOs, glucuronidase, and sulfatase¹⁴².

4. Conclusions

For several of the parameters summarized in Table 2, there were insufficient data to make conclusions, particularly in the elderly. However, these should not necessarily be considered data gaps. Much of the data provided in this report were obtained from studies with pharmaceutical agents. The pharmacokinetics of pharmaceutical agents have been studied extensively. Consequently, the absence of published data in a particular area is likely an indication that no differences have been observed that would justify further study.

As discussed previously, the pharmacokinetics of a chemical is determined by the interaction of complex biological systems. For some of the parameters, inconsistent findings (e.g., increases and decreases) have been reported, which is not surprising for such a wide array of chemicals. However, it is also possible that the investigators were misled by the unstated assumption that they were actually measuring a single pharmacokinetic parameter, such as absorption. Although it is easier to describe and to think of absorption, distribution, metabolism and excretion as isolated events, they are in fact interrelated and should be evaluated collectively. In order to do this, PBPK modeling is an

essential tool. Moreover, the application of a PBPK model allows for a more accurate estimation of the quantitative factors determining the dose to a particular tissue. Consequently, data-derived adjustments could be made to risk estimates. The next phase of this work will consist of using PBPK models to develop examples of approaches through the development of specific case studies to investigate quantitatively incorporating information on age- and gender-specific pharmacokinetic differences in risk assessments for chemicals.

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Chapter 7

Evaluation of the Potential Impact of Age- and Gender-Specific Pharmacokinetic Differences on Tissue Dosimetry

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ABSTRACT

The physiological and biochemical processes that determine the tissue concentration time courses (pharmacokinetics) of xenobiotics vary, in some cases significantly, with age and gender. While it is known that age- and gender-specific differences have the potential to affect tissue concentrations, and hence individual risk, the relative importance of the contributing processes and the quantitative impact of these differences for various life-stages are not well characterized. The objective of this study was to identify age- and gender-specific differences in physiological and biochemical processes that affect tissue dosimetry and integrate them into a predictive physiologically based pharmacokinetic (PBPK) life-stage model. The life-stage model was exercised for several environmental chemicals with a variety of physicochemical, biochemical and mode-of-action properties. In general, predictions of average pharmacokinetic dose metrics for a chemical across life-stages were within a factor of two; although larger transient variations were predicted, particularly during the neonatal period. The most important age-dependent pharmacokinetic factor appears to be the potential for decreased clearance of a toxic chemical in the perinatal period due to the immaturity of many metabolic enzyme systems, although this same factor may also reduce the production of a reactive metabolite. Given the potential for age-dependent pharmacodynamic factors during early life, there may be chemicals and health outcomes for which decreased clearance over a relatively brief period could have a substantial impact on risk.

INTRODUCTION

Several recent initiatives by the U. S. Environmental Protection Agency (USEPA) have increased the emphasis on research to investigate age-specific risk issues. One such initiative, the Voluntary Children's Chemical Evaluation Program (VCCEP), is designed to provide data to improve understanding of potential health risks to children associated with chemical exposures (USEPA, 2000). This program also provides for the development of methods specific to exposure assessment in children. In 2002, USEPA also announced the first coordinated effort by the agency to examine and prioritize environmental health threats to the elderly (USEPA 2002). In order to adequately address the concerns raised in these initiatives, it will be necessary to make optimal use of the resources available to obtain toxicological data on the early life period. Prioritization of toxicity study requirements can be aided by an understanding of age-related differences in pharmacokinetics and pharmacodynamics and their potential impact on the internal exposure to environmental chemicals. Methods for incorporating this pharmacokinetic information into the risk assessment process are also needed in order to assess whether exposure to a given chemical may be of greater concern during a particular life-stage.

In response to this heightened interest in estimating age-related risks, a comprehensive review was conducted to identify the available quantitative

information in humans related to age- and gender-dependent differences in physiological, biochemical, and pharmacokinetic parameters that may impact risk from chemical exposure (Clewell *et al.*, 2002). This information was reviewed from a risk assessment perspective and the key factors that are likely to have a significant impact on susceptibility, as it relates to estimates of target tissue exposure, were identified. Much of the available data were obtained from studies with pharmaceutical agents. The majority of the differences in pharmacokinetics identified were between neonates/children and adults, with fewer differences identified between young adults and the elderly. The results of this study are consistent with the results of studies that have been conducted to evaluate the pharmacokinetic differences between children and adults (Renwick *et al.*, 2000; Ginsberg *et al.*, 2002; Hattis *et al.*, 2003).

However, in all of these studies it has been necessary to rely upon pharmacokinetic data for pharmaceuticals, which are more readily available than similar data for environmentally relevant chemicals. Unfortunately, the application of the results for pharmaceuticals to environmentally relevant compounds is problematic, due to the significant differences in the typical physicochemical and biochemical properties of pharmaceutical and environmental compounds. Pharmaceuticals tend to be water soluble, while environmental chemicals of concern are frequently lipophilic. The two classes of chemicals also tend to be substrates for different, although overlapping, subsets of the metabolic enzyme systems. For example, much of the information on age-dependent metabolic clearance of pharmaceuticals is for CYP3A4 substrates (Ginsberg *et al.* 2002), but this isozyme has not frequently been associated with the metabolism of environmental contaminants. On the other hand, CYP2E1 is associated with the toxicity of many environmental contaminants (Guengerich *et al.* 1991), but was not identified as a common drug metabolizing isozyme.

The primary objective of this study was to identify age- and gender-specific differences in physiological and biochemical processes that affect tissue dosimetry, and integrate them into a predictive physiologically based pharmacokinetic (PBPK) life-stage model that could be exercised for environmental chemicals with a variety of physicochemical, biochemical and mode-of-action properties in order to determine the interaction between the pharmacokinetic processes and the properties of the chemical.

PBPK modeling has been routinely used in risk assessment when extrapolating across route and species. The same qualities that make PBPK modeling attractive for these extrapolations also make it a useful platform for predicting age-dependent pharmacokinetics. Specifically, a PBPK model can provide a quantitative structure for incorporating into the risk assessment process information on the various age- and gender-specific pharmacokinetic factors that can impact the relationship between the external (environmental) exposure and the internal (biologically effective) target tissue exposure. Recent guidance from the International Programme on Chemical Safety (IPCS, 2001) provides an approach for replacing default uncertainty factors with chemical-specific adjustment factors (CSAFs). This approach divides the animal-to-human

interspecies and human intraspecies uncertainty factors into toxicokinetic and toxicodynamic components¹⁰, each of which can be replaced by a CSAF if data are available. For example, the magnitude of the factor for human variability in toxicokinetics (HK_{AF}) may be calculated based on an evaluation of human variability in the area under the tissue concentration-time curve (AUC) or clearance. A PBPK model provides an excellent basis for performing such an evaluation (Pelekis *et al.* 2001; Lipscomb *et al.* 2003). This paper describes the initial development of a generic PBPK model that can be used to estimate age-specific CSAFs for any chemical, exposure route, and life-stage, dependent only on the availability of adequate chemical-specific partitioning and metabolism information. Child-adult CSAFs predicted with such model could in principle be used in the context of the VCCEP initiative to replace or inform a child-specific uncertainty factor, and the same approach could also be applied to obtain CSAFs for other life-stages, such as the elderly.

The purpose of the study presented here is to provide a proof-of-principle demonstration of the potential of PBPK modeling to address age-specific dosimetry issues quantitatively. It is not the intent of the authors to suggest that the predictions of this initial model for specific chemicals should be used quantitatively in support of risk assessments. The chemical-specific parameterization of this initial model is based on previously published PBPK descriptions for young adults. Predictions of the model for other lifestages, while based on reasonable physiological and biochemical principles, should be considered to represent no more than exploratory dosimetry estimates. Further age-specific parameter refinement and chemical-specific model validation will be required before PBPK-based age extrapolation can be performed with confidence.

METHODS

Selection of Chemical Class Surrogates

Six chemical classes were targeted with different physicochemical properties, and surrogate chemicals were selected to represent each class. The surrogates selected were based on the availability of chemical-specific information critical for PBPK modeling: metabolic parameters and partition coefficients. Examples were conducted for two non- or semi-volatile classes and four volatile classes (Table 1). For the nonvolatile classes, one example was selected to represent highly lipophilic, nonvolatile compounds, for which 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) served as the surrogate. Nicotine was selected as a representative for water soluble, non- or semi-volatile chemicals.

¹⁰ IPCS (2001) 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."

Table 1. List of Surrogate Chemicals and Important Characteristics

Compound	Physicochemical Characteristics	Active Compound
Isopropanol	water soluble/volatile	Parent, Circulating Metabolite
Vinyl Chloride	Lipophilic/volatile	Reactive Metabolite
Methylene Chloride	Lipophilic/volatile	Parent, Reactive Metabolite
Perchloroethylene	Lipophilic/ volatile	Parent, Circulating Metabolite
TCDD	Lipophilic/nonvolatile	Parent
Nicotine	Water soluble/nonvolatile	Parent

The chemicals selected to serve as surrogates for the volatile classes represented not only differences in physicochemical properties, but also differences in metabolic characteristics, including metabolic production of stable metabolites or reactive intermediates, as well as single or competing metabolic pathways. Four volatile chemicals were selected with decreasing water solubility and increasing lipophilicity: isopropanol, vinyl chloride, methylene chloride, and perchloroethylene. Of these volatiles, vinyl chloride produces reactive intermediates via a single oxidative metabolic pathway, while methylene chloride has two competing metabolic pathways, oxidation and glutathione conjugation, that both produce reactive intermediates. Isopropanol and perchloroethylene are both metabolized to stable compounds: acetone and trichloroacetic acid (TCA), respectively.

Model Structure and Parameters

The life-stage PBPK model used in this study is an elaboration of a previously published adult model for isopropanol (Clewell *et al.*, 2001a) (Figure 1). The primary feature of the life-stage model is that it allows for the simulation of the time-dependence of physiological and biochemical parameters due to growth and aging. All physiological and biochemical parameters in the model were allowed to change with time, using either equations or interpolation between discrete data points, based on available age-related quantitative information, with only the chemical-specific parameters remaining constant. Since the adult model has been documented in previous publications (Clewell *et al.*, 2001a; Gentry *et al.*, 2002), only the modifications to the model necessary to support age-dependent simulations will be discussed. A description of the

age- and gender-related information used to describe the physiological and biochemical parameters in the life-stage model is provided in the following sections, and the model equations are provided in Appendix A. The model was coded using Advanced Continuous Simulation Language (ACSL, Aegis Technologies Group, Inc., Huntsville, AL).

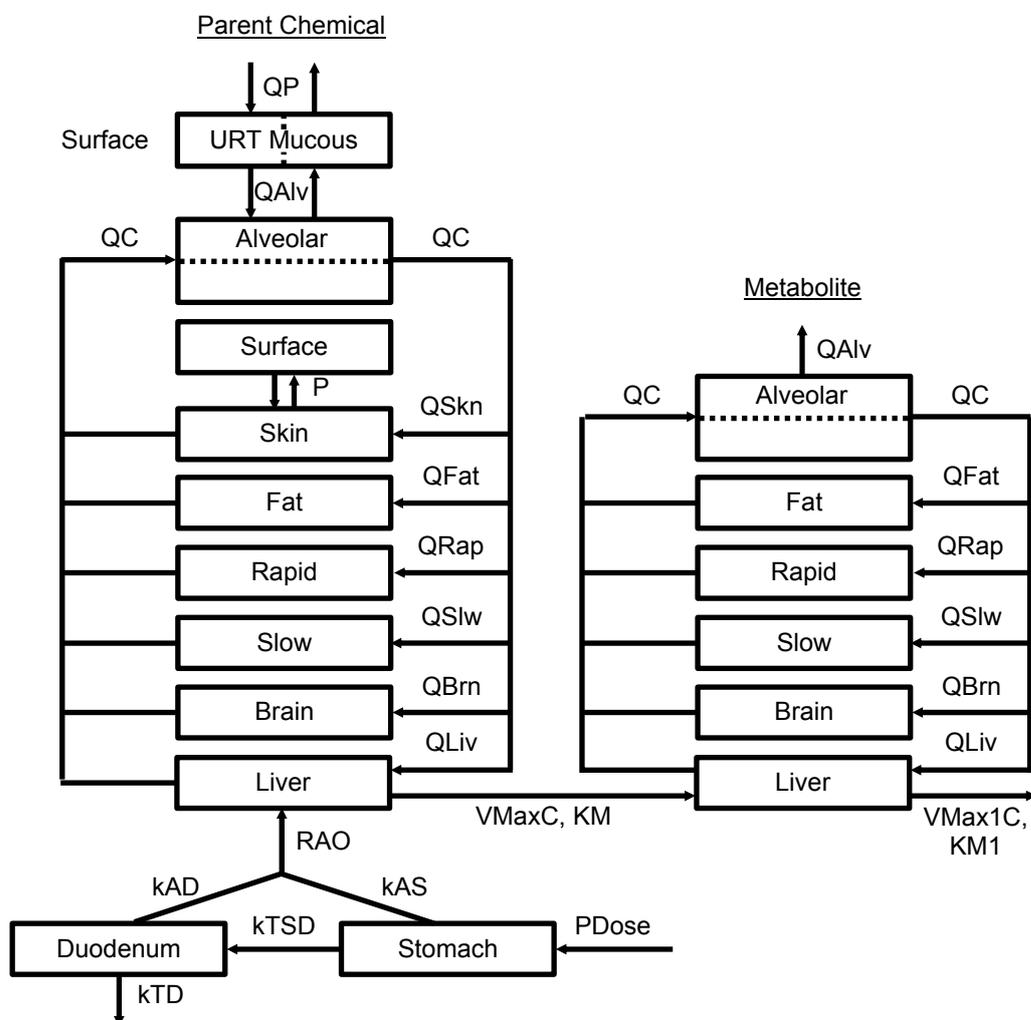


Figure 1. Schematic of the life-stage PBPK model.

If there were sufficient data, an equation was used in the model to describe the age- or gender-specific changes in a parameter; otherwise, linear interpolation between available data points was used to define the time evolution of the parameter. Where data were available for males and females, separate equations or data points were used to describe each gender. Equations were used to describe age-dependent changes in body weight, skin surface area, cardiac output, rapidly perfused tissue volume, and glomerular filtration rate (Supplementary Data,¹¹ Appendix A). For some of these parameters, multiple equations were necessary to adequately describe the changes observed in the parameter throughout the entire period of life. Data on alveolar ventilation;

¹¹ Supplementary Data are available on the Toxicological Sciences website (and at the end of this chapter).

metabolic capacity; brain, liver, and fat weight were used for interpolation (Supplementary Data, Tables A, B, C, and D). As a check of the resulting model's structural framework, the predictions of the age-dependent model for isopropanol were compared with the predictions of the validated adult human PBPK model on which it was based (Clewell *et al.*, 2001a). This comparison was performed at several ages, using the appropriate age-specific parameters in each model, to assure that the age-dependent model could accurately reproduce the output of the original model for the same life-stage. However, these comparisons do not serve as validation of the predictions of the model for early life-stages; the predictions of the published isopropanol model have only been validated against human kinetic data for the adult. The results of this comparison are shown in Figures 2A and 5A.

Physiological Parameters

Information on monthly body weights for birth and age 1 month were obtained from USEPA (1997), and weights for age 2 months to 90 years were obtained from NHANES (1995). A separate equation was needed for each of four different time periods (three Gompertz equations and one cubic equation), and the equations were fit to the data such that the curve was continuous. Separate sets of equations were developed to fit the data for males and females (Supplementary Data, Figure A).

Total surface areas (cm^2) corresponding to age-specific body weights were obtained from nomograms provided in ICRP (1975). Two separate cubic equations were necessary to adequately describe the data (Supplementary Data, Figure B). Because the equation is a function of body weight, the same equation was used to describe age-related changes in surface area for both males and females.

Data describing pulmonary ventilation (m^3/day) for various ages are provided in USEPA (1997). These values were converted to alveolar ventilation rates for use in the model based on the assumption that alveolar ventilation is approximately two-thirds of pulmonary ventilation. The values reported in USEPA (1997), which consider typical activity levels at different ages, were matched to age-specific body weights calculated as above. Because the resulting ventilation rates did not follow a smooth curve, alveolar ventilation is described as a function of time, in months, using linear interpolation between discrete data points (Supplementary Data, Table A).

Data on the relationship of cardiac output and pulmonary ventilation to level of activity (oxygen consumption) were taken from Åstrand (1983) (Supplementary Data, Figure C). For various values of oxygen uptake, corresponding values for cardiac output and pulmonary ventilation rate were extracted from these figures. As with the USEPA (1997) ventilation rates, these rates were adjusted by a ratio of 2/3 to obtain alveolar ventilation rates for use in the model. Based on the resulting relationship, cardiac output was described

as a function of alveolar ventilation using a Gompertz curve (Supplementary Data, Figure D).

Ogiu *et al.* (1997) provided age-related data on brain and liver weights as fractions of body weight for a Japanese population. Data on fat content as a fraction of body weight through age 20 was obtained from Hattis (2003), and was combined with data from ICRP (1975). These data were used in the model to calculate fractional tissue volumes as a function of time in months (Supplementary Data, Table B). These calculated fractional values were then multiplied by body weight to get the necessary tissue volumes.

Data on intestinal weight by age was used to estimate relative age-dependence of the volume of GI tract tissue (ICRP, 1975). Three separate Gompertz equations were used to describe the available data (Supplementary Data, Figure E). Due to a lack of data on adults, it was assumed that the fractional volume of the GI tract remained constant after age 22.

As in most simplified PBPK descriptions, the rapidly perfused tissue compartment in the life-stage model is a loosely-defined composite of a large number of tissues, most of which represent a very small fraction of the body weight. Age-dependent data were not available for many of these tissues. Since the volume of the GI tract is by far the largest component of the rapidly perfused tissue volume, data on the weight of this tissue (ICRP, 1975) was used as a surrogate for the age-dependent changes in this lumped compartment. The age-dependent equation describing fractional GI tract volume was multiplied by a constant to produce a fractional volume of rapidly perfused tissues at age 25 that was consistent with the previously published adult model (Clewell *et al.*, 2001a). The skin volume was modeled in the same manner as in the published adult model (Clewell *et al.*, 2001a).

The volume of slowly perfused tissues was modeled as 84% of the total body weight minus the volume of the other tissues. This assures that the total weight of perfused tissues for which the model accounts is 84% of the body weight (Clewell *et al.*, 2001a). The rest of the body (16%) is assumed to be non-perfused tissue (cortical bone, hair, gut contents, etc.).

Due to the lack of data on tissue blood flow changes with age, the blood flows were assumed to change proportionally with the tissue volumes. The adult fractional tissue blood flows from the published adult model (Clewell *et al.*, 2001a) were used along with the age-specific tissue volumes and the tissue volumes at age 25 years. To maintain mass balance for the blood flows, the age-specific fractional blood flows were normalized to always sum to unity.

Metabolic Parameters

Quantitative information on the age-related changes in the relevant metabolic pathways was used in modeling each of the surrogate chemicals (Supplementary Data, Table C). For the oxidative metabolism of vinyl chloride,

methylene chloride, and perchloroethylene, data on the development of CYP2E1 was used (Vieira *et al.*, 1996). For the metabolism of TCDD, data for CYP1A2 (Sonnier and Cresteil, 1998) were used, while for isopropanol, both alcohol dehydrogenase (ADH) data (Pikkarainen and R ih a, 1967) and CYP2E1 data (for acetone metabolism) were used. No information on age-related differences was available for the CYP2A6 pathway, which is the primary metabolic pathway for nicotine; therefore, age-related information available on the development of CYP2C (Treluyer *et al.*, 1997) was used as a surrogate for the purposes of this case study. Only limited quantitative information is available on the development of the glutathione-S-transferase (GST) pathway. Based on these limited data (Pacifici *et al.* 1981; Mendrala *et al.* 1993), the trend associated with the development of this pathway is similar to that observed for ADH. Therefore, age-related information on the metabolic capacity for ADH was used as a surrogate to predict age-related changes in GST for methylene chloride.

Due to the lack of gender-specific metabolism data, the same data was used for both males and females. Metabolism by all enzyme systems was initiated at zero at birth, with the exception of ADH, for which data were available that demonstrated prenatal activity; available data for the other metabolic pathways indicates no fetal metabolic activity (Vieira *et al.*, 1996; Sonnier and Cresteil, 1998; and Pikkarainen and R ih a, 1967). For ADH, linear interpolation between the last prenatal value and the first post-natal value was used to estimate a value for ADH at birth.

Age-specific metabolism rates were calculated using the adult metabolism rate (mg/hr), the adult liver volume (kg), the age-specific liver volume (kg), and the appropriate linearly interpolated fractional activity. Scenarios were also evaluated in which metabolism was artificially delayed for the first six months of life in order to evaluate the potential impact of late development of an enzyme system. In these cases, enzyme activity was assumed to be zero until six months of age, after which it was assumed to increase linearly from zero to the first fractional value available after six months.

Urinary Clearance Parameters

Data on glomerular filtration rate (GFR) at different ages were obtained from several sources (Braunlich, 1977; Milsap and Jusko, 1994; and Plunkett *et al.*, 1992). It was determined that, as a function of age, GFR is best described in several distinct phases. GFR ranges from 0.12 L/hr to 0.24 L/hr at birth, and then increases by a factor of about 4 within 72 hours (Milsap and Jusko, 1994). Between days 3 and 10 of life, GFR continues to increase until the normalized value is approximately that of an adult (Braunlich, 1977; Milsap and Jusko, 1994; and Plunkett *et al.*, 1992). After reaching normalized adult values, GFR scales as a constant function (per 1.73 m²) of total body surface area until approximately 30 years of age, after which GFR declines from the adult value at 30 years of age by 0.66% per year. These phases were modeled using five separate equations (Supplementary Data, Appendix A). The first equation sets GFR to be 0.12 L/hr until 1 day of age. The second equation increases GFR

linearly by a factor of four over the next 3 days, and the third equation linearly increases GFR to normalized adult levels by the end of 10 days of age. The fourth equation was developed using data on body weight and total body surface area, and calculates GFR as a function (per 1.73 m²) of total body surface area until 30 years of age. The last equation decreases GFR from the adult value at 30 years of age by 0.66% each year.

Renal blood flow data were available for ages up to 50 days (Braunlich, 1977), (Supplementary Data, Figure F) and for ages 25 to 85 years (Mayersohn, 1994) (Supplementary Data, Table D). The data from Braunlich (1977) indicated that by 50 days of age, fractional renal blood flow has reached adult levels. Age-specific urinary clearance rates were calculated using the adult urinary clearance rate (L/hr) and the ratio of the linearly interpolated age-specific GFR or renal blood flow to the corresponding adult GFR or renal blood flow.

Model Simulations for Surrogate Chemicals

The conditions of the model simulations are summarized in Table 2. For each surrogate chemical, continuous lifetime oral exposure was simulated (birth to 75 years) for both males and females at a daily dose rate of 1 µg/kg/d (with the exception of TCDD, for which a daily dose of 1 ng/kg/d was used). For TCDD, the gestational period was also simulated to account for bioaccumulation prior to birth; the rapid clearance of the other surrogate chemicals makes this additional step unnecessary. A comparison of *in utero* and neonatal exposure with these same chemicals was performed in a separate study (Gentry et al. 2003). Oral exposure was modeled using a constant intake rate, rather than attempting to simulate a time-varying diurnal ingestion pattern, since the impact of widely varying ingestion patterns on average daily internal dose metrics, such as those used in this analysis, has been shown to be relatively small (NRC, 1986).

The dose metrics of concern (Table 2) were estimated continuously, as well as at specific ages (1, 3, and 6 months and 1, 5, 10, 15, 25, 50, and 75 years). It is important to note that the age-dependent values of the oral dose metrics obtained with these simulations only provide a basis for comparing the relative internal exposure associated with the same nominal daily intake, and do not reflect age-dependent differences in intake.

Table 2. Age- and Chemical-Specific Information for Model Simulations

Chemical	Reference for Chemical-Specific Parameter Values	Age-Specific Metabolic Enzyme Activity	Continuous Exposure Scenarios Simulated	Dose Metrics
Isopropanol	Clewell et al. (2001a)	ADH (Vieira et al. 1996) CYP2E1 (Pikkarainen and Raiha 1967)	Drinking water (1 µg/kg/day) Inhalation (1ppb) Dermal (0.07 mg/l over 18.5% body surface area for males and 20% body surface area for females)	Arterial Blood concentration of parent and its metabolite acetone
Vinyl Chloride	Clewell et al. (2001b)	CYP2E1 (Pikkarainen and Raiha 1967)	Drinking water (1 µg/kg/day)	Arterial Blood concentration of parent Rate of Metabolism of parent/kg liver volume
Methylene Chloride	Andersen et al. (1987)	CYP2E1 (Pikkarainen and Raiha 1967) GST (Pacifci et al. 1981; Mendrala et al. 1993) ¹	Drinking water (1 µg/kg/day)	Arterial Blood concentration of parent Rate of Metabolism of parent/kg liver volume
Perchloro-ethylene	Gearhart et al. (1993)	CYP2E1 (Pikkarainen and Raiha 1967)	Drinking water (1 µg/kg/day)	Arterial Blood concentration of parent and its metabolite trichloroacetic acid
TCDD	Murphy et al. (1995) Andersen et al. (1997)	CYP2A1 (Sonnier and Cresteil 1998)	Drinking water (1 ng/kg/day)	Arterial Blood concentration of parent
Nicotine	Robinson et al. (1992)	CYP2A6 ²	Drinking water (1 µg/kg/day)	Arterial Blood concentration of parent and metabolite cotinine

¹ The cited references provided limited quantitative information on the development of GST; however, the information was sufficient to determine that the pattern of development was similar to ADH, for which sufficient information was available. Therefore, age-related information on the metabolic capacity for ADH was used as a surrogate for GST.

² No age-related quantitative information was available for CYP2A6. Information was available for CYP2C, which was used as a surrogate for CYP2A6.

In particular, no attempt was made to evaluate life-stage dependent exposure, such as breast- *versus* bottle-fed infants. This age-independent daily intake scenario was selected to provide information on the nature of the variation in the internal, or biologically effective, dose associated with exposure at different life-stages to a specified acceptable daily intake (ADI) or reference dose (RfD).

For each of the surrogate chemicals, partition coefficients from the literature or from a previously published adult human model for that chemical were used (Table 2); adult metabolic and urinary clearance parameters were also based on the previously developed models. However, except as already described for the case of isopropanol, the resulting age-dependent model was not directly validated against the corresponding published adult model or against data for the surrogate chemicals.

Isopropanol: Parameters for isopropanol were taken from the model of Clewell *et al.* (2001b). Age-dependent metabolism of isopropanol was based on data for ADH, while age-dependent metabolism of acetone was based on data for CYP2E1. In the case of isopropanol, predictions of age-dependent dosimetry were conducted for three routes of exposure: oral, dermal, and inhalation. Artificial continuous exposure scenarios for dermal exposure (0.07 mg/L over 18.5% body surface area for males and 20% body surface area for females) and inhalation exposure (1 ppb continuous) were selected to result in values of the dose metrics on the same order of magnitude as the oral exposure, so comparisons could readily be made of the impact of route of exposure on age-dependent behavior. However, while the oral exposure was characterized as a constant daily intake, as discussed above, the inhalation and dermal exposures were characterized as a constant media concentration (in the air or in a water vehicle on a constant fraction of the total skin surface area). Thus the body-weight, and hence age-dependence, of the exposure scenarios varies significantly across routes. A more complete evaluation of age-dependent pharmacokinetics from inhalation exposures was performed in a separate study (Sarangapani *et al.* 2003).

Vinyl chloride: The parameters for vinyl chloride were taken from a published model (Clewell *et al.*, 2001b), and the age-dependence of metabolism was based on data for CYP2E1.

Methylene chloride: The parameters for methylene chloride were taken from the model of Andersen *et al.* (1987). The age-dependence of oxidative metabolism was based on data for CYP2E1, while the age-dependence of glutathione conjugation was based on data for ADH.

Perchloroethylene: The parameters for perchloroethylene were taken from the model of Gearhart *et al.* (1993), and the age-dependence of metabolism was based on data for CYP2E1. The Gearhart *et al.* (1993) model used a single-compartment model for the metabolite. Therefore, the tissue:blood partitions for

the metabolite in the life-stage model were set to a uniform value producing the same volume of distribution as the Gearhart *et al.* (1993) model. Similarly, the Gearhart *et al.* (1993) model described the amount of TCA produced as 60% of the total metabolized amount of perchloroethylene; therefore, the life-stage model was parameterized such that 60% of perchloroethylene is metabolized to TCA. The adult urinary clearance value for TCA was also taken from Gearhart *et al.* (1993), and the age-dependent urinary clearance for TCA was modeled as a fraction of the adult urinary clearance value, where the fraction was determined by the age-dependent renal blood flow data, assuming active secretion.

Nicotine: The parameters for nicotine were taken from the model of Robinson *et al.* (1992). The metabolism of nicotine is mainly via CYP2A6, but, due to the lack of age-related data on CYP2A6, metabolism was modeled using available data on age-specific activity of CYP2C (Treluyer *et al.* 1997). The same was true for the metabolite, cotinine, which is also metabolized via the CYP2A6 pathway. Because cotinine accounts for 80% of the metabolized amount of nicotine (Robinson *et al.*, 1992), the model was parameterized such that 80% of nicotine was metabolized to cotinine. Urinary clearance of both nicotine and cotinine was modeled as a fraction of the adult urinary clearance value from Robinson *et al.* (1992), where the fraction was determined by the equations for GFR.

TCDD: Partition coefficients for TCDD were obtained from Murphy *et al.* (1995), and the adult metabolism parameter was adjusted to obtain a half-life of 7.5 years for a young adult, after Andersen *et al.* (1997). Age-dependent metabolism was modeled using data for CYP1A2. Time-series sensitivity coefficients were also calculated for TCDD to demonstrate the dynamic age-dependent impact of the most sensitive parameters, the fat volume and the metabolic rate constant.

RESULTS

In general, the simulations of the different surrogate chemicals with the life-stage model did not produce significant differences in dose metrics between males and females; therefore, only the male results are presented for most of the chemicals. For those cases for which a significant difference was observed, both the male and female results are presented. A cross-chemical comparison of the average internal dose metrics predicted during each of the selected life-stages, normalized to the 25 year old adult value, is provided in Table 3. The entries under each life-stage represent the relative sensitivity, from a pharmacokinetic viewpoint, for exposure during that life-stage as compared to exposure of a 25 year old adult. The entries for overall HK_{AF} for each chemical represent the highest ratio of the internal dose metric for any of the life-stages to the internal dose metric for a 25 year old adult. These results can be compared with the default value for HK_{AF} of 3.2. In general, the greatest departures from the 25 year old adult internal dose metrics tended to occur during the infant life-stage (birth to six months).

Table 3. Ratio of Average Daily Dose During Different Life-Stages to Average Daily Dose for (25 year old) Adult

Chemical		Birth to 6 months	6 months to 5 years	5 years to 25 years	25 years to 75 years	Overall HK _{AF}
IPA	Parent	1.9	1.5	1.2	0.83	1.9
	Circulating Metabolite	1.1	0.88	0.95	1.0	1.1
Vinyl Chloride	Parent	1.4	0.99	0.99	0.99	1.4
	Reactive Metabolite	0.46	0.54	0.88	0.98	1.0
Methylene Chloride	Parent	1.4	1.0	0.98	0.99	1.4
	Reactive Metabolite	0.053	0.12	0.60	1.3	1.3
Perc	Parent	0.33	0.42	0.76	1.2	1.2
	Circulating Metabolite	0.057	0.16	0.59	1.4	1.4
TCDD	Parent	0.50	0.45	0.84	0.89	1.0
Nicotine	Parent	3.4	2.9	1.6	0.93	3.4
	Circulating Metabolite	2.7	2.8	1.6	0.92	2.8

Table 4 provides a similar comparison for one of the chemicals, isopropanol, across all three routes of exposure. For isopropanol exposure, the predicted internal dose metrics during the infant life-stage are greater than the adult values for all exposure routes, with inhalation showing the greatest difference.

In the case of chronic toxicity, the contribution of each life-stage to the cumulative internal dose over a lifetime depends on the value of the internal dose metric and the duration of the life-stage. Table 5 provides the predicted contributions of each lifestage to the lifetime cumulative internal dose for each chemical. Although the infant life-stage demonstrated the greatest departures from the 25 year old adult, the short duration of this life-stage limits its contribution to a small percentage of the lifetime cumulative internal dose. Of course, this comparison only reflects predicted pharmacokinetic differences. Age-dependent pharmacodynamic differences should also be considered in determining the relative contribution of exposure during a particular life-stage to cumulative lifetime risk for a particular effect.

Table 4. Ratio of Average Daily Dose During Different Life-Stages to Average Daily Dose for (25 year old) Adult Resulting from Exposure to Isopropanol via Different Routes

Chemical		Birth to 6 months	6 months to 5 years	5 years to 25 years	25 years to 75 years	Overall HK _{AF}
Drinking Water	Parent	1.9	1.5	1.2	0.83	1.9
	Circulating Metabolite	1.1	0.88	0.95	1.0	1.1
Inhalation	Parent	2.0	1.6	1.2	0.90	2.0
	Circulating Metabolite	3.9	2.6	1.4	0.81	3.9
Dermal	Parent	1.1	0.89	0.89	1.1	1.1
	Circulating Metabolite	2.2	1.4	1.1	0.98	2.2

Table 5. Fraction of Total Cumulative Lifetime Dose Contributed by Cumulative Doses During Individual Life-Stages

Chemical		Birth to 6 months	6 months to 5 years	5 years to 25 years	25 years to 75 years
IPA	Parent	1.3e-2	9.4e-2	0.33	0.56
	Circulating Metabolite	7.3e-3	5.4e-2	0.26	0.68
Vinyl Chloride	Parent	9.5e-3	6.0e-2	0.27	0.66
	Reactive Metabolite	3.3e-3	3.5e-2	0.25	0.71
Methylene Chloride	Parent	9.5e-3	6.1e-2	0.26	0.67
	Reactive Metabolite	3.5e-4	7.3e-3	0.16	0.84
Perc	Parent	2.2e-3	2.5e-2	0.20	0.77
	Circulating Metabolite	3.5e-4	8.7e-3	0.14	0.85
TCDD	Parent	4.0e-3	3.2e-2	0.26	0.70
Nicotine	Parent	1.8e-2	0.14	0.34	0.50
	Circulating Metabolite	1.5e-2	0.14	0.34	0.51

Oral Exposure to Volatiles

The results of the model simulations for isopropanol (Figure 2A) indicate that, for a constant daily intake, blood concentrations in the first decade of life decrease by nearly a factor of two, after which a slight rise is observed over the

next decade. A slight decline in blood concentration is again predicted from age 20 until approximately 40, after which the blood concentration remains fairly constant. Changes in blood concentrations of the metabolite acetone parallel the initial decline for isopropanol, but return completely to perinatal levels by age 20, after which they remain relatively constant before rising slightly late in life. Predicted blood concentrations of isopropanol and acetone vary approximately 2.5- and 2-fold, respectively, across all life-stages, with peak concentrations of both occurring in early life; a second peak for acetone occurs between the ages of 15 and 20 years. The more rapid fluctuations in blood concentrations superimposed over these trends reflect transient changes in hepatic clearance, principally the result of changes in liver clearance associated with growth spurts for that organ. The open circles and squares in Figure 2A represent the concentrations predicted with the published adult model (Clewell *et al.*, 2001a), using the appropriate physiological and biochemical parameters for several discrete ages, as a check on the predictions of the continuous age-dependent simulations performed with the life-stage model.

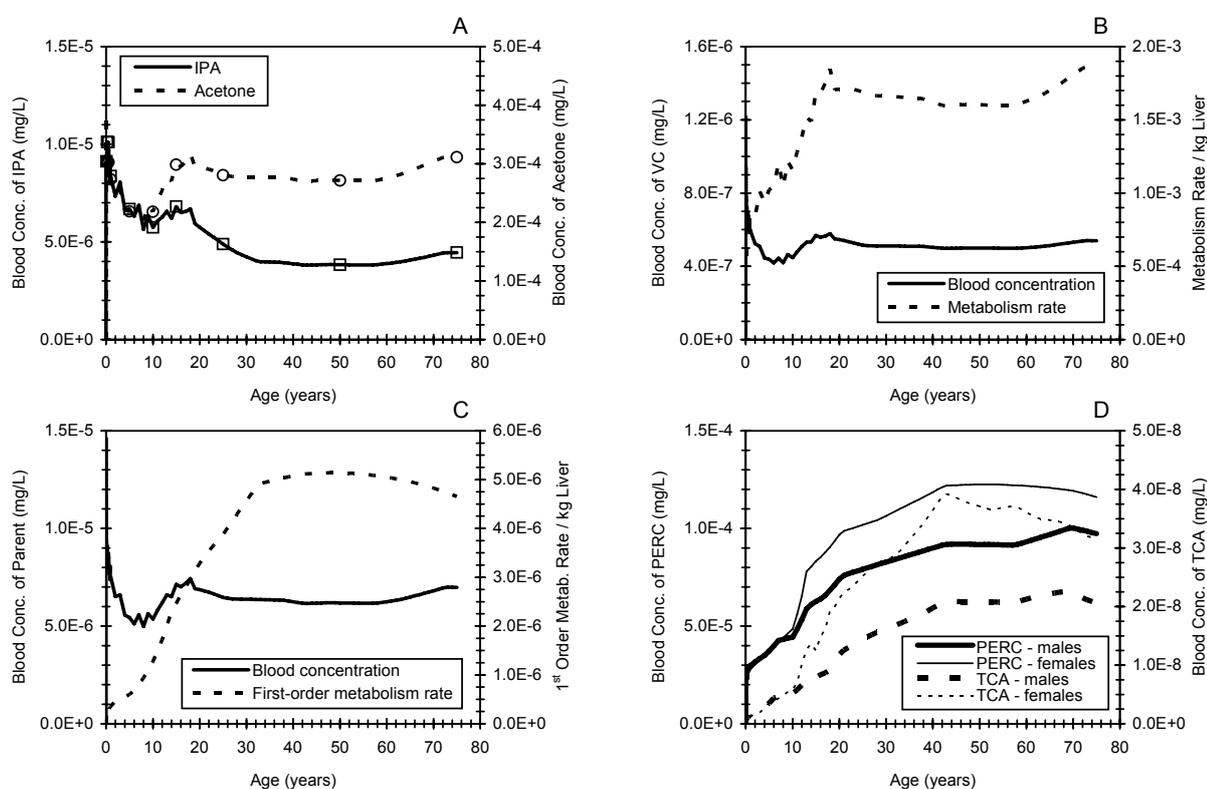


Figure 2. Blood concentrations as a function of age for continuous lifetime oral exposure at a constant daily intake of 1 $\mu\text{g}/\text{kg}/\text{d}$ for (A) isopropanol (IPA) and its metabolite acetone, and (D) perchloroethylene (PERC) and its primary metabolite TCA. Blood concentration and the rate of metabolite production per kg of liver as a function of age for continuous oral exposure at a constant daily intake of 1 $\mu\text{g}/\text{kg}/\text{d}$ for (B) vinyl chloride (VC) and (C) methylene chloride (MC). Circles and squares in panel A indicate the predictions of the published IPA model (Clewell *et al.* 2001a) using the corresponding age-specific parameters.

The profile over time predicted for vinyl chloride arterial blood concentrations (Figure 2B) is similar to that for isopropanol. Predicted blood concentrations decrease during the first 5 years of life, rising slightly thereafter until the age of 16, after which they decrease slightly before plateauing. Estimated vinyl chloride concentrations vary roughly 3-fold over the lifetime, with

the highest concentrations occurring in the first month of life. The estimated rate of reactive metabolite production per volume of liver, on the other hand, rises rapidly from birth until about age 16, after which it remains relatively constant before rising again late in life. The rate of metabolite production per volume of liver, which is the dose metric used in the cancer risk assessment for vinyl chloride (Clewell *et al.* 2001b) varies 4-fold from birth to 75 years of age, with peak values estimated in adolescence at age 14 to 16 and again at the end of the simulation.

The temporal profile for methylene chloride arterial blood concentrations (Figure 2C) is very similar to that for vinyl chloride. Methylene chloride concentrations vary 3-fold over the lifetime with the highest concentrations occurring in the first month of life. At the low concentration used in these simulations, the first-order metabolic clearance of methylene chloride by GSH conjugation competes with a higher affinity clearance by CYP2E1. The GSH conjugation rate per volume of liver, which is the dose metric used in the cancer risk assessment for methylene chloride (Andersen *et al.*, 1987) varies 31-fold with age, increasing consistently until age 25, after which it remains stable with only a slight decline after age 60.

In contrast with the results for isopropanol, vinyl chloride, and methylene chloride, blood concentrations of perchloroethylene were predicted to rise consistently from birth to approximately age 40, followed by a plateau between ages 40 and 50, and a decline from age 68 to 75 (Figure 2D). Similar trends are predicted for both males and females, but female blood concentrations are predicted to be as much as 30% higher than male values. Female blood concentrations rise more rapidly between the ages of 10 and 20, a period during which females typically experience larger increases in the fractional volume of fat, an important storage compartment for the highly lipophilic perchloroethylene, as compared with males. Across the lifespan of both males and females, perchloroethylene blood concentrations were predicted to vary approximately 5 to 7-fold, with peak values occurring between the ages of 50 and 70.

The general rise in perchloroethylene blood concentrations, as contrasted with the relatively flat profile for the other volatiles, can be ascribed two factors: (1) the much lower metabolic and pulmonary clearance of perchloroethylene relative to the other volatiles, and (2) its much higher lipophilicity. These characteristics of perchloroethylene result in greater storage of perchloroethylene in fat and other tissues.

TCA metabolite concentrations in males and females generally parallel blood perchloroethylene concentrations (Figure 2D); however, the range in metabolite blood concentrations across age is significantly larger than that of the parent compound.

Oral Exposure to Non- or Semi-Volatiles

After a substantial decline immediately following birth, TCDD concentrations were estimated to return to perinatal levels during the first 7 years of life, followed by a second rise during adolescence, a decline until age 50, and a final rise late in life (Figure 3A). At birth the concentration of TCDD in the neonate reflects trans-placental exposure to maternal stores of the chemical. The initial drop in TCDD concentration during the neonatal period results from dilution of TCDD stores by the rapid growth of the neonate. This prediction of the model, while somewhat non-intuitive, is consistent with experimental data showing that TCDD concentrations in neonates are below adult levels (Kreuzer *et al.* 1997). As pointed out by Kreuzer *et al.* (1997), the rapid growth during the neonatal period, and the resulting dilution of TCDD stores, produces an apparent half-life for TCDD in infants on the order of 5 months, as opposed to a half-life observed in the adult (resulting primarily from metabolic clearance) on the order of 10 years.

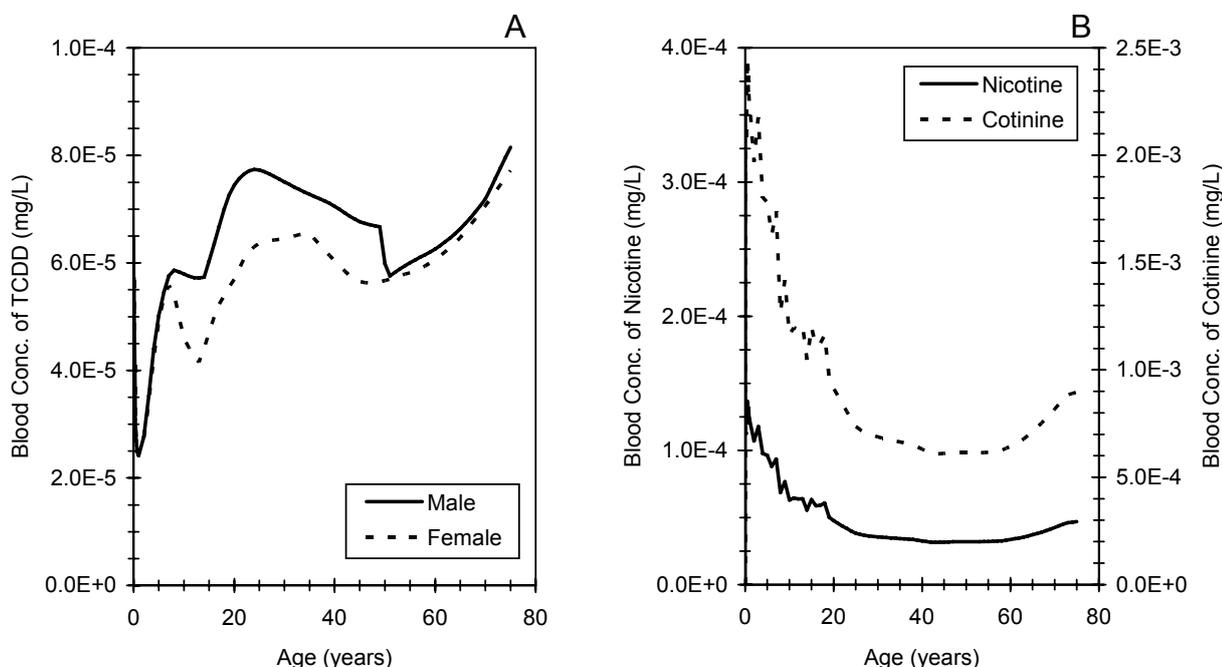


Figure 3. Blood concentrations as a function of age for (A) TCDD for continuous oral exposure of males (solid line) or females (dashed line) at a constant daily intake of 1 ng/kg/d, and (B) nicotine and its metabolite cotinine for continuous oral exposure to nicotine at a constant daily intake of 1 μ g/kg/d.

The rise in TCDD blood concentrations during childhood and adolescence can be attributed to the accumulation of the highly lipophilic, poorly metabolized compound in fat as the growth rate declines and the apparent half-life decreases. Male and female blood concentrations diverge somewhat after the 7-year point,

because the fractional volume of fat in females rises more rapidly than in males and rises to values that are about 50% larger than those for males (Supplementary Data, Table B). The larger storage capacity for TCDD in females results in blood concentrations that are generally lower than those for males.

Time-dependent sensitivity coefficients demonstrate the dependence of blood concentrations on metabolic clearance and the size of the fat compartment (Figure 4). Blood TCDD concentrations are more sensitive to fat compartment size than clearance during childhood and adolescence, while the rate of clearance exerts more control over blood concentrations during adulthood. This result demonstrates that, where a large number of parameters change with age, the relative importance of the parameters can also change with age, resulting in different expectations regarding risk factors in different age groups. The investigation of these age-dependent sensitivities would be impossible with the standard "reference man", young-adult PBPK models typically used in risk assessment.

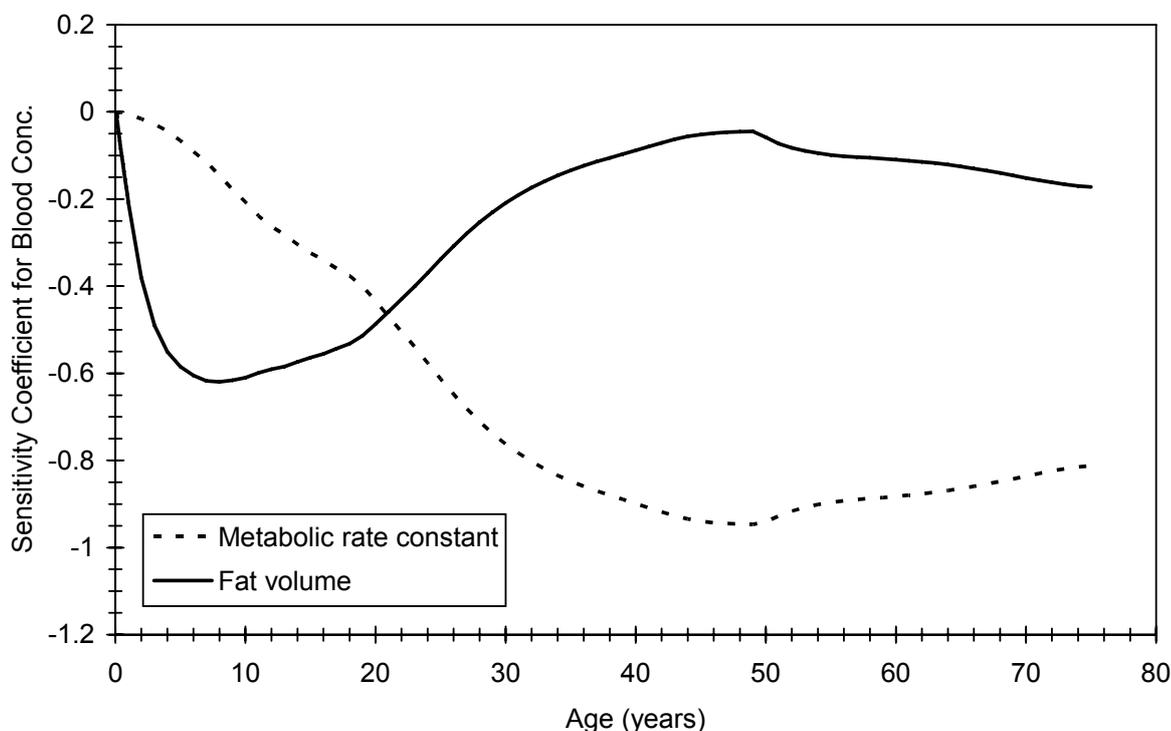


Figure 4. Age-dependent sensitivity coefficients for blood TCDD concentrations resulting from constant oral exposure. Arterial blood concentration is more sensitive to changes in fat volume (solid line) than metabolism for the first several years of life, after which it becomes more sensitive to the metabolic rate constant (dotted line). Other parameters showed lower sensitivity.

In contrast to the behavior of the highly lipophilic TCDD, estimated blood concentrations for the water soluble compound nicotine decline steadily from birth until approximately age 20 when the concentrations plateau (Figure 3B). This trend correlates with increases in total hepatic metabolic capacity, with the

spikes in blood concentration reflecting changes in liver blood flow. With the exception of an initial rise in concentration during early life driven by an increase in metabolic capacity in the liver, the trend for the cotinine metabolite blood concentration is similar to the trend for nicotine. Blood concentrations of both the parent and metabolite rise slightly after 60 years of age, as metabolic and renal clearance diminish.

Cross-Route Comparison

Comparison of dose metrics for isopropanol across oral, inhalation and dermal exposures confirms the expectation that the age-dependent pattern of internal exposure is different for each of the exposure routes (Table 4). For inhalation (Figure 5A), higher concentrations of both parent and metabolite were predicted to occur during early life. This higher exposure during early life was much more pronounced for inhalation than for ingestion (Figure 2a). For the dermal route (Figure 5B), on the other hand, peak concentrations of parent were predicted to occur late in life, although peak concentrations of metabolite were still predicted to occur early in life.

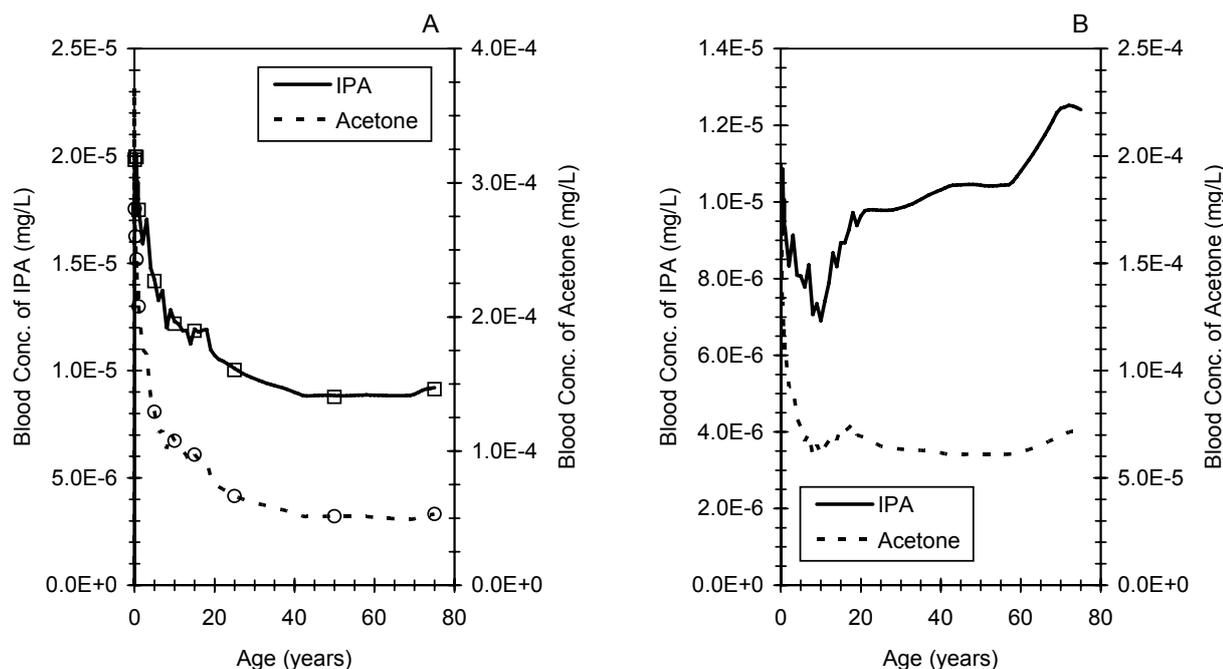


Figure 5. Blood concentrations of isopropanol (IPA) and its metabolite acetone as a function of age for (A) continuous inhalation exposure at a concentration of 1 ppb and (B) continuous dermal exposure of 18.5% of the total skin surface area to isopropanol in water at a concentration of 70 µg/L. Circles and squares in panel A indicate the predictions of the published IPA model (Clewell et al. 2001a) using the corresponding age-specific parameters.

The variation in IPA blood concentrations across age was less than 2-fold regardless of route of exposure, but the variation in acetone blood concentrations was much greater, ranging from 2- to 8-fold. The open circles and squares in Figure 5A represent the concentrations predicted with the published adult model (Clewell *et al.*, 2001a), using the appropriate physiological and

biochemical parameters for several discrete ages, as a check on the predictions of the time-dependent life-stage model.

The comparison of the age-dependent concentrations for isopropanol and acetone across exposure routes provides a classic demonstration of the first pass effect of presystemic hepatic metabolism. Following oral exposure, all of the administered isopropanol goes to the liver before distribution, resulting in the generation of greater blood concentrations of acetone, compared with inhalation or dermal exposure.

DISCUSSION

The results of the simulations conducted with the life-stage PBPK model (Tables 3 and 4) indicate that, in general, variations in pharmacokinetic dose metrics for a chemical, averaged over different life-stages, were within a factor of 2 of the young adult values. As discussed in the results section, larger variations were observed, particularly for exposures very early in life, but these were associated with relatively short durations. For all but one of the surrogate chemicals studied, estimated variation in the dose metrics over the lifespan of an individual following drinking water exposure is less than the IPCS (2001) default factor for human pharmacokinetic variability of 3.2 (Table 3). For nicotine, the estimated average dose metric for the parent chemical during the birth to six month life-stage is approximately 3.4 times greater than the parent concentration estimated at 25 years of age.

Similar trends in parent chemical dose metrics were observed for all of the volatiles, with the exception of perchloroethylene (Table 3). For most of the volatile chemicals, the average dose metrics peaked early in life, with decreasing trends over the lifespan. For these parent chemicals, the adjustment factor for pharmacokinetic variability (HK_{AF}) is based on the ratio for the neonatal (birth to six months) life-stage. For perchloroethylene, however, accumulation of the parent chemical over time results in increased concentrations later in life, rather than early. A similar trend was also observed with TCDD.

Impact of Exposure Scenario

The direction and magnitude of the age-related changes in isopropanol arterial blood concentration as a function of route of exposure can best be understood in terms of age-related changes in the ratio of (Dose Rate)/(Clearance) resulting from differential body-weight, and hence age-dependent, scaling of these terms. For each exposure route, dose rate is a different function of body weight, and therefore of age. In these case studies, the dose rate for oral exposure is assumed to be a zero-order intake scaled by body weight. That is, the exposure comparison across ages is made on the basis of equal intake of the chemical in mg/kg/d, rather than equal media (e.g., drinking water) concentration. Thus, the oral dose rate scales linearly with body weight. An alternative comparison could be made assuming equal drinking water concentration and adding age-specific drinking water consumption data to the

model. Since infants and children consume up to twice as much drinking water per kg body weight as adults (USEPA 1997), this assumption would tend to increase the dose metrics predicted for these earlier life-stages by a factor of up to 2 for oral exposure.

The inhalation exposures in these case studies were conducted at a constant inhalation concentration. For inhalation exposure to isopropanol, which is highly soluble in the blood, the dose rate is approximately equal to the exposure concentration multiplied by alveolar ventilation rate (Supplementary Data, Appendix B). Alveolar ventilation rate scales less than linearly with body weight, in part because of the nature of the scaling of basal oxygen consumption and in part because of the higher activity level in children relative to adults. Therefore, the inhalation dose rate for isopropanol is a somewhat smaller function of body weight than the oral dose rate. After an initial loading period, the uptake from inhalation of less soluble chemicals is roughly independent of ventilation rate, depending instead primarily on metabolic clearance. For these chemicals, blood concentration is primarily determined by the blood:air partition coefficient, which is assumed in these case studies to be independent of age. Thus "inhaled dose" is not a well-defined concept for poorly soluble, highly volatile chemicals such as vinyl chloride.

For dermal exposure, the dose rate is the exposure concentration x surface area x permeability coefficient. In these case studies, it has been assumed that (1) exposure is to a constant concentration, (2) exposed skin area is a constant fraction of the total body skin area, and (3) there is no change in the permeability of the skin to the chemicals with age. Thus the dose rate for dermal exposure in these case studies scales with surface area, which is a smaller function of body weight than ventilation rate. If, instead, the dermal comparison were made on the basis of equal dermal uptake in mg/kg/day, the concentrations in the infant and child would be increased by a factor of up to about 2.

Clearance, whether metabolism or blood-flow limited, tends to scale roughly with surface area over most of the lifetime, regardless of the route of exposure. However, in the first decade of life, the development of metabolic and excretory systems can lead to a substantial increase in clearance. The combination of these dose rate and clearance factors leads to the complex differences in the age-dependent profiles, such as those shown in Figures 2A, 5A, and 5B for the concentration of isopropanol in arterial blood for the different exposure routes.

Additional Considerations and Uncertainties

It is important to note that the purpose of this investigation was to develop a methodology for using PBPK modeling to evaluate the potential impact of age- and gender-specific differences on risk from chemical exposure, and to demonstrate that methodology with representative chemicals. It is not the intent of the authors to suggest that the current predictions of this initial model

for the specific chemicals addressed in the case studies should be used quantitatively in support of risk assessments. A number of simplifying assumptions have been necessary to perform these case studies in the face of significant uncertainties regarding the age-dependence of the clearance of the chemicals concerned. The main criterion for the selection of the surrogate chemicals was the existence of a validated human PBPK model in the published literature. The available human PBPK models, however, were strictly for adults, and the available age-specific metabolic information was extremely limited.

For example, for chemicals metabolized by the CYP enzymes, we have assumed that a single isoform is solely responsible for metabolic clearance regardless of age. However, other CYP isoforms can contribute to the metabolism of these chemicals, and the age-dependence of these other isoforms could vary considerably from the isoform selected for a particular case study. Many chemicals, like nicotine, have complex metabolic profiles in which minor pathways could play an important role in specific tissues at certain ages. In conducting an age-dependent risk assessment for a specific chemical, these possibilities would have to be explored.

In the case of nicotine it was even necessary to use data on the ontogeny of CYP2C as a surrogate for the development of CYP2A6. This was also a limitation for evaluating age and gender-related changes in glutathione-S-transferase. Very little is known about the development of this enzyme, although the limited data (Pacifici et al. 1981; Mendrala et al. 1993) suggests a pattern similar to that for ADH (Pikkarainen and R ih a 1967). Therefore, it was necessary to use the quantitative information on ADH to characterize the development of GST.

Assumptions were also made regarding the stoichiometry of the metabolism of the parent compound. For both PERC and nicotine, the percentage of parent chemical that is metabolized to the major metabolite (TCA and cotinine, respectively) was expressed as a constant fraction across the lifespan, due to a lack of age-specific information to the contrary. However, this yield may not be constant throughout the lifespan. Another metabolic issue that was not addressed in these case studies was detoxification of reactive metabolites (e.g., the epoxide formed from vinyl chloride). For some chemical intermediates, the rate of clearance may also be subject to age-specific variation.

There is even greater uncertainty regarding the nature of clearance processes for highly lipophilic chemicals such as TCDD. The clearance of TCDD has been ascribed to metabolism and biliary/fecal excretion (Rohde et al. 1999). For this case study, we have assumed that the age-dependence of the clearance of TCDD is primarily dependent on the development of metabolic clearance by CYP1A2. This assumption was based on data showing that fecal clearance of non-metabolized TCDD contributes on the order of 37% to total elimination in the adult (Rohde et al. 1999). To the extent that fecal clearance of non-metabolized chemical contributes to the age-dependent clearance of TCDD, the results of this case study could be somewhat misleading. However, the most

striking feature of the age-dependent kinetics of TCDD, the rapid decrease in body burden during the first year of life, is not sensitive to this uncertainty. Regardless of the nature of TCDD clearance, the observed kinetics in infants is consistent with complete absorption and negligible elimination during the first year of life (Abraham *et al.* 1996), with an apparent half-life on the order of 5 months (as compared to greater than 5 years in the adult) that reflects dilution by the growth of tissues rather than actual elimination (Kreuzer *et al.* 1997).

Conclusions

The results of the present analysis for environmental contaminants are in general agreement with the findings of quantitative analyses of data on pharmaceutical chemicals (Renwick *et al.*, 2000; Ginsberg *et al.*, 2002; Hattis *et al.*, 2003), which have suggested that the largest difference in pharmacokinetics observed between children and adults is for the early postnatal period. These results are also consistent with work conducted by Alcorn and McNamara (2003) in which the general pattern of postnatal development of selected CYP P450 enzyme pathways were quantified based on *in vitro* activity in fetal or infant hepatic microsomes as a fraction of adult activity. The differences observed in the pharmacokinetics between children and adults, which are reflected in a slower clearance of the chemicals in the infant, appear to be related primarily to the immaturity of the metabolic enzyme systems responsible for clearance of these chemicals from the body. However, these enzyme systems mature rapidly, resulting in smaller differences in pharmacokinetics as compared to adults (generally on the order of a factor of 2 or 3) by the time children reach 6 months of age. Changes observed in later childhood, which can result in faster clearance than in the adult, appear to be related to the physiological changes associated with growth (*i.e.*, changes in organ to body weight ratios and accompanying changes in organ blood flows) and activity (which impacts the ventilation rate).

For selected classes of chemicals, mainly the more lipophilic compounds (perchloroethylene and TCDD), there is a relative increase in dose metrics in the later life-stages (25 years to 75 years) for either the circulating metabolite or the parent compound. This increase reflects the capability of these chemicals, due to their physical/chemical properties, to accumulate in the body over the duration of the lifetime, possibly combined later in life with a decrease in function of clearance systems.

The results of the current analyses provide some insight into potential differences in pharmacokinetics across life-stages and how these differences may result in differences in internal dose metrics following chemical exposure. The most important factor appears to be the potential for decreased clearance of a toxic chemical in the perinatal period due to the lower activity of many metabolic enzyme systems, although this same factor may also reduce the production of a toxic metabolite. In general, as long as large differences in dosimetry are restricted to the early childhood period their contribution to the lifetime average daily dose, the metric used for cancer risk assessment and many chronic non-cancer effects, is limited by the relatively small portion of the lifetime over which

they occur (Table 5). However, due to the potential for age-dependent pharmacodynamic factors associated with growth and development, there may be chemicals and health outcomes for which the maintenance of a higher internal dose over a relatively brief period during early life may have a substantial impact on risk (Ginsberg, 2003). Thus the immaturity of clearance systems during early life may represent an important window of pharmacokinetic sensitivity for exposures to toxic chemicals.

The results presented here illustrate a methodology for using PBPK modeling to evaluate the potential impact of age- and gender-specific differences on risk from chemical exposure. This modeling approach establishes a basis for forming expectations about the magnitude and range of differences in dosimetry resulting from age-dependent differences in various biochemical and physiological parameters at different life-stages. This work represents an initial attempt to provide a predictive pharmacokinetic framework that could be used to characterize the effect of age and gender differences on tissue dosimetry for a chemical or class of chemicals. In particular, only young adult data has been used for validation of chemical-specific model predictions; therefore, extrapolations with the model to other life-stages, while based on a reasonable description of age-dependent physiological and biochemical processes, have not yet been validated. At this stage, the model predictions for early and late life-stages should be considered to represent reasonable expectations rather than predictive extrapolations.

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SUPPLEMENTARY DATA

APPENDIX A

Growth and Age-Related Equations

Body Weight

For males:

$$BW = \begin{cases} (13.642 \cdot (1.0 - e^{-0.0555 \cdot Months})) + 3.512, & \text{if } Months \leq 40.0 \\ (312.608 \cdot (1.0 - e^{-0.0389 \cdot e^{0.0102 \cdot Months}})) - 2.218, & \text{if } 40.0 < Months \leq 162.0 \\ 2.346 \cdot e^{3.533 \cdot (1.0 - e^{-0.0142 \cdot Months})}, & \text{if } 162.0 < Months \leq 303.0 \\ (2.19e-8 \cdot Months^3) - (1.22e-4 \cdot Months^2) + (0.120 \cdot Months) + 50.824, & \text{if } Months > 303.0 \end{cases}$$

(A-1)

and for females:

$$BW = \begin{cases} (14.291 \cdot (1.0 - e^{-0.0466 \cdot Months})) + 3.319, & \text{if } Months \leq 32.0 \\ (280.684 \cdot (1.0 - e^{-0.0236 \cdot e^{0.0139 \cdot Months}})) + 4.30, & \text{if } 32.0 < Months \leq 154.0 \\ 1.097 \cdot e^{4.118 \cdot (1.0 - e^{-0.0198 \cdot Months})}, & \text{if } 154.0 < Months \leq 268.0 \\ (6.17e-9 \cdot Months^3) - (9.68e-5 \cdot Months^2) + (0.109 \cdot Months) + 43.790, & \text{if } Months > 268.0 \end{cases}$$

(A-2)

where BW is body weight in kg and $Months$ is the age in months.

Surface Area

$$SA = \begin{cases} (0.1165 \cdot BW^3) - (9.442 \cdot BW^2) + (460.997 \cdot BW) + 288.329, & \text{if } BW < 35.0 \\ (0.002573 \cdot BW^3) - (1.127 \cdot BW^2) + (289.137 \cdot BW) + 1000.0, & \text{if } BW \geq 35.0 \end{cases} \quad (\text{A-3})$$

where SA is total body surface area in cm^2 and BW is body weight in kg.

Ventilation

$$QAlv = QAlvC(Months) \bullet BW^{0.75} \quad (A-4)$$

where $QAlv$ is the alveolar ventilation rate in L/hr, $QAlvC$ is the table function for body-weight adjusted alveolar ventilation rate in L/hr/kg^{3/4}, $Months$ is the age in months, and BW is body weight in kg.

Cardiac Output

$$QC = \left(\left(56.906 \bullet \left(1.0 - e^{-0.681 \bullet e^{0.0454 \bullet QAlvC(Months)}} \right) \right) - 29.747 \right) \bullet BW^{0.75} \quad (A-5)$$

where QC is cardiac output in L/hr, $QAlvC$ is the table function for body-weight adjusted alveolar ventilation in L/hr/kg^{3/4}, $Months$ is age in months, and BW is body weight in kg.

Tissue Volumes

$$VTissue = VTissueC(Months) \bullet BW \quad (A-6)$$

where $Tissue$ is brain, fat, or liver; $VTissue$ is the volume of the specified tissue in L; $VTissueC$ is the table function for the specified tissue volume as a fraction of body weight; $Months$ is age in months; and BW is body weight in kg.

For males:

$$VGI = \begin{cases} \left(10.497 \bullet \left(1.0 - e^{-4.033 \bullet e^{0.0118 \bullet Months}} \right) \right) - 10.266, & \text{if } Months \leq 59.0 \\ \left(0.533 \bullet \left(1.0 - e^{-0.00116 \bullet e^{0.0422 \bullet Months}} \right) \right) + 0.226, & \text{if } 59.0 < Months \leq 158.0 \\ \left(1.645 \bullet \left(1.0 - e^{-0.110 \bullet e^{0.0153 \bullet Months}} \right) \right) - 0.624, & \text{if } 158.0 < Months \leq 265.0 \\ \left(1.645 \bullet \left(1.0 - e^{-0.110 \bullet e^{0.0153 \bullet 265.0}} \right) \right) - 0.624, & \text{if } Months > 265.0 \end{cases} \quad (A-7)$$

and for females:

$$VGI = \begin{cases} \left(10.950 \cdot \left(1.0 - e^{-4.075 \cdot e^{0.0117 \cdot Months}}\right)\right) - 10.718, & \text{if } Months \leq 58.0 \\ \left(0.780 \cdot \left(1.0 - e^{-0.00104 \cdot e^{0.0397 \cdot Months}}\right)\right) + 0.224, & \text{if } 58.0 < Months \leq 147.0 \\ \left(1.012 \cdot \left(1.0 - e^{-0.0376 \cdot e^{0.0199 \cdot Months}}\right)\right) - 0.525, & \text{if } 147.0 < Months \leq 265.0 \\ \left(1.012 \cdot \left(1.0 - e^{-0.0376 \cdot e^{0.0199 \cdot 265.0}}\right)\right) - 0.525, & \text{if } Months > 265.0 \end{cases} \quad (\text{A-8})$$

where VGI is the volume of GI tract tissue in L, and $Months$ is age in months.

For males:

$$VRap = 2.596 * VGI \quad (\text{A-9})$$

and for females:

$$VRap = 2.464 * VGI \quad (\text{A-10})$$

where $VRap$ is the volume of rapidly perfused tissue in L and VGI is the volume of GI tract tissue in L.

$$VSkn = \frac{((0.001 \cdot SA) \cdot Depth)}{1000.0} \quad (\text{A-11})$$

where $VSkn$ is the volume of skin in L, SA is the total body surface area in cm^2 , and $Depth$ is the skin depth in cm.

If dosing via dermal exposure:

$$VSlw = \left(0.84 - VAlvC - VBrnC(Months) - VFatC(Months) - VLivC(Months) - VMucC - \left(\frac{VRap}{BW}\right) - \left(\frac{VSkn}{BW}\right)\right) \cdot BW \quad (\text{A-12})$$

If not dosing via dermal exposure:

$$VSlw = \left(0.84 - VAlvC - VBrnC(Months) - VFatC(Months) - VLivC(Months) - VMucC - \left(\frac{VRap}{BW}\right)\right) \cdot BW \quad (\text{A-13})$$

where VS/w is the volume of slowly perfused tissue in L; $VALvC$, $VBrnC(Months)$, $VFatC(Months)$, $VLivC(Months)$, $VMucC$ are the volumes of alveolar blood, brain, fat, liver, and mucous, respectively, as a fraction of body weight; $VRap$ is the volume of rapidly perfused tissues in L; $VSkn$ is the volume of skin in L; and BW is the body weight in kg.

Blood Flows

$$QTissueFrac = \frac{VTissueC(Months)}{VTissueC(300.0)} \cdot QAdultTissue \quad (A-14)$$

where *Tissue* is for brain or fat; $QTissueFrac$ is the blood flow to the specified tissue as a fraction of cardiac output; $VTissueC(Months)$ is the volume for the specified tissue as a fraction of body weight; $VTissueC(300.0)$ is the volume for the specified tissue as a fraction of body weight at 25 years old; and $QAdultTissue$ is the standard fractional blood flow for the specified tissue as a fraction of cardiac output for an adult.

$$QLivFrac = \frac{VLivC(Months) + \frac{VGI}{BW}}{VLivC(300.0) + \frac{VGI25}{BW25}} \cdot QAdultLiv \quad (A-15)$$

where $QLivFrac$ is the blood flow to the liver as a fraction of cardiac output; $VlivC(Months)$ is the liver volume as a fraction of body weight; VGI is the GI tract volume in kg; BW is the body weight in kg, $VLivC(300.0)$ is the liver volume as a fraction of body weight at 25 years old; $VGI25$ is the GI tract volume in kg at 25 years old; $BW25$ is the body weight in kg at 25 years old; and $QAdultLiv$ is the standard fractional blood flow for the specified tissue as a fraction of cardiac output for an adult.

$$QRapFrac = \frac{\left(\frac{VRap}{BW}\right)}{VRapC25} \cdot QAdultRap \quad (A-16)$$

where $QRapFrac$ is the blood flow to the rapidly perfused tissues as a fraction of cardiac output, $VRap$ is the volume of rapidly perfused tissue in L, $VRapC25$ is the volume of rapidly perfused tissue as a fraction of body weight at 25 years old, BW is the body weight in kg, and $QAdultRap$ is the standard fractional blood flow for rapidly perfused tissues as a fraction of cardiac output for an adult.

$$Q_{SknFrac} = \begin{cases} \left(\frac{V_{Skn}}{BW} \right) \cdot Q_{AdultSkn} \cdot 0.001, & \text{if dosing dermally} \\ 0.0, & \text{if not dosing dermally} \end{cases} \quad (\text{A-17})$$

where $Q_{SknFrac}$ is the blood flow to skin as a fraction of cardiac output, V_{Skn} is the volume of skin in L, V_{SknC25} is the volume of skin as a fraction of body weight at 25 years old, BW is the body weight in kg, and $Q_{AdultSkn}$ is the standard fractional blood flow for skin as a fraction of cardiac output for an adult.

$$Q_{SlwFrac} = \left(\frac{\left(\frac{V_{Slw}}{BW} \right) \cdot Q_{AdultSlw}}{V_{SlwC25}} \right) - Q_{SknFrac} \quad (\text{A-18})$$

where $Q_{SlwFrac}$ is the blood flow to the slowly perfused tissues as a fraction of cardiac output, V_{Slw} is the volume of slowly perfused tissue in L, V_{SlwC25} is the volume of slowly perfused tissue as a fraction of body weight at 25 years old, BW is the body weight in kg, $Q_{AdultSlw}$ is the standard fractional blood flow for slowly perfused tissues as a fraction of cardiac output for an adult, and $Q_{SknFrac}$ is the blood flow to skin as a fraction of cardiac output.

$$Q_{Sum} = Q_{BrnFrac} + Q_{FatFrac} + Q_{LivFrac} + Q_{RapFrac} + Q_{SknFrac} + Q_{SlwFrac} \quad (\text{A-19})$$

where Q_{Sum} is the sum of the blood flows as a fraction of cardiac output; and

$Q_{BrnFrac}$, $Q_{FatFrac}$, $Q_{LivFrac}$, $Q_{RapFrac}$, $Q_{SknFrac}$, and $Q_{SlwFrac}$ are the blood flow to the brain, fat, liver, rapidly perfused tissues, skin, and slowly perfused tissues, respectively, as a fraction of cardiac output.

$$Q_{Tissue} = \frac{Q_{TissueFrac}}{Q_{Sum}} \cdot QC \quad (\text{A-20})$$

where $Tissue$ is for all of the tissues, Q_{Tissue} is the blood flow to the specified tissue in L/hr, $Q_{TissueFrac}$ is the blood flow to the specified tissue as a fraction of cardiac output, Q_{Sum} is the sum of the blood flows as a fraction of cardiac output, and QC is cardiac output in L/hr.

Metabolism

$$V_{Max} = \left(\frac{V_{MaxC} \cdot BW_{25}^{0.75}}{V_{Liv25}} \right) \cdot V_{Liv} \cdot FracMet(Months), \quad (A-21)$$

where V_{Max} is the rate of oxidative metabolism in mg/hr, V_{MaxC} is the body-weight adjusted rate of oxidative metabolism in mg/hr/kg^{3/4}, BW_{25} is body weight in kg at 25 years old, V_{Liv25} is the liver volume in L at 25 years old, V_{Liv} is the liver volume in L, and $FracMet(Months)$ is the table function that gives enzyme activity as a fraction of the adult level.

$$KF = \left(\frac{KFC}{BW_{25}^{0.25} \cdot V_{Liv25}} \right) \cdot V_{Liv} \cdot FracMet1(Months), \quad (A-22)$$

where KF is the rate constant for 1st order metabolism in /hr, KFC is the body-weight adjusted rate constant for 1st order metabolism in kg^{1/4}/hr, BW_{25} is body weight in kg at 25 years old, V_{Liv25} is the liver volume in L at 25 years old, V_{Liv} is the liver volume in L, and $FracMet1(Months)$ is the table function that gives enzyme activity as a fraction of the adult level.

Urinary Clearance

$$GFR = \begin{cases} 0.12, & \text{if Days} \leq 1 \\ 0.12 \cdot (1.0 + Days), & \text{if } 1 \leq \text{Days} \leq 3 \\ 0.48 + (0.0571 \cdot (Days - 3)), & \text{if } 3 < \text{Days} \leq 10 \\ 7.343 \cdot \left(\frac{(-5.33e-5 \cdot BW^2) + (2.547e-2 \cdot BW) + 0.113}{1.73} \right), & \text{if Days} > 10 \\ \left(7.343 \cdot \left(\frac{(-5.33e-5 \cdot BW^2) + (2.547e-2 \cdot BW) + 0.113}{1.73} \right) \right) - (0.0066 * 7.62 * (Years - 30)), & \text{if Years} > 30 \end{cases} \quad (A-23)$$

where $Days$ is age in days, BW is body weight in kg, and $Years$ is age in years.

$$CI_{Urine} = (CI_{UrineC} \cdot BW^{0.75}) \cdot \left(\left(Use_{GFR} \cdot \frac{GFR}{GFR_{25}} \right) + \left(Use_{Renal} \cdot \frac{Renal(Months)}{Renal(300.0)} \right) \right) \quad (A-24)$$

where CI_{Urine} is the urinary clearance rate in L/hr, CI_{UrineC} is the body-weight adjusted urinary clearance rate in L/hr/kg^{3/4}, BW is body weight in kg, Use_{GFR} and Use_{Renal} are switches to indicate whether to use GFR or renal blood flow for scaling of urinary clearance, GFR is the glomerular filtration rate in L/hr, GFR_{25} is the GFR at 25 years old, $Renal(Months)$ is the renal blood flow in L/hr from the table function, and $Renal(300.0)$ is the renal blood flow in L/hr at 25 years old from the table function.

APPENDIX B

Steady State Analysis

A. Inhalation

Using a steady-state analysis, we can compute the arterial blood concentration of the inhaled parent chemical in terms of the physiological and kinetic model parameters such as minute ventilation, hepatic clearance, hepatic blood flow, etc. The mass balance in the system at steady-state requires that the net extraction of the compound from the respiratory tract is equal to hepatic clearance by metabolism (Equation B-1). The liver mass balance requires that the rate of loss of the chemical species from the blood perfusing the liver is equal to its metabolic clearance (Equation B-2). For inhaled compounds that are metabolized at the portal of entry (i.e., respiratory tract), the metabolic capacity of the respiratory tract tissues is ignored when computing the arterial blood concentration. This simplification is justified because the volume of the respiratory tract tissues expressing enzyme activity is very small compared to the liver, and hence the total metabolic capacity of the respiratory tract is small compared to the metabolic capacity of the liver. The governing mass balance equations for this simplified system at steady-state are:

$$\dot{V}_a \left(C_{inh} - \frac{C_{art}}{H_{ba}} \right) = Cl_l C_{vl} \quad (\text{B-1})$$

$$Q_l (C_{art} - C_{vl}) = Cl_l C_{vl} \quad (\text{B-2})$$

Here H_{ba} is the styrene blood:air partition coefficient, V_a is the minute ventilation rate, C_{inh} and C_{art} are the inhaled concentration and arterial concentration of the parent compound, respectively, C_{vl} is the parent chemical concentration in the venous blood exiting the liver, and Cl_l is the metabolic clearance of styrene in the liver. By rearranging the above equations arterial blood concentration can be represented in terms of system-independent parameters as follows:

$$C_{art} = C_{inh} \left[\frac{1}{\dot{V}_a} \left(\frac{Q_l Cl_l}{Q_l + Cl_l} \right) + \frac{1}{H_{ba}} \right]^{-1} \quad (\text{B-3})$$

Although expressed differently, this equation is equivalent to that derived previously (Andersen, 1981).

For insoluble compounds with very low hepatic clearance where $Cl_{li} \ll Q_{li}$ and $Cl_{li} \ll V_a/H_{ba}$, the system is metabolism limited and the steady-state arterial concentration simplifies to $H_{ba}C_{inh}$. For soluble compounds with very high hepatic clearance where $Cl_{li} \gg Q_{li}$ and $Q_{li} \gg V_a/H_{ba}$, the system is flow limited and the steady-state arterial concentration simplifies to $V_a C_{inh}/Q_{li}$. Thus, for poorly metabolized lipophilic chemicals (e.g., perchloroethylene), the blood

concentration is chiefly determined by the blood:air partition coefficient, while for well metabolized, water-soluble chemicals (e.g., isopropanol), the arterial concentration is dependent on ventilation rate and liver blood flow. For intermediate chemical properties, changes in metabolic clearance will also impact blood concentration.

B. Oral

The steady-state condition for oral exposure to volatile compounds can be derived similarly to the inhalation case, to the extent that it is meaningful to consider the case of a continuous oral exposure. In fact, even though ingestion of a chemical contaminant in drinking water is obviously sporadic rather than continuous, a simplification that is often adequate (NRC 1986), and the one used in this study, is to assume a continuous zero-order ingestion rate (Drink, mg/hr) based on the daily ingested Dose (mg/kg/day):

$$Drink = \frac{Dose * BW}{24.0} \quad (B-4)$$

where BW is the body weight (kg) and 24 is the number of hours in a day. The mass balance for the liver can then be expressed as follows:

$$Drink + (Q_l * C_{art}) = (Q_l * C_{vl}) + (Cl_l * C_{vl}) \quad (B-5)$$

For continuous oral dosing of a highly volatile chemical, the chemical leaving the liver is transported to the lung where it is rapidly exhaled. Assuming that $C_{art} \ll C_{vl}$ yields:

$$C_{vl} = \frac{Drink}{Q_l + Cl_l} \quad (B-6)$$

At the same time, the mass balance on the arterial blood requires that:

$$(Q_c * C_{ven}) - (Q_c * C_{art}) = V_a * \frac{C_{art}}{H_{ba}} \quad (B-7)$$

or:

$$C_{art} = \frac{Q_c * C_{ven}}{Q_c + \frac{V_a}{H_{ba}}} \quad (B-8)$$

where C_{ven} is the mixed venous blood concentration and Q_c is the cardiac output. But C_{ven} is just the flow-weighted average of the tissue venous blood concentrations:

$$C_{ven} = \frac{\sum Q_i * C_{vi}}{Q_c} \quad (B-9)$$

and:

$$C_{art} = \frac{\sum Q_i * C_{vi}}{Q_c + \frac{V_a}{H_{ba}}} \quad (\text{B-10})$$

Thus the main age-dependent drivers for C_{art} in this case are the blood flows and ventilation rate. However, one term in the sum is $Q_l * C_{vl}$, which introduces a dependence on Cl_l . At steady state, the venous blood leaving the rest of the tissues will be at the same concentration as the arterial blood, while the venous blood leaving the liver will be reduced by hepatic clearance:

$$C_{art} = \frac{((Q_c - Q_l) * C_{art}) + (Q_l * C_{vl})}{Q_c + \frac{V_a}{H_{ba}}} \quad (\text{B-11})$$

Solving for C_{art} and substituting for C_{vl} from Equation B-6:

$$C_{art} = \frac{Drink * Q_l}{(Q_l + Cl_l) * \left(Q_l + \frac{V_a}{H_{ba}} \right)} \quad (\text{B-12})$$

For high hepatic clearance ($Cl_l \gg Q_l$) and volatility ($V_a/H_{ba} \gg Q_l$):

$$C_{art} = \frac{Drink * Q_l}{Cl_l * \frac{V_a}{H_{ba}}} \quad (\text{B-13})$$

Thus the age-dependent parameters of importance for determining the arterial blood concentration resulting from ingestion of a well-metabolized, highly volatile chemical (e.g., vinyl chloride) are the metabolism parameters, the liver blood flow, and the ventilation rate.

The case of oral exposure to non-volatile chemicals that are cleared by hepatic metabolism is more simply derived, since there is no exhalation clearance. Thus Equation B-11 becomes:

$$C_{art} = \frac{((Q_c - Q_l) * C_{art}) + (Q_l * C_{vl})}{Q_c} \quad (\text{B-14})$$

Therefore, at steady state, $C_{art} = C_{vl}$. Substituting C_{art} for C_{vl} in Equation B-5 results in an inverse dependence of arterial blood concentration on hepatic clearance:

$$C_{art} = \frac{Drink}{Cl_l} \quad (\text{B-15})$$

References

- Andersen M. 1981. A physiologically based toxicokinetic description of the metabolism of inhaled gases and vapors: analysis at steady state. *Toxicol Appl Pharmacol* 60:509-526.

Table A. Table Function for Normalized Alveolar Ventilation Rates (L/hr/kg^{3/4})

Months	Alveolar Ventilation (L/hr/kg ^{3/4})	
	Males	Females
0	28.06	29.83
6	28.06	29.83
18	29.20	30.54
48	27.60	27.78
84	24.76	25.11
120	26.18	24.01
156	21.46	16.38
198	20.74	15.13
246	18.04	13.36
342	16.94	12.70
510	15.20	10.97
690	15.25	10.98
834	13.71	11.11
900	14.07	11.35
1080	14.07	11.35

Table B. Table Function for Relative Tissue Volumes (fraction of body weight)

Months	Brain		Liver		Fat	
	Male	Female	Male	Female	Male	Female
0	0.133	0.106	0.0445	0.0417	0.2147	0.2147
1	0.128	0.121	0.0414	0.0426		
2	0.114	0.122	0.0383	0.0418	0.2147	0.2147
3	0.0986	0.116	0.0363	0.0388		
4	0.115	0.112	0.0379	0.0359	0.2565	0.2565
5	0.118	0.113	0.0390	0.0370		
6	0.110	0.112	0.0354	0.0370	0.2583	0.2583
7	0.103	0.126	0.0366	0.0465		
8	0.113	0.123	0.0395	0.0405	0.2525	0.2525
9	0.117	0.119	0.0416	0.0399		
10	0.113	0.110	0.0376	0.0373	0.2470	0.2470
11	0.117	0.119	0.0424	0.0469		
12	0.112	0.109	0.0406	0.0408	0.2416	0.2416
18					0.2265	0.2265
24	0.0997	0.103	0.0420	0.0382	0.2126	0.2126
36	0.0965	0.0866	0.0350	0.0325		
48	0.0890	0.0767	0.0390	0.0350	0.1711	0.1712
60	0.0771	0.0774	0.0361	0.0383	0.1585	0.1585
72	0.0694	0.0638	0.0356	0.0324	0.1518	0.1515
84	0.0670	0.0608	0.0311	0.0306	0.1486	0.1516
96	0.0607	0.0504	0.0361	0.0310	0.1490	0.1597
108	0.0494	0.0491	0.0307	0.0322		
120	0.0496	0.0408	0.0324	0.0300	0.1543	0.1952
132	0.0436	0.0355	0.0294	0.0281		
144	0.0373	0.0397	0.0273	0.0287	0.1575	0.2128
156	0.0364	0.0300	0.0251	0.0243		
168	0.0287	0.0257	0.0256	0.0244	0.1600	0.2278
180	0.0252	0.0281	0.0225	0.0197		
192	0.0255	0.0274	0.0227	0.0225	0.1624	0.2410
204	0.0256	0.0262	0.0218	0.0229		
216	0.0248	0.0273	0.0207	0.0258	0.1643	0.2524
228	0.0224	0.0271	0.0226	0.0242		
240					0.1661	0.2625
270	0.0239	0.0272	0.0227	0.0236		
330	0.0237	0.0269	0.0235	0.0247		

Months	Brain		Liver		Fat	
	Male	Female	Male	Female	Male	Female
390	0.0232	0.0263	0.0237	0.0239		
421.2						0.2874
450	0.0224	0.0254	0.0239	0.0249		
510	0.0232	0.0251	0.0248	0.0261		
536.4						0.3533
570	0.0233	0.0253	0.0246	0.0246		
588					0.213	
606					0.258	
630	0.0235	0.0246	0.0247	0.0237		
670.8						0.4188
690	0.0246	0.0249	0.0247	0.0244		
750	0.0256	0.0255	0.0239	0.0231		
774						0.4456
810	0.0247	0.0276	0.0226	0.0232		
840					0.305	
870	0.0253	0.0269	0.0212	0.0225		
930	0.0256	0.0278	0.0212	0.0231		
990	0.0279	0.0284	0.0203	0.0218		
1080	0.0249	0.0281	0.0182	0.0228	0.305	0.4456

Table C. Table Function for Enzyme Activity (fraction of adult value)

Months	Relative Activity			
	2E1	1A2	2C	ADH
0	0	0	0	0.2318
0.0164	0.1009	0.0105	0.0382	
0.1281	0.1429	0.0199		
0.1314			0.2209	
0.5552			0.3150	
0.5881	0.2378	0.0356		
1.15				0.2513
1.9877	0.3163	0.1245		
2.0041			0.3860	
5.2238			0.3221	
5.4538	0.3579	0.2458		
72				0.4188
74.6776	0.8113	0.5178		
300	1	1	1	
390				1
1080	1	1	1	1

Table D. Table Function for Renal Blood Flow (L/hr)

Months	Renal Blood Flow (L/hr)
0	7.482
0.0667	19.677
0.133	31.871
0.200	39.005
0.267	44.066
0.333	47.992
0.400	51.199
0.467	53.911
0.533	56.261
0.600	58.333
0.667	60.186
0.733	61.863
0.800	63.394
0.867	64.802
0.933	66.106
1.000	67.320
1.067	68.455
1.133	69.522
1.200	70.528
1.267	71.479
1.333	72.381
1.400	73.240
1.467	74.058
1.533	74.840
300	74.840
420	81.802
540	69.366
660	58.967
780	54.419
900	40.625
1020	32.915
1080	32.915

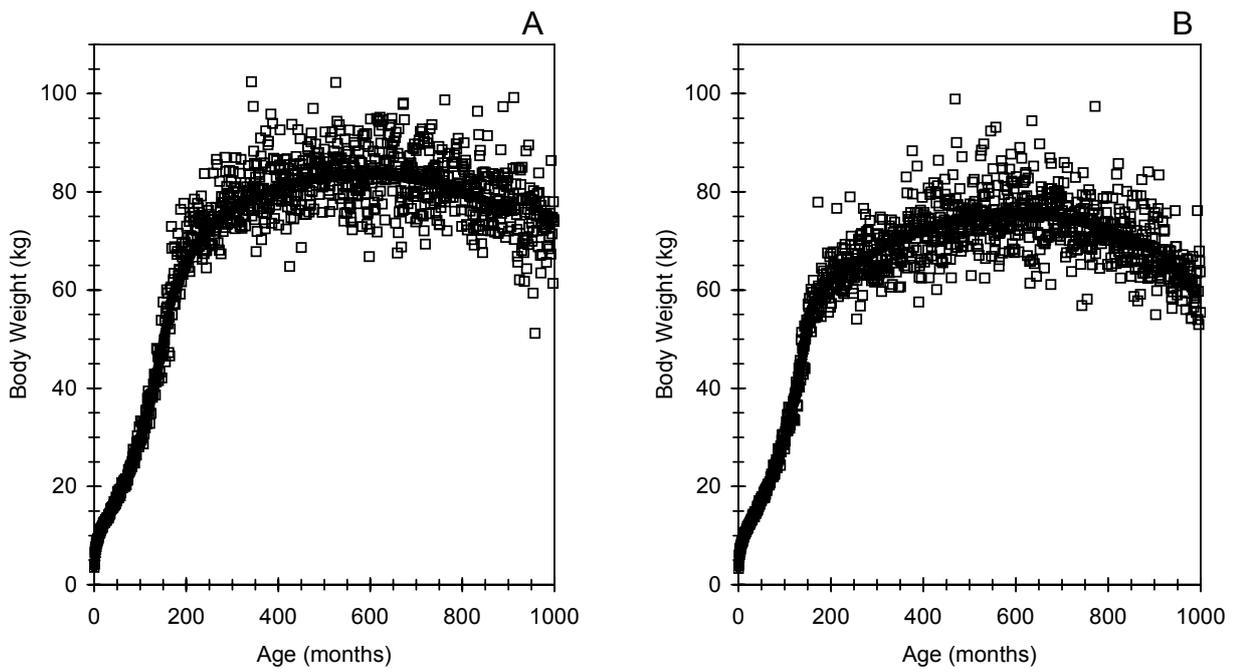


Figure A. Male (A) and female (B) body weight data (squares) from NHANES (1995) and equations (lines) fit to the data.

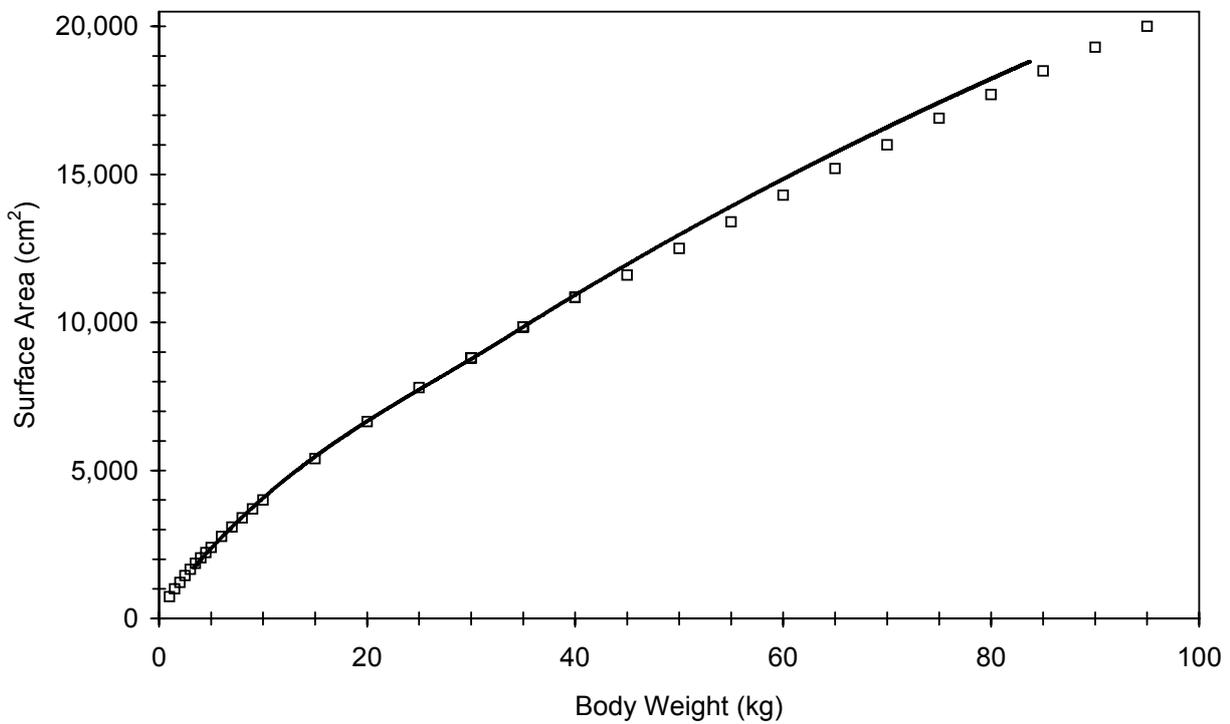


Figure B. Total body surface area data (squares) from ICRP (1975) and equations (line) fit to the data.

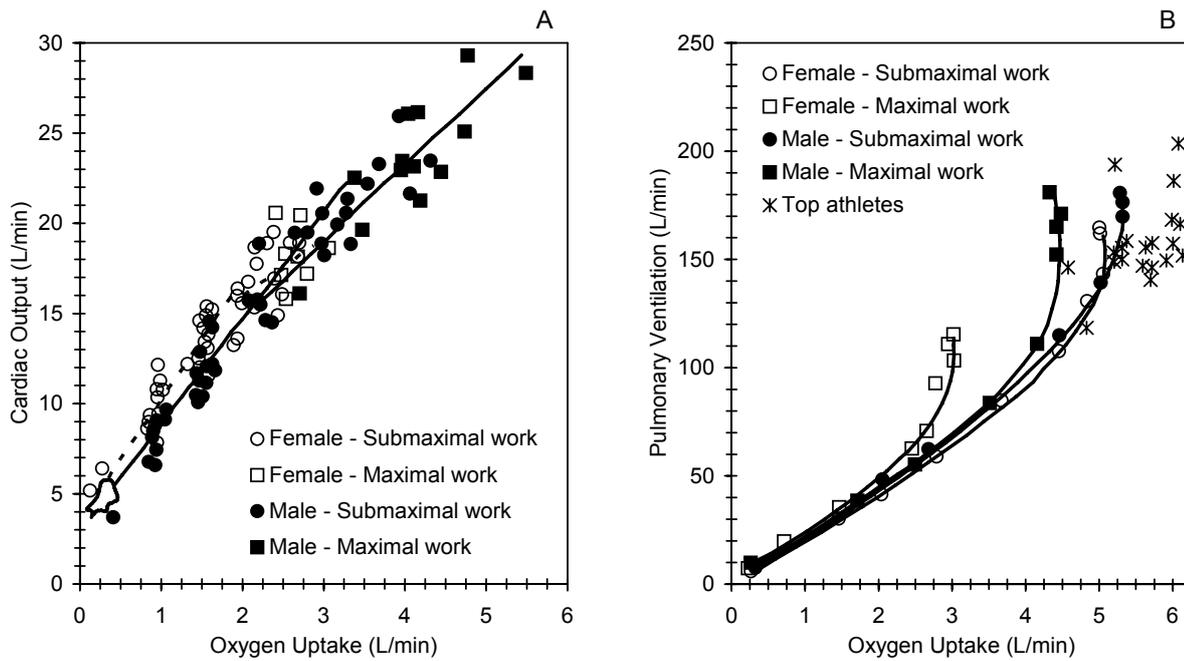


Figure C. (A) Individual values on cardiac output in relation to oxygen uptake at rest, during submaximal, and during maximal exercise on 23 subjects sitting on a bicycle ergometer. Regression lines (broken lines for women) were calculated for experiments where the oxygen uptake was (1) below 70 percent and (2) above 70 percent of the individual's maximum. (From P.-O. Åstrand et al., 1964). (B) Pulmonary ventilation at rest and during exercise (running or cycling). Four individual curves are presented. Several work loads gave the same maximal oxygen uptake. Work time from 2 to 6 minutes. Stars denote individual values for top athletes measured when maximal oxygen uptake was attained. (Data from Saltin and P.-O. Åstrand, 1967.) Individuals with maximal oxygen uptake of 3 L/min or higher usually fall within the shadowed area. Note the wide scattering at high oxygen uptakes. (Figure is reproduced from I. Astrand, 1983)

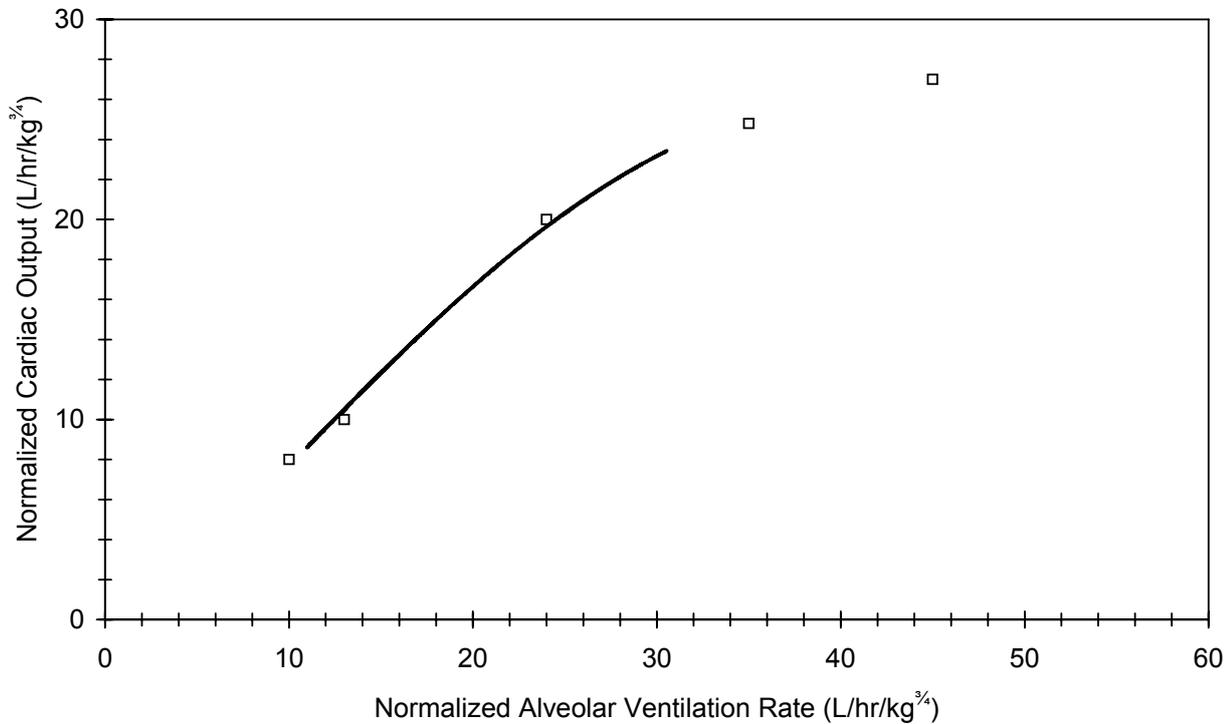


Figure D. Cardiac output *versus* alveolar ventilation (squares) computed from Astrand (1983) and equation (line) fit to the data.

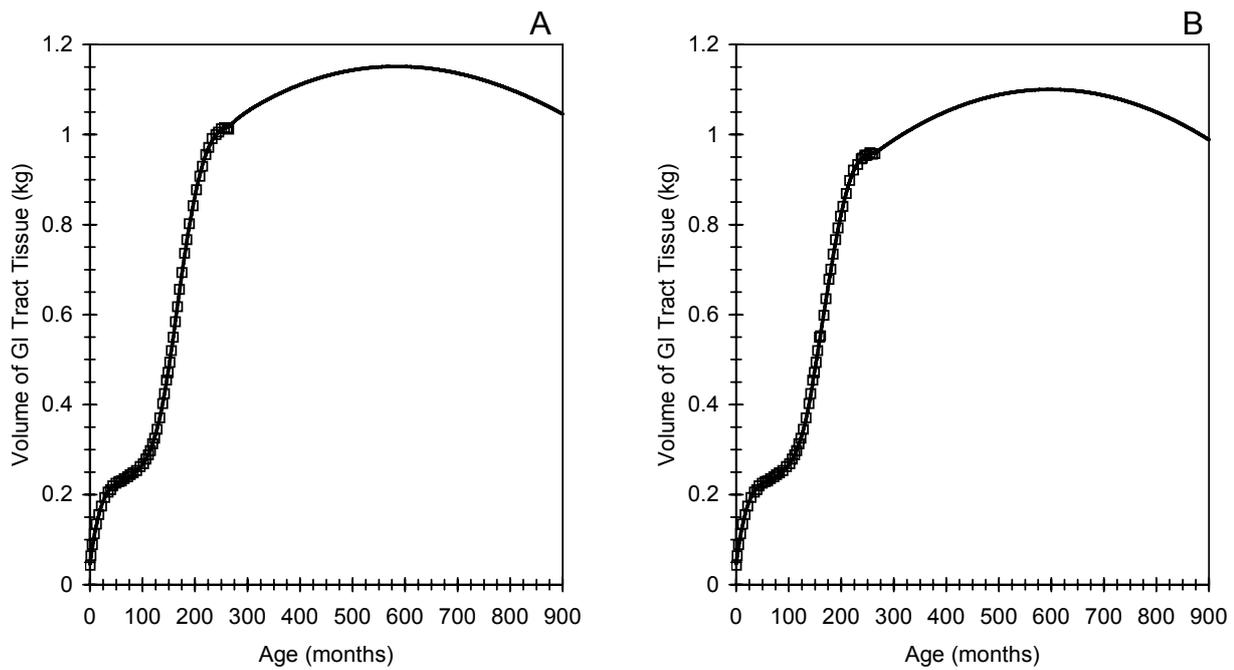


Figure E. Data (squares) for male (A) and female (B) intestinal weight from ICRP (1975) and equations (lines) fit to the data.

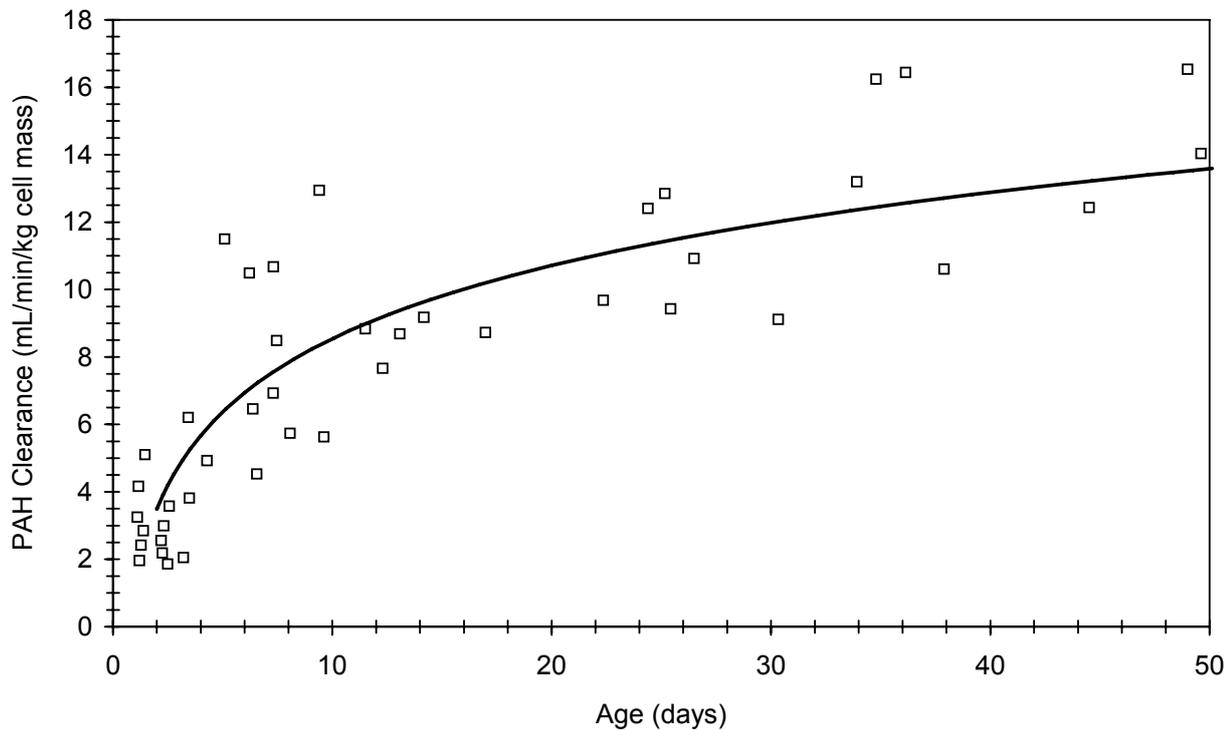


Figure F. Data (squares) for development of tubular secretion from Braunlich (1977) and the equation (line) fit to the data.

Part III

Use of Biokinetic and Mechanistic Data in Modeling for Risk Assessments of the Chloroethylenes

Chapter 8

Comparison of Cancer Risk Estimates for Vinyl Chloride Using Animal and Human Data with a PBPK Model

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Abstract

Vinyl Chloride (VC) is a trans-species carcinogen, producing tumors in a variety of tissues, from both inhalation and oral exposures, across a number of species. In particular, exposure to VC has been associated with a rare tumor, liver angiosarcoma, in a large number of studies in mice, rats, and humans. The mode of action for the carcinogenicity of VC appears to be a α -phenobarbital straightforward example of DNA adduct formation by a reactive metabolite, leading to mutation, mistranscription, and neoplasia. The objective of the present analysis was to investigate the comparative potency of a classic genotoxic carcinogen across species, by performing a quantitative comparison of the carcinogenic potency of VC using data from inhalation and oral rodent bioassays as well as from human epidemiological studies. A physiologically based pharmacokinetic (PBPK) model for VC was developed to support the target tissue dosimetry for the cancer risk assessment. Unlike previous models, the initial metabolism of VC was described as occurring via two saturable pathways, one representing low capacity-high affinity oxidation by CYP2E1 and the other (in the rodent) representing higher capacity-lower affinity oxidation by other isozymes of P450, producing in both cases chloroethylene oxide (CEO) and chloroacetaldehyde (CAA) as intermediate reactive products. Depletion of glutathione by reaction with CEO and CAA was also described. Animal-based risk estimates for human inhalation exposure to VC using total metabolism estimates from the PBPK model were consistent with risk estimates based on human epidemiological data, and were lower than those currently used in environmental decision-making by a factor of 80.

Introduction

Vinyl chloride (VC) has been produced commercially since the 1920s (Maltoni et al. 1981). It has been used as a refrigerant, an extraction solvent, an aerosol-propellant, and even as an ingredient in drug and cosmetic products. However, when it became evident that VC was carcinogenic both in animals and in humans, these uses were discontinued; the current use of VC is limited to serving as a chemical precursor in the production of such materials as polyvinyl chloride (PVC) and copolymer resins (USEPA 1985). Nevertheless, VC is also produced from the biodegradation of trichloroethylene by bacteria in the soil. Thus past spills of trichloroethylene may lead to current or future exposures of the public to VC in drinking water or other environmental media. The current potency estimates for VC published by the Environmental Protection Agency (EPA) do not quantitatively incorporate pharmacokinetic information on VC into the risk calculations (USEPA 1985). The purpose of the study reported here was to re-evaluate the quantitative risk to humans of cancer from exposure to VC, using pharmacokinetics to describe the dose-response for carcinogenicity in both animals and humans.

Evidence for the Carcinogenicity of VC

In 1967, Viola and co-workers initiated the first long-term bioassays using VC, in which male Wistar rats were exposed via inhalation (Viola et al. 1971). They reported a significant increase in the incidence of skin and lung carcinomas and osteochondromas. Since that time, the carcinogenicity of VC has been well established in several animal species by a number of routes of exposure. Frequently observed tumor sites in animals following VC exposure include the liver, kidney, lung, brain, mammary glands, and Zymbal glands. Of the many different tumor types which have been reported in animal bioassays of VC, three are of greater concern because they have been seen reproducibly at low concentrations (250 ppm and below): liver angiosarcoma, nephroblastoma, and mammary gland adenocarcinoma (Purchase et al. 1985). Hepatocellular carcinomas have also been occasionally observed at these low concentrations, particularly in studies of exposure to young animals (Bolt et al. 1980). Of these four low-concentration tumors, two are particularly notable in that they are rarely seen in unexposed animals: liver angiosarcoma and nephroblastoma.

In 1974, Creech and Johnson reported for the first time an association between exposure to VC and cancer in man: three cases of liver angiosarcoma were reported in men employed in a PVC plant (Maltoni et al. 1981). Angiosarcoma of the liver is considered to be a very rare type of cancer, with only 20-30 cases per year reported in the U.S. (Gehring et al. 1978, ATSDR 1993). Greater than expected incidences of angiosarcoma of the liver have since been reported in a number of other cohorts of workers occupationally exposed to VC (Bryen et al. 1976, Fox and Collier 1977, Infante 1976, Jones et al. 1988, Monson et al. 1974, Pirastu et al. 1990, Rinsky et al. 1988, Teta et al. 1990, Waxweiler et al. 1976, Weber et al. 1981, Wong et al. 1986, Wu et al. 1989). Increased death due to cancer associated with VC exposure has also been reported for brain, lung, and hematopoietic systems (USEPA 1985), as well as for other tissues, but several analyses have concluded that liver angiosarcomas show the clearest evidence for causal association and also demonstrate the highest relative risk (Purchase et al. 1985). The correspondence across species for liver hemangiosarcoma is quite striking and has made this tumor the primary focus for VC risk assessments in recent years. In particular, the correspondence across species for this tumor suggests a unique opportunity for comparing quantitative cancer risk estimates based on epidemiological data with those derived from animal bioassay data.

Previous Risk Assessments for VC

The EPA has performed a series of risk assessments on VC as new information has become available regarding its carcinogenic potency (USEPA 1985). In 1980, a carcinogenic potency estimate for ingestion of contaminated drinking water was determined to be $1.74 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$, based on the incidence of total tumors among rats exposed by inhalation at 50 to 10,000 ppm, with a correction for the relationship between inhalation and oral dose levels and a cube-root body-weight-ratio correction to convert from animal to human

potency. This latter correction is commonly referred to as the body-surface-area (BSA) adjustment for cross-species extrapolation. In 1984, this oral potency was revised on the basis of an oral intubation study in rats; the revised oral potency was $1.4096 \times 10^{-1} \text{ (mg/kg/day)}^{-1}$, based specifically on liver angiosarcoma rather than total tumors, and again using the BSA adjustment.

In 1985, data on the total incidence of lung and liver tumors in female rats treated with diets containing VC-fortified PVC (Feron et al., 1981) was used to calculate an oral potency of $1.9 \text{ (mg/kg/day)}^{-1}$, which yields a unit risk of $5.4 \times 10^{-5} \text{ (}\mu\text{g/L)}^{-1}$. At the same time, an inhalation potency was calculated based on the studies of Maltoni et al. (1981, 1984) in which rats were exposed to concentrations of VC ranging from 1 to 30,000 ppm, 4 hrs/day, 5 days/wk, for 52 weeks. The resulting inhalation potency, based on liver angiosarcoma and applying the BSA adjustment, was $2.95 \times 10^{-1} \text{ (mg/kg/day)}^{-1}$, yielding a unit risk of $8.4 \times 10^{-5} \text{ (}\mu\text{g/m}^3\text{)}^{-1}$. The increase in the oral potency estimate from previous values was presumed to result from the availability of better tumor incidence data below the level of saturation of metabolism (USEPA 1985). It was also noted at that time that the oral potency, expressed in (mg/kg/day)^{-1} , was higher than the inhalation potency by roughly a factor of eight, suggesting that VC was more effective by the oral route. A subsequent study of the effect of exposure route on potency of carcinogens (Pepelko, 1991) also found that VC appeared to be more potent by the oral route when the comparison was made on the basis of mg/kg/day, and suggested that pharmacokinetic differences could underlie at least part of the discrepancy.

A pharmacokinetically-based risk assessment was proposed for VC as early as 1978 (Gehring et al., 1978). In this study it was demonstrated that the incidence of angiosarcoma correlated with amount of VC metabolized, which demonstrated Michaelis-Menten saturable kinetics, rather than with the exposure concentration of VC itself. Based on this observation and mechanistic arguments, it was proposed that daily amount metabolized should be used as the dose measure in a risk assessment for VC. Based on the limited pharmacokinetic and bioassay data available at that time, a pharmacokinetic human risk estimate for liver angiosarcoma was attempted. Their estimates of human risk at occupational exposure levels greatly overestimated the risks compared to estimates from epidemiological studies (Fox and Collier, 1977), while their predicted low-dose risks were many orders of magnitude lower than those estimated by the EPA. However, interpretation of this risk estimate is complicated by the authors' use of the probit (log probability) dose-response model rather than a linear model.

A more complete pharmacokinetic risk analysis for VC was performed (Chen and Blancato 1989) using a physiologically-based pharmacokinetic (PBPK) description of VC kinetics and total metabolism, patterned after the PBPK model for styrene (Ramsey and Andersen 1984). Metabolism of VC was modeled with a single saturable pathway, and the kinetic constants were estimated from measurements of whole body clearance (e.g. Filser and Bolt, 1979), although no attempt was made to validate the model against data on blood time-courses or

total metabolism in rodents or humans. The model was used to calculate total metabolism of VC (representing total production of reactive metabolites) as the dose metric in a carcinogenic risk assessment for liver tumors from exposure to VC. Human inhalation potency estimates based on the internal dose metric (mg VC metabolized/kg/day) were derived from the incidence of liver tumors in rat bioassays of VC performed by Maltoni et al. (1981, 1984).

The same PBPK model was then used to estimate total metabolism for oral bioassays of VC (Maltoni et al. 1981, 1984, Feron et al. 1981). Liver tumor incidence for the oral route predicted using the PBPK dose metric (mg metabolized/kg/day) to extrapolate from the inhalation potency agreed well with observed tumor incidence in the oral bioassays. This correspondence across routes based on metabolized dose contrasted with the apparently higher potency of VC by the oral route when the comparison was based on @inhaled@ versus Aingested@ dose in mg/kg/day.

Chen and Blancato (1989) also used the PBPK model to calculate the human inhalation potency for VC induced liver cancer based on epidemiological data (Fox and Collier, 1977). Using the same pharmacokinetic dose measure (mg metabolized/kg/day), the inhalation potency estimated from epidemiological data (3.8×10^{-3} /ppm) was essentially identical to the potency estimated from rat inhalation data ($1.7 - 3.7 \times 10^{-3}$ /ppm), using body weight scaling (that is, without applying the BSA adjustment). However, a second, more complicated comparison performed in the same analysis, which involved time-to-tumor modeling, suggested that the BSA adjustment might be necessary to accurately predict lifetime-exposure human cancer fatality from animal incidence data.

More recently, a PBPK model of VC was developed by Reitz et al. (1996) and applied to compare cancer potency in mice, rats, and humans. The structure of the model was similar to that of Chen and Blancato (1989), providing a description of parent chemical kinetics and total metabolism based on the styrene model of Ramsey and Andersen (1984). Metabolism of VC was modeled with a single saturable pathway, and the kinetic constants were estimated from fitting of closed chamber gas uptake studies with rats. The model was then validated against data on total metabolism in the rat (Watanabe et al., 1976b), gas uptake data in the mouse, and inhalation data in the human (Baretta et al., 1969). The model was used to calculate total metabolism of VC as the dose metric in a carcinogenic risk assessment. Based on the rat inhalation bioassay of Maltoni et al. (1981, 1984), and using the linearized multistage model, they estimated that lifetime continuous human exposure to $1.75 \mu\text{g}/\text{m}^3$ VC is associated with an increased lifetime risk of one in a million. This estimate equates to a lifetime risk of approximately 1.5×10^{-3} /ppm, in good agreement with the results of Chen and Blancato (1989). The potency estimates from rats were then shown to be consistent with tumor incidence data in mice and humans when the pharmacokinetic dose metric was used.

Pharmacokinetics and Metabolism of VC

Numerous studies on the pharmacokinetics and metabolism of VC have been conducted, with the majority of these studies conducted in rats (Withey 1976, Hefner et al. 1975, Guengerich and Watanabe 1979, Bolt et al. 1976, 1977, Watanabe et al. 1976a, 1976b, 1978, Jedrychowski et al. 1984, 1985, Tarkowski et al. 1980). A simplified diagram of the metabolism of VC is shown in Figure 1. The primary route of metabolism of VC is by the action of the mixed function oxidase (MFO) system, now referred to as Cytochrome P450 or CYP, on VC to form chloroethylene oxide (CEO) (Bolt et al. 1977, Plugge and Safe 1977). Chloroethylene oxide (CEO) is a highly reactive, short-lived epoxide that rapidly rearranges to form chloroacetaldehyde (CAA), a reactive α -halocarbonyl compound; this conversion can also be catalyzed by epoxide hydrolase (Pessayre et al. 1979).

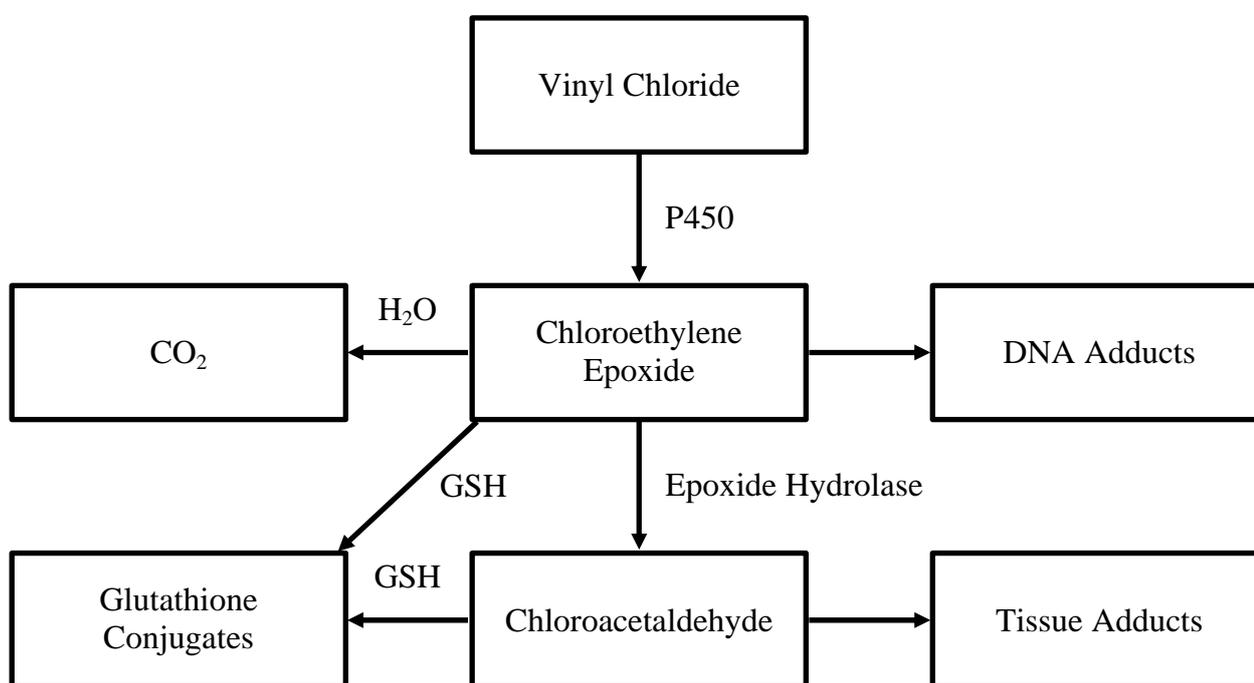


Figure 1. Diagram of the metabolism of vinyl chloride (VC). Abbreviations: P450 – Cytochrome P450 (CYP); GSH – glutathione.

The main detoxification of these two metabolites is conjugation binding with glutathione (Jedrychowski et al. 1985, Leibman 1977, Tarkowski et al. 1980). This hypothesis is supported by the observation of decreased non-protein sulfhydryl concentrations at high VC exposure concentrations (Jedrychowski et al. 1985, Tarkowski et al. 1980), as well as by the excretion of glutathione (GSH) conjugated metabolites in the urine, observed in rats following exposure to VC (Watanabe et al. 1976c, Hefner et al. 1975). CAA may also combine directly or enzymatically via glutathione transferase (GST) with GSH to form S-formylmethylglutathione. S-Formylmethylglutathione, through direct interaction with GSH derived cysteine, can be excreted as N-acetyl-S-(2-hydroxyethyl)cysteine, another major urinary metabolite of VC (Green and

Hathway 1975). The GSH conjugates are then subject to hydrolysis resulting in excretion of cysteine conjugates in the urine (Hefner et al. 1975). Two of the three major urinary metabolites of VC in rats have been identified as N-acetyl-S-(2-hydroxyethyl)cysteine and thiodiglycolic acid (Watanabe et al. 1976b).

Based on the elimination of VC observed following administration by various routes of exposure, the metabolism of VC appears to be a dose-dependent, saturable process (Green and Hathway 1975, Bolt 1978, Hefner et al. 1975, Gehring et al. 1977, 1978). Following exposure via oral or inhalation routes to low doses of VC, metabolites are excreted primarily in the urine. However, once the saturation point for metabolism is reached, VC is eliminated via other routes, primarily exhalation of the parent compound (Gehring et al. 1977, Watanabe et al. 1976b, Watanabe and Gehring 1976). The route of elimination of VC also depends on the route of administration, with urinary excretion favored more following oral or intraperitoneal administration, indicating a first-pass effect due to liver metabolism (Bolt 1978).

The specific isozymes of the P450 system involved in the metabolism of VC have not yet been unequivocally established. However, it is clear from both *in vitro* and *in vivo* studies that several isozymes can play a role. High affinity, low capacity oxidation by CYP 2E1 is probably responsible for essentially all of the metabolism of VC at low concentrations in uninduced animals and humans (Guengerich et al. 1991). There is also evidence for a significant increase in metabolism in animals pretreated with α -phenobarbital (Ivanetich et al. 1977), suggesting that CYP 2B1 also metabolizes VC. At high concentrations *in vivo*, the metabolism of VC in rats leads to a destruction of P450 enzyme (Reynolds et al. 1975), which is greatly enhanced in α -phenobarbital- or Arochlor- induced animals (Arochlor induces CYP 1A2). The loss of P450 has been suggested to result from the production of reactive intermediates during the metabolism of VC (Guengerich and Strickland 1977), and is inhibited by GSH *in vitro* (Ivanetich et al. 1977). Induction of P450 by α -phenobarbital or Arochlor was also necessary to produce acute hepatotoxicity from VC in rats (Jaeger et al. 1977).

The contribution of several P450 isozymes to the metabolism of the related compound trichloroethylene has been studied in the male Wistar rat and male B6C3F1 mouse (Nakajima et al. 1993). Using monoclonal antibodies specific to each isozyme, the investigators were able to determine that: (1) CYP2E1 contributes more to the metabolism of TCE in mice than in rats, (2) CYP2C11/6 contributes more to the metabolism of TCE in rats than in mice (CYP2C11/6 is a constitutive, non-inducible isozyme present only in male rodents), (3) CYP1A1/2 contributes to the uninduced metabolism of TCE in mice but not in rats, and (4) CYP2B1 does not contribute to the metabolism of TCE in naive animals of either species.

Summarizing the above observations, it appears that at low concentrations the metabolism of VC is primarily due to CYP2E1, but that at higher concentrations, where CYP2E1 becomes capacity-limited, other isozymes may contribute to its metabolism. The extent of this higher-capacity metabolism is

likely to vary across animal species, strain, and sex. To the extent that such higher capacity, lower affinity metabolism (referred to in future as "non-2E1" metabolism) may be important in conducting a risk assessment for VC, it will have to be characterized separately for each species, strain, and sex of interest. From a pharmacokinetic modeling perspective, non-2E1 metabolism would be handled as a second saturable metabolic pathway with a larger value for the Michaelis-Menten constant (KM). For example, it has been demonstrated that the metabolism of the related compound, vinyl bromide, is best described with two distinct saturable pathways having different affinities (Gargas and Andersen, 1982). Of major importance for human risk assessment, some of the low-affinity, high capacity constitutive (2C11/6) and inducible (2B1/2) P450 isozymes in the rodent may have no human correspondents (Guengerich, 1987).

Pharmacokinetic Modeling of VC

The pharmacokinetic models that have previously been used in risk assessments for VC (Gehring et al. 1978, Chen and Blancato, 1989, Reitz et al. 1996) have in common the assumption of a single saturable pathway for the metabolism of VC. However, another published model of VC (Gargas et al., 1990) differed from the models discussed above by the incorporation of a second, linear metabolic pathway (presumed by the authors to be GSH conjugation) in parallel with the saturable (oxidative) pathway. Based on gas uptake studies in the male F344 rat, both a saturable and a linear metabolic component were postulated for VC.

These alternative descriptions of metabolism in the published models of VC were examined in a more in-depth study of VC pharmacokinetics performed for the U.S. Air Force by several of the present authors (Clement, 1990). The one- and two-pathway descriptions were refit to gas uptake data and then compared with measurements of total metabolism by Gehring et al. (1978) and Watanabe et al. (1976b). Although the two-pathway description provided a significantly better fit to the gas uptake data (adding parameters nearly always improves a fit), the resulting parameters tended to overpredict total metabolism at higher concentrations owing to the presence of the first-order component. In addition, it was not possible to explain the continued increase in glutathione (GSH) depletion measured at the highest exposure levels (where the saturable component was above saturation) because only products of the oxidative metabolism of VC have been shown to react with GSH. In an attempt to provide a better correspondence to the data on both total metabolism and glutathione depletion, two possible refinements to the model were investigated. In the first, direct reaction of VC with GSH was postulated, and in the second, the products of both the saturable and the linear pathways were assumed to react with GSH. Unfortunately, neither description was able to provide a satisfactory correspondence to both total metabolism and GSH depletion data. The analysis suggested that a different formulation featuring two saturable oxidative pathways, both producing reactive metabolites, might provide the required behavior.

The PBPK models previously used to conduct a human risk assessment for VC (Chen and Blancato, 1989, Reitz et al. 1996) certainly provide a more biologically plausible basis for estimating human carcinogenic risk than default measures of VC exposure. However, a 2-saturable pathway model structure would have the potential advantage of being able to reproduce experimental data on both total metabolism and GSH depletion, based on a reasonable hypothesis: only saturable oxidative metabolism is involved (no other metabolic pathway for VC has been demonstrated), and only products of oxidative metabolism react with GSH (neither direct reaction nor GST-mediated conjugation of VC with GSH has been demonstrated). This metabolic hypothesis formed the basis for the model development conducted in this study. The model, which will be described in more detail later, is similar to the PBPK model developed by D'Souza and Andersen (1988) to describe vinylidene chloride (VDC) kinetics and toxicity, including the depletion of GSH by the products of VDC metabolism. The chief difference is the use of two saturable pathways instead of one to describe metabolism. The use of a low affinity pathway in parallel with the high affinity pathway is necessary to provide the continued increases in total metabolism and GSH depletion observed with VC in rats.

Mechanism of Carcinogenicity of VC

Many of the results of the pharmacokinetic and metabolism studies discussed above indicate that like other chlorinated alkenes, VC must be metabolized to cause carcinogenicity (Bartsch and Montesano 1975). A reactive, short-lived metabolite, which achieves only low steady-state concentrations, is thought to be responsible for the toxic effects of VC (Bolt 1978). That the toxicity of VC is mediated by the production of reactive metabolites is suggested by the results of *in vitro* studies in which enhanced mutagenicity was observed if microsomal enzymes of fortified liver homogenates were present (Bartsch et al. 1975, Malavielle et al. 1975, Rannug et al. 1974). The rapid elimination of VC and its major metabolites is also consistent with the theory that a shortly lived, reactive metabolite occurring at concentrations too low for direct observation could be responsible for the carcinogenicity of VC (Bolt et al. 1977).

Both CEO and CAA have been evaluated as possible carcinogenic metabolites of VC, because both compounds can react with tissue nucleophiles (Guengerich et al. 1981). However, comparative studies have shown that CEO is carcinogenic in skin, and acts as an initiator in the initiation/promotion protocol, but that CAA does not (Zajdela et al. 1980). Moreover, CEO has been found to display 400-fold greater mutagenic potency than CAA in bacterial mutagenicity assays (Perrard 1985). Comparative experiments have also been conducted with VC and 2,2'-dichlorodiethylether which support the conclusion that CEO is the carcinogenic metabolite (Bolt 1986, Gwinner et al. 1983). These experiments made use of an important difference between the metabolism of 2,2'-dichloroethylether and VC: only the metabolism of VC leads to an intermediary epoxide. While both 2,2'-Dichloroethylether and VC are metabolic precursors of CAA, only VC is a precursor of CEO. After administration of 2,2'-dichloroethylether to rats, protein alkylation in the liver was comparable to that

observed with VC; however, no adduct formation was observed. Preneoplastic hepatocellular ATP-deficient foci were also reported in rats following exposure to VC, but not 2,2'-dichlorodiethylether (Gwinner et al. 1983). These results support the conclusion that CEO is the ultimate carcinogenic metabolite of VC.

The metabolism of VC to products irreversibly bound to DNA and protein was examined *in vitro* with rat liver microsomes to investigate the possible role of CAA in the carcinogenicity of VC. Inhibition studies were performed with alcohol dehydrogenase, which is the enzyme involved in the breakdown of CAA, and epoxide hydrolase, which is the enzyme involved in the breakdown of CEO. Alcohol dehydrogenase was effective in inhibiting the binding of VC metabolites to protein, while epoxide hydrolase was effective in inhibiting the binding of VC metabolites to DNA. These results also support the conclusion that the epoxide is the carcinogenic moiety (Guengerich et al., 1981). More recent studies have also demonstrated that all of the major identified DNA adducts formed from VC exposure are derived solely from CEO (Laib 1986, Guengerich 1992). One possible explanation for the ability of CEO, rather than CAA, to form adducts with DNA is that CEO is lipophilic, while CAA is hydrophilic; this difference could impact the compound's relative abilities to cross the nuclear membrane (Bolt et al. 1980).

Adduct Formation from VC

It has long been a tenet of carcinogenic risk assessment that the mechanism of carcinogenicity for "genotoxic" carcinogens (sometimes referred to as initiators) involves reaction with DNA, leading to mistranscription during subsequent cell division, causing a loss or change in heritable information which results in a neoplastic daughter cell (Van Duuren 1988, Singer 1985). As early as 1978, it was demonstrated that binding of VC to liver macromolecules following inhalation exposure of rats correlated well with both total metabolism and the observed incidence of angiosarcoma (Watanabe et al. 1978). It was suggested that the carcinogenicity of VC was due to binding with DNA and subsequent miscoding during cell reproduction, although no DNA or RNA binding was actually observed in that study. Subsequently, a DNA adduct produced by VC exposure was identified: 7-(2-oxoethyl)guanine (OEG); however, although this adduct represents roughly 98% of all VC adducts (Swenberg et al. 1992), it is very rapidly repaired and does not appear to lead to miscoding during DNA replication. Therefore it is not considered important for carcinogenesis (Laib 1986, Swenberg et al. 1992).

The *in vivo* formation of four etheno-DNA adducts have since been demonstrated following exposure of animals to VC (Laib 1986, Fedtke et al. 1990, Dosanjh et al. 1994): 1,N²-ethenoguanine (1,N²-EG); N²,3-ethenoguanine (N²,3-EG); 1,N⁶-etheno-2'-deoxyadenosine (EDA), and 3,N⁴-etheno-2'-deoxycytidine (EDC). These etheno-adducts, although produced at much lower concentrations than OEG, are highly persistent (Swenberg et al. 1992) and can lead to defective transcription (Singer et al. 1987); for example, EG produces a base pair mismatch (G->A transition) in bacterial assays (Cheng et al. 1990). Single-

strand breaks (SSBs) have been detected in liver DNA following inhalation exposure of mice to VC (Wallis et al. 1988). (It is generally assumed that SSBs represent an intermediate stage in the excision repair of DNA adducts.) The occurrence of SSBs reached a maximum at exposures of 500 ppm, consistent with saturation of metabolism. It was found that 20% of the SSBs remained after 20 hours.

It is still not possible to determine which, if any, of the DNA-adducts identified from VC exposure may be responsible for the observed carcinogenicity of VC. The likelihood that a given DNA-adduct will lead to a neoplastic transformation depends on many factors, including its persistence as well as the consequences of its repair or failure to be repaired. The persistence of a given adduct depends on both the rate of formation and the rate of repair (Singer 1985); in humans, all of the etheno adducts appear to be repaired by the same DNA glycosylase, but not at the same rate (Dosanjh et al. 1994). In particular, the repair of the ethenoguanines (1,N²-EG and N²,3-EG) appears to be much slower than that of the other etheno-adducts (EDA and EDC) in humans (Dosanjh et al. 1994). This is in contrast to the results of a similar study in rats, where N²,3-EG was repaired with a half-life of about 30 days, while there was no evidence that EDA and EDC were repaired at all (Swenberg et al. 1992).

In summary, although there is strong circumstantial evidence linking etheno-DNA adducts with the observed carcinogenicity of VC, there is not yet sufficient information to provide a quantitative link between the tissue concentrations of a specific adduct and the risk of cancer in that tissue. The ratio of the concentrations of the various etheno adducts in the rat is only marginally consistent across tissues and studies (Fedtke et al. 1990, Swenberg et al. 1992), and there are no data on relative adduct levels in VC exposed humans. Amount of metabolism would still appear to provide the best dose metric for comparison with tumor incidence.

Other Considerations in the Carcinogenicity of VC

The majority of the DNA adduct studies conducted with VC have been conducted on or related to the hepatocyte. However, although VC is primarily metabolized in the hepatocyte (Ottenwalder and Bolt 1980), the primary target cell for carcinogenicity in the liver is the sinusoidal cell, as indicated by the incidence of liver angiosarcoma in both animals and humans. Sinusoidal cells show a relatively low activity for transforming VC into reactive, alkylating metabolites, roughly 12% of the activity of hepatocytes (Ottenwalder and Bolt 1980). Therefore, it has been suggested that the carcinogenic metabolites of VC may have to migrate from the hepatocytes to produce tumors in the sinusoidal cells (Laib and Bolt 1980). This possibility was suggested by Laib and Bolt (1980) following their observation that alkylating metabolites of VC were capable of diffusing through an artificial semipermeable membrane in a model *in vitro* system. In studies conducted *in vitro* with rat hepatocytes by Guengerich et al. (1981), more than 90% of the hexane-insoluble metabolites were found to migrate out of the cell, with more than 70% of the total irreversibly bound

species found outside the cell. These results were interpreted to indicate that the majority of the reactive metabolites can leave the intact hepatocyte. On the other hand, sinusoidal cells do possess the ability to produce reactive metabolites from VC, albeit at a slower rate than the hepatocyte (Ottenwalder and Bolt 1980). In either case, the greater susceptibility of the sinusoidal cells to the carcinogenic effects of VC may result from an inability of the sinusoidal cells to repair one or more of the DNA adducts produced by VC as efficiently as the hepatocytes.

Another concern with regard to the carcinogenicity of VC is the relative sensitivity of young animals. Newborn rats treated with VC respond with both angiosarcoma and hepatocellular carcinoma, in contrast with adult animals, which generally evidence only angiosarcoma (Maltoni et al. 1981). Consistent with this observation, VC was found to induce preneoplastic foci in newborn rats, but not in adults (Laib et al. 1979). Interestingly, in the same study it was found that VC did induce preneoplastic foci in adult rats after partial hepatectomy, indicating that the appearance of foci, and presumably of hepatocellular carcinoma, in neonatal animals was a consequence of the increased rate of cell proliferation at that age. Support for this hypothesis was obtained in studies with 11-day old Wistar rats (Laib et al. 1989). Following inhalation exposure to VC, roughly 5-fold higher levels of OEG were found in the livers of the 11-day old rats as compared to similarly treated adult rats. It was also determined that the rate of cell proliferation in the livers of the 11-day old rats was roughly 8-fold higher than in the adults. These results are consistent with the suggestion that the apparent sensitivity of young animals to VC-induced liver cancer is related to enhanced DNA alkylation and by increased cellular proliferation at an early age (Laib et al. 1989). In a similar study, roughly 4-fold greater concentrations of both OEG and EG were also seen in preweanling rats exposed to VC (Fedtke et al. 1990).

This apparent increased sensitivity of newborn animals occurs in spite of a much lower metabolic capability at birth: during the first week of life, the P450 activity in the liver of rats increases from about 4% to about 80% of adult levels (Filser and Bolt 1979). However, the low metabolic capability at birth probably explains why VC is not a transplacental carcinogen (Bolt et al. 1980). The potential implications of the higher cell proliferation rates found in newborn animals provide sufficient cause for extra prudence regarding exposure of infants to VC or any other DNA-reactive carcinogen. Nevertheless, cell proliferation and DNA adduct data alone is not adequate to demonstrate a quantitative differential between acceptable adult and infant exposures. In comparative studies of partial lifetime exposure of rats to VC (Drew et al. 1983), exposure from 0 to 6 months showed an overall similar potency to exposure from 6 to 12 months of life. In particular, for exposure to VC from 0 to 6 months the incidence of hepatocellular carcinoma and hemangiosarcoma was 4.0% and 5.3%, respectively, while for exposure from 6 to 12 months, the incidence was 11.5% and 3.8%. Thus this comparative bioassay does not provide support for an increased sensitivity due to early age exposure for hepatocellular carcinomas specifically, or for VC-induced carcinogenicity in general.

Selection of a Risk Assessment Approach

Based on the information described above on the pharmacokinetics, metabolism, and mechanism of carcinogenicity of VC, it is necessary to determine the appropriate approach for conducting a human risk assessment. Clearly, the evidence is strong that the carcinogenicity of VC is related to the production of reactive metabolic intermediates. The most appropriate pharmacokinetic dose metric for a reactive metabolite is the total amount of the metabolite generated divided by the volume of the tissue into which it is produced (Andersen et al. 1987a). In the case of VC, reasonable dose metrics for angiosarcoma would include the total amount of metabolism divided by the volume of the liver (RISK), or the total amount of metabolism not detoxified by reaction with glutathione, again divided by the volume of the liver (RISKM). A third, less likely possibility, that the GSH conjugate of VC is subsequently metabolized to a reactive species which is responsible for the carcinogenicity, can also be considered by using a dose metric based on the total amount of reaction with GSH divided by the volume of the liver (RISKG). The assumption underlying the use of these dose metrics is that the concentration of the actual carcinogenic moiety, or the extent of the crucial event associated with the cellular transformation, is linearly related to this pseudo-concentration of reactive intermediates, and that the relationship of the actual carcinogenic moiety or crucial event to the dose metric is constant across concentration and species. Specifically, the average amount generated in a single day is used, averaged over the lifetime (i.e., the lifetime average daily dose, or LADD). The use of a dose rate, such as the LADD, rather than total lifetime dose, has been found empirically to provide a better cross-species extrapolation of chemical carcinogenic potency (USEPA, 1992).

Subsequent steps in the carcinogenic mechanism related to specific adduct formation, detection, and repair, as well as to the consequences of DNA mistranscription and the potential impact of increased cell proliferation, have only been sketchily outlined and have not yet reached the point where they could be incorporated into a risk assessment in any quantitative form. However, there appears to be sufficient evidence to justify the assumption that VC acts as a classic initiator, producing genetic transformations through direct reaction of its metabolites with DNA. Therefore the traditional assumption of low-dose linearity of risk appears to be warranted, and the linearized multistage (LMS) model would seem to be the most appropriate for low-dose extrapolation.

Description of PBPK Model for VC

The PBPK model for VC developed in this study is shown in Figure 2. As mentioned earlier, the model is basically an adaptation of a previously developed PBPK model for vinylidene chloride (D'Souza and Andersen 1988). For a poorly soluble, volatile chemical like VC, only four tissue compartments are required: a richly perfused tissue compartment which includes all of the organs except the liver, a slowly perfused tissue compartment which includes all of the muscle and skin tissue, a fat compartment which includes all of the fatty tissues, and a liver

compartment. All metabolism is assumed to occur in the liver, which is a good assumption in terms of the overall kinetics of VC, but which would have to be revised to include target-tissue-specific metabolism if a serious attempt were to be made to perform a VC risk assessment for a tissue other than the liver (Andersen et al. 1987a). The model also assumes flow-limited kinetics, or venous equilibration; that is, that the transport of VC between blood and tissues is fast enough for steady state to be reached within the time it is transported through the tissues in the blood.

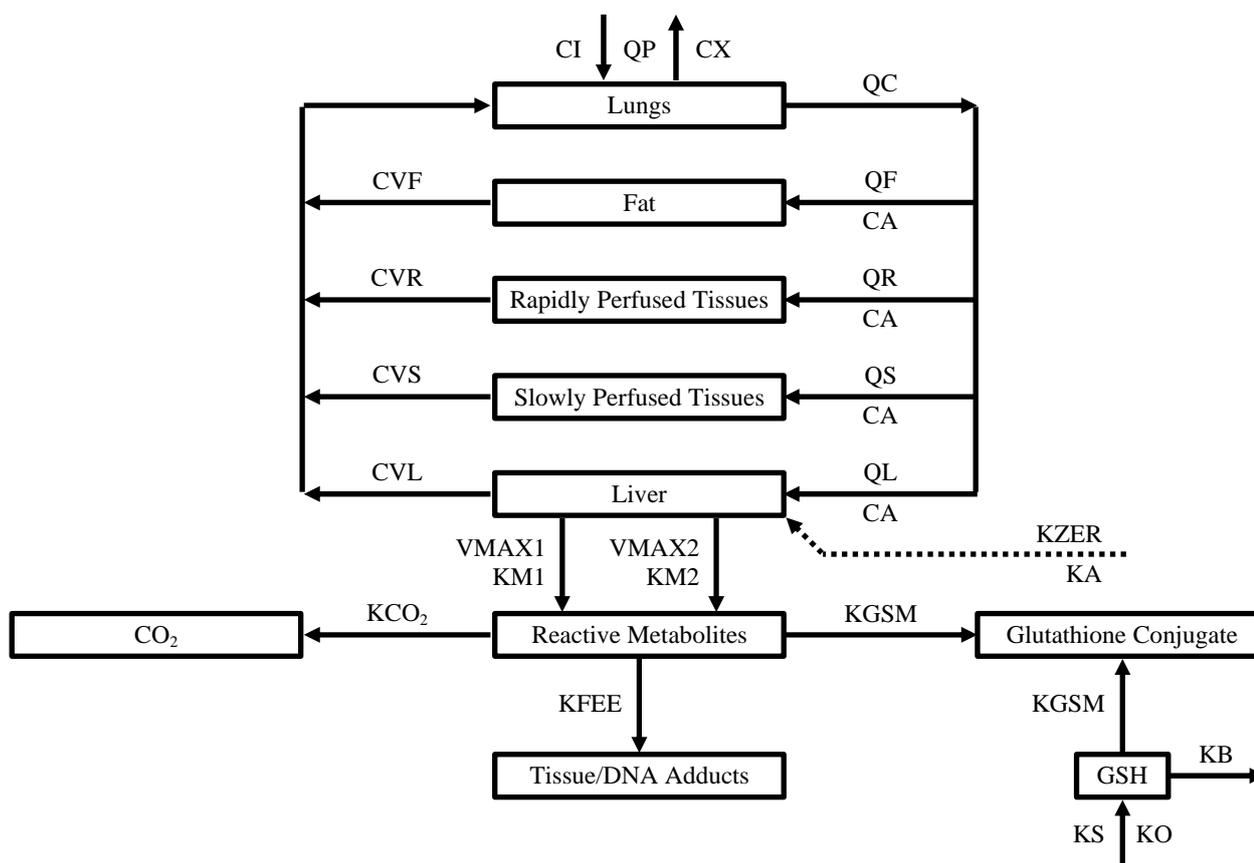


Figure 2. Diagram of the PBPK model of VC. Abbreviations: QP – alveolar ventilation; CI – inhaled concentration; CX – exhaled concentration; QC – cardiac output; QF, CVF – blood flow to, and venous concentration leaving, the fat; QR, CVR – blood flow to, and venous concentration leaving, the richly perfused tissues (most organs); QS, CVS – blood flow to, and venous concentration leaving, the slowly perfused tissues (e.g., muscle); QL, CVL – blood flow to, and venous concentration leaving, the liver; VMAX1, KM1 – capacity and affinity for the high affinity oxidative pathway enzyme (CYP 2E1); VMAX2, KM2 – capacity and affinity for the lower affinity oxidative pathway enzymes, (e.g., CYP 2C11/6); KZER – zero-order rate constant for uptake of VC from drinking water; KA – first-order rate constant for uptake of VC from corn oil; KCO₂ – first-order rate constant for metabolism of VC to CO₂; KGSM – first-order rate constant for reaction of VC metabolites with GSH; KFEE – first-order rate constant for reaction of VC metabolites with other cellular materials, including DNA; KB – first-order rate constant for normal turnover of GSH; KO – zero-order rate

constant for maximum production of GSH; KS – parameter controlling rate of recovery of GSH from depletion.

Metabolism of VC is modeled by two saturable pathways, one high affinity, low capacity (with parameters VMAX1C and KM1) and one low affinity, high capacity (with parameters VMAX2C and KM2). Subsequent metabolism is based on the metabolic scheme shown in Figure 1: the reactive metabolites (whether CEO, CAA, or other intermediates) may then either be metabolized further, leading to CO₂, react with GSH, or react with other cellular materials, including DNA. Because exposure to VC has been shown to deplete circulating levels of GSH, a simple description of GSH kinetics was also included in the model.

The parameters for the model are listed in Tables 1 and 2. The physiological parameters are EPA reference values (USEPA 1988), except for the alveolar ventilation (QPC) in the human, which was calculated from the standard EPA value for the ventilation rate in the human, 20 m³/day, assuming a 33% pulmonary dead-space (USEPA 1988). The value for the cardiac output (QCC) in the human was selected to correspond to the same workload as the standard EPA ventilation using data from Astrand and Rodahl (1970). For modeling of the closed chamber studies with human subjects, more typical resting values of 15 for cardiac output (QCC) and 18 for alveolar ventilation (QPC) were used. In some cases, it was also necessary to slightly vary the alveolar ventilation and cardiac output of the animals in the closed chamber studies in order to obtain acceptable simulations of those experiments. The partition coefficients for Fisher-344 (F344) rats were taken from Gargas et al. (1989), and those for Sprague Dawley rats were taken from Barton et al. (1995). The Sprague Dawley values were also used for modeling of Wistar rats. Blood/air partition coefficients for the other species were obtained from Gargas et al. (1989), and the corresponding tissue/blood partition coefficients were estimated by dividing the Sprague Dawley rat tissue/air partition coefficients by the appropriate blood/air value.

Table 1
Model Parameters and Dose Metrics for the Vinyl Chloride Model

		Mouse (CV-%) ¹	Rat (CV-%)	Human (CV-%)
BW	Body Weight (kg)	-- ² (11)	-- (11)	70.0 (30)
QPC	Alveolar Ventilation (L/hr, 1 kg animal)	30.0 (58)	21.0 (58)	24.0 (16)
QCC	Cardiac Output (L/hr, 1 kg animal)	18.0 (9)	18.0 (9)	16.5 (9)

Tissue Blood Flows (Fraction of Cardiac Output):

QRC	Rapidly Perfused Tissues	0.51 (50)	0.51 (50)	0.5 (20)
QFC	Fat	0.09 (60)	0.09 (60)	0.05 (30)
QSC	Slowly Perfused Tissues	0.15 (40)	0.15 (40)	0.19 (15)
QLC	Liver	0.25 (96)	0.25 (96)	0.26 (35)

Tissue Volumes (Fraction of Body Weight):

VSC	Slowly Perfused Tissues	0.77 (30)	0.75 (30)	0.63 (30)
VFC	Fat	-- (30)	-- (30)	0.19 (30)
VRC	Richly Perfused Tissues	0.035 (30)	0.05 (30)	0.064 (10)
VLC	Liver	0.055 (6)	0.04 (6)	0.026 (5)

Partition Coefficients:

PB	Blood/Air	2.26 (15)	2.4 (15)	1.16 (10)
PF	Fat/Blood	10.62 (30)	10.0 (30)	20.7 (30)
PS	Slowly Perfused/Blood	0.42 (20)	0.4 (20)	0.83 (20)
PR	Richly Perfused/Blood	0.74 (20)	0.7 (20)	1.45 (20)
PL	Liver/Blood	0.74 (20)	0.7 (20)	1.45 (20)

¹ CV-%: Coefficient of Variation = 100 * Standard Deviation / Mean

² See Table 2

Table 1 (cont.)

Model Parameters And Dose Metrics For The Vinyl Chloride Model

		Mouse (CV-%)	Rat (CV-%)	Human (CV-%)
Metabolic Parameters:				
VMAX1 C	Maximum Velocity of First Saturable Pathway (mg/hr, 1 kg animal)	-- (20)	-- (20)	4.0 (30)
KM1	Affinity of First Saturable Pathway (mg/L)	0.1 (30)	0.1 (30)	1.0 (50)
VMAX2 C	Maximum Velocity of Second Saturable Pathway (mg/hr, 1 kg animal)	-- (20)	-- (20)	0.1 (0)
KM2	Affinity of Second Saturable Pathway (mg/L)	10.0 (30)	10.0 (30)	10.0 (50)
GSH Parameters:				
KCO2C	First Order CEO Breakdown to CO ₂	1.6 (20)	1.6 (20)	1.6 (20)
KGSMC	Conjugated Rate Constant with Metabolite	0.13 (20)	0.13 (20)	0.13 (20)
KFEEC	Conjugated Rate Constant with Non-GSH	35.0 (20)	35.0 (20)	35.0 (20)
GSO	Initial GSH Concentration	5800.0 (20)	5800.0 (20)	5800.0 (20)
KBC	First Order Rate Constant for GSH Breakdown	0.12 (20)	0.12 (20)	0.12 (20)
KS	Constant Controlling Resynthesis	2000.0 (20)	2000.0 (20)	2000.0 (20)
KOC	Zero Order Production of GSH	28.5 (20)	28.5 (20)	28.5 (20)
Dosing Parameters:				
KA	Oral Uptake Rate (/hr)	3.0 (50)	3.0 (50)	3.0 (50)

Table 2
Strain/Study-Specific Parameter Values

		BW	VFC	VMAX1 C	VMAX2 C
Swiss Albino Mice (Inhalation Study)	Male	0.044	0.13	8.0	0.1 ¹²
	Female	0.040	0.12	5.0	3.0
Sprague-Dawley Rats (Inhalation Study)	Male – Low Dose	0.638	0.19	4.0	2.0
	Male – High Dose	0.433	0.13	4.0	2.0
	Female – Low Dose	0.485	0.20	3.0	0.1 ¹
	Female – High Dose	0.321	0.14	3.0	0.1 ¹
Sprague-Dawley Rats (Gavage Study)	Male – Low Dose	0.632	0.19	4.0	2.0
	Male – High Dose	0.405	0.12	4.0	2.0
	Female – Low Dose	0.445	0.18	3.0	0.1 ¹
	Female – High Dose	0.301	0.13	3.0	0.1 ¹
Wistar Rats (Dietary Study)	Male	0.436	0.14	4.0	2.0
	Female	0.245	0.11	3.0	0.1 ¹

The affinity for the 2E1 pathway (KM1) in the rat, mouse, and hamster was set to 0.1 on the basis of studies of the competitive interactions between CYP2E1 substrates in the rat (Barton et al. 1995, Andersen et al. 1987b). The affinity used for the non-2E1 pathway (KM2) in the mouse and rat was set during the iterative fitting of the rat total metabolism, glutathione depletion, and rate of metabolism data, described below. The capacity parameters for the two oxidative pathways (VMAX1C and VMAX2C) in the mouse, rat, and hamster were estimated by fitting the model to data from closed-chamber exposures with each of the species and strains of interest (Barton et al. 1995, Bolt et al. 1977, Clement 1990, Gargas et al. 1990), holding all of the other model parameters fixed and requiring a single pair of values for VMAX1C and VMAX2C to be used for all of the data on a given sex/strain/species. Figures 3 and 4 show examples of the results of this fitting process for the male B6C3F1 mouse and male Wistar rat, respectively.

¹² The value of this parameter was normally set to zero. It was only set to 0.1 for the PBPk_SIM runs. The variance for this parameter was set to zero in the PBPk_SIM runs.

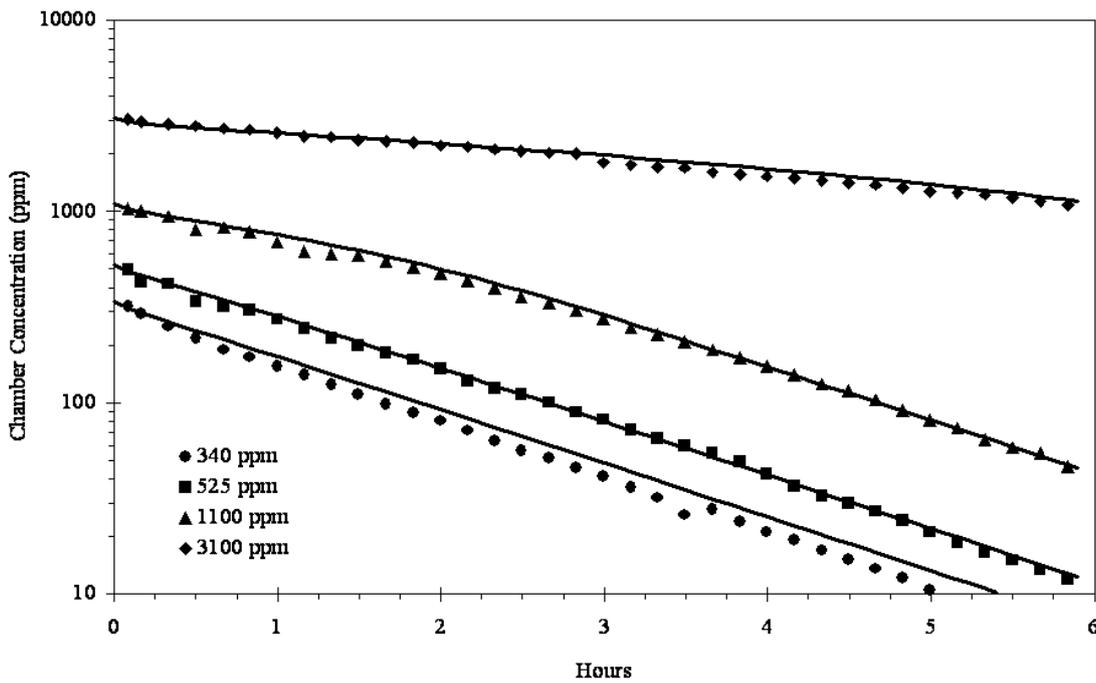


Figure 3. Model predictions (lines) and experimental data (symbols) for the chamber concentration during exposure of male B6C3F1 mice to VC in a closed, recirculated chamber (Data taken from Clement 1990).

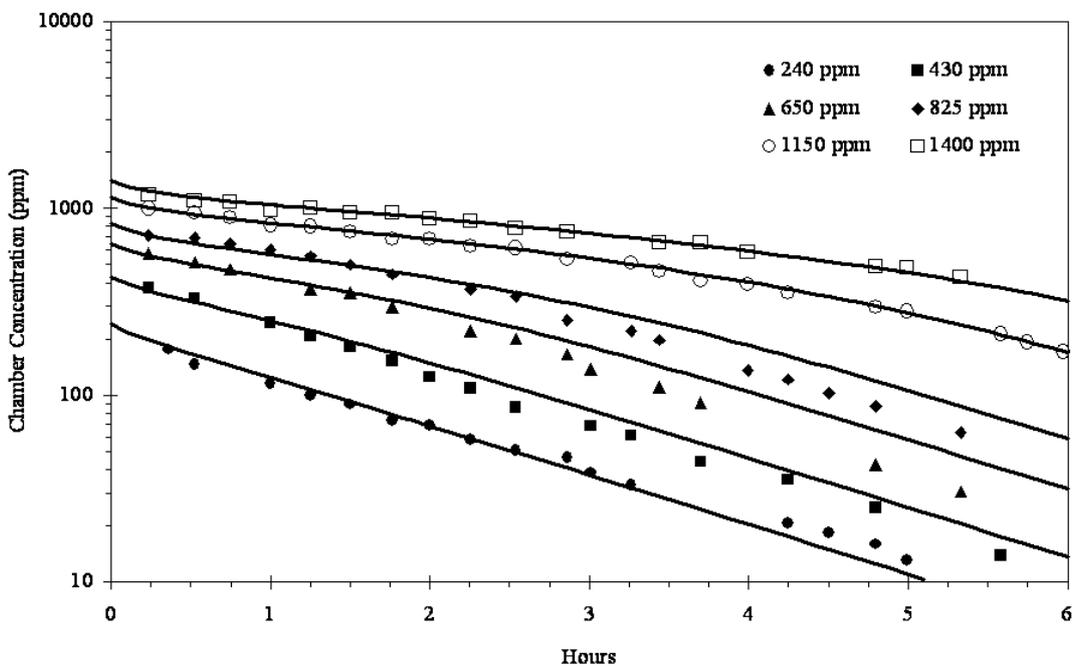


Figure 4. Model predictions (lines) and experimental data (symbols) for the chamber concentration during exposure of male Wistar rats to VC in a closed, recirculated chamber (Data taken from Bolt et al. 1977).

Initial estimates for the subsequent metabolism of the reactive metabolites and for the glutathione submodel in the rat were taken from the model for vinylidene chloride (D'Souza and Andersen, 1988). These parameter estimates, along with the estimates for VMAX2C and KM2, were then refined for the case of VC in the Sprague Dawley rat using an iterative fitting process which included the closed chamber data for the Sprague Dawley and Wistar rat (Barton et al. 1995, Bolt et al. 1977, Clement 1990) along with data on glutathione depletion (Jedrichowski et al. 1985, Watanabe et al., 1976c), total metabolism (Gehring et al. 1978), and CO₂ elimination (Watanabe and Gehring 1976). Since this last data set was obtained for oral dosing with VC in corn oil, a value for KA, the oral uptake rate from corn oil, was estimated from fitting of separate data on blood concentrations following dosing of rats with VC in corn oil (Withey 1976). The results of this iterative process are illustrated in Figures 4 through 8, and the resulting parameters are listed in Tables 1 and 2. The parameters obtained for the rat were used for the other species with appropriate allometric scaling (e.g., body weight to the -1/4 for the first order rate constants).

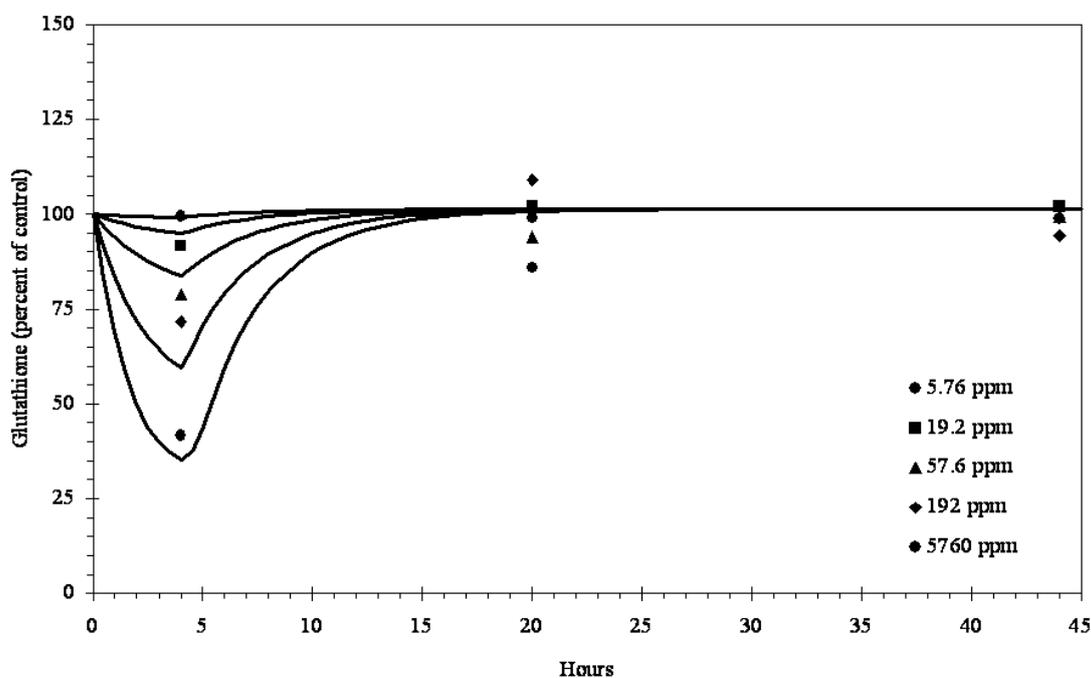


Figure 5. Model-predicted (lines) and experimentally determined (symbols) glutathione concentrations (as percent of control animal levels) 0, 16, and 40 hours following 4-hr inhalation exposures to VC at concentrations of (top to bottom) 15, 50, 150, 500, and 15000 mg/m³ (Data taken from Jedrichowski et al. 1985).

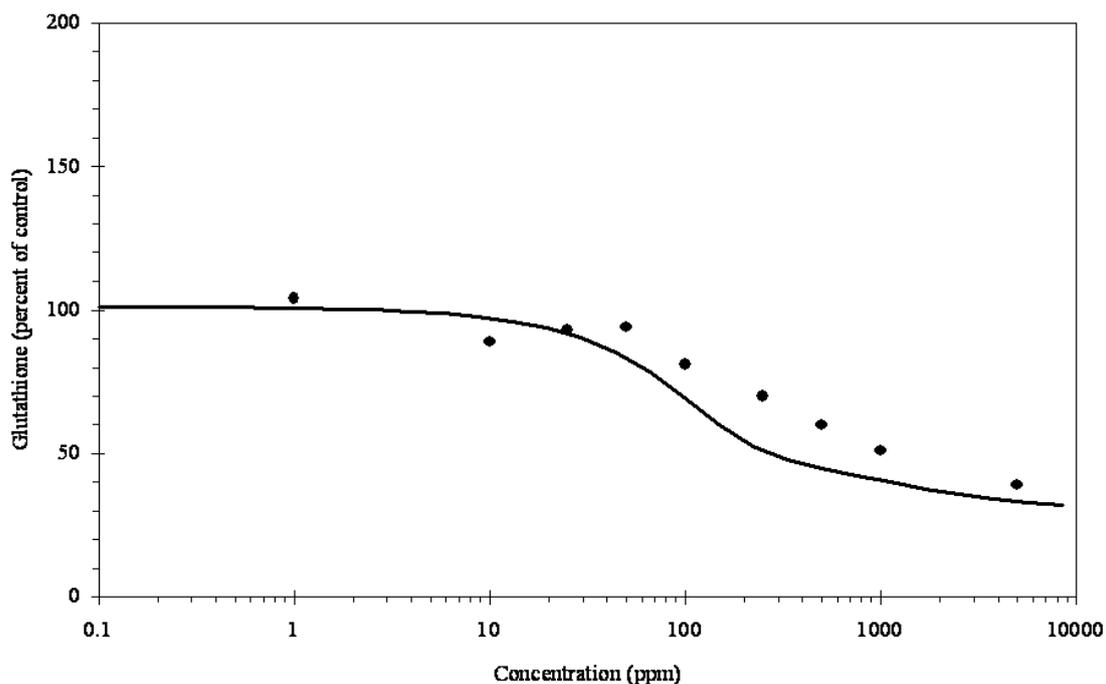


Figure 6. Model-predicted (lines) and experimentally determined (symbols) glutathione concentrations (as percent of control animal levels) immediately following 6-hr inhalation exposures to VC (Data taken from Watanabe et al. 1976c).

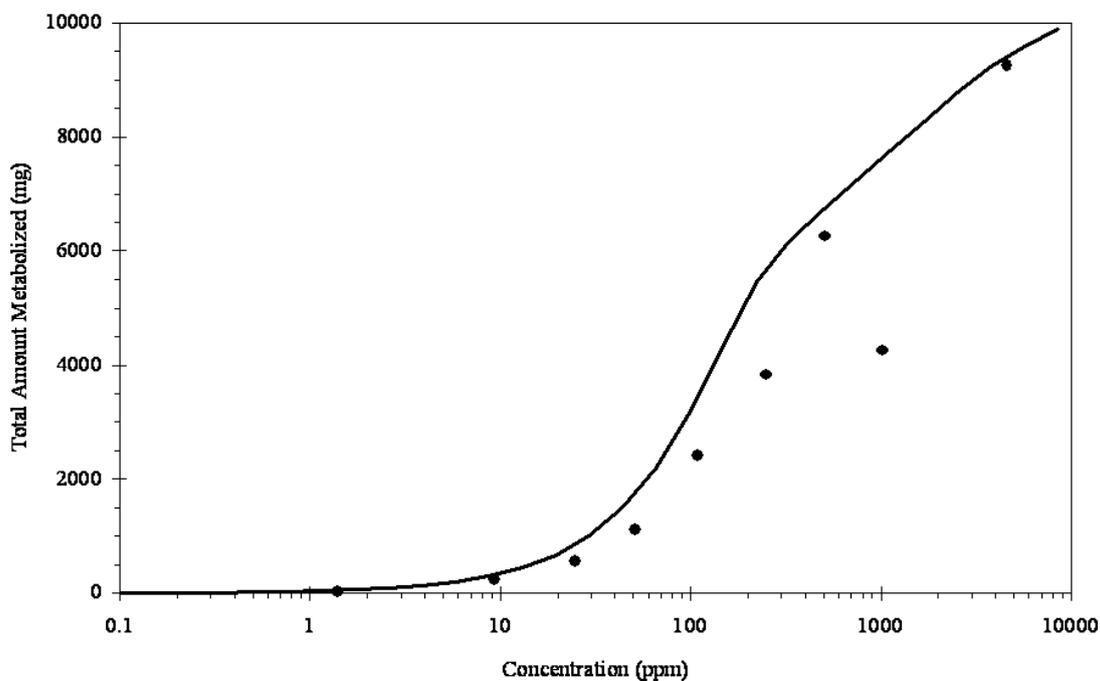


Figure 7. Model-predicted (lines) and experimentally determined (symbols) total amount metabolized during 6-hr inhalation exposures to VC (Data taken from Gehring et al. 1978).

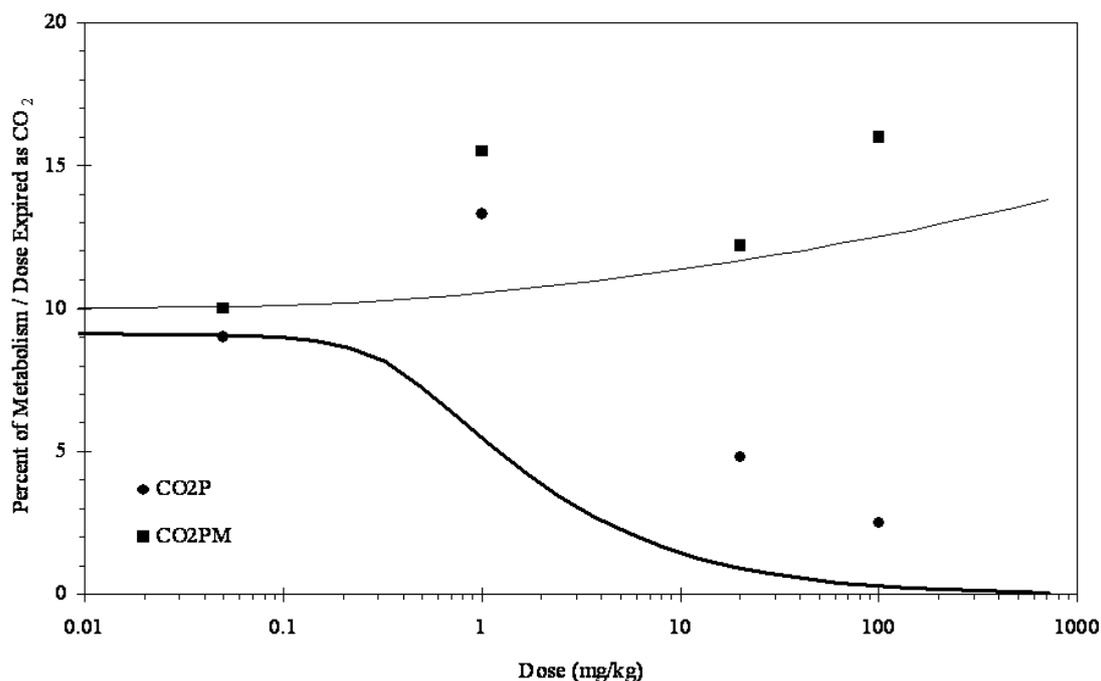


Figure 8. Model-predicted (lines) and experimentally determined (symbols) total expired CO₂, as a percent of total metabolism (upper line and symbols) and as a percent of dose (lower line and symbols), following oral dosing with VC in corn oil (Data taken from Watanabe and Gehring 1976).

Parameterization of the P450 metabolism pathways in the human was accomplished as follows: There is no evidence of high capacity, low affinity P450 metabolism for chlorinated ethylenes in the human; therefore, VMAX2C in the human was set to zero. The ratio of VMAX1C to KM1 could be estimated by fitting the model to data from closed chamber studies with two human subjects (Buchter et al. 1978), in a manner entirely analogous to the method used for the animal closed chamber analysis. The result of fitting the data on one of the two subjects is shown by the upper curve in Figure 9a, and the model prediction using the value estimated from this subject is compared with the data from the second subject in Figure 9b. The precision of the estimate of VMAX1C/KM1 can be evaluated by a comparison of the two model runs shown in these figures, for KM1=1.0 and KM1=0.1. It can be seen that the ratio of VMAX1C/KM1 varies between the two subjects. This variability of CYP2E1 activity in the human is not surprising; several studies have demonstrated a variability of human CYP2E1 activity of roughly an order of magnitude (Reitz et al. 1989, Sabadie et al. 1980). Much lower variability is observed in the inbred strains typically used in animal studies; for example, the coefficient of variation (standard deviation divided by the mean) for CYP2E1 activity in rats in one study of rodents was only 14% (Sabadie et al. 1980). The wide variability in human CYP2E1 activity is an important consideration for estimating the potential difference between average population risk and individual risk in a human cancer risk assessment for materials like VC, whose carcinogenicity depends on metabolic activation.

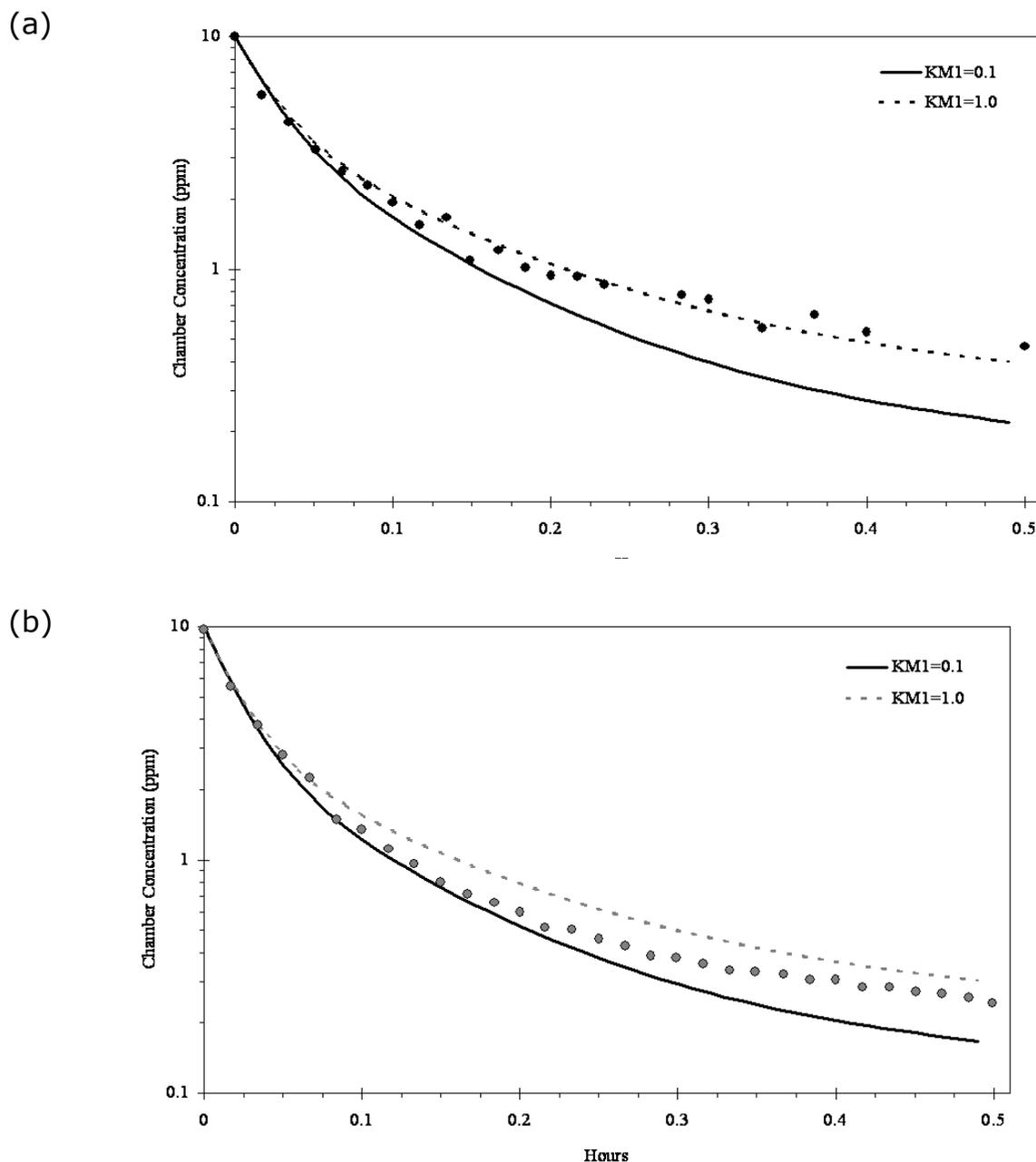


Figure 9. Model predictions (lines) and experimental data (symbols) for the chamber concentration during exposure of human subjects to VC in a closed, recirculated chamber (Data taken from Buchter et al. 1978). (a) Subject A – the lines show the model predictions for (top to bottom) $KM1 = 1.0$ and 0.1 ; (b) Subject B – the lines show the model predictions for (top to bottom) $KM1 = 1.0$ and 0.1 . The rest of the model parameters are those shown for the human in Tables 3 and 4.

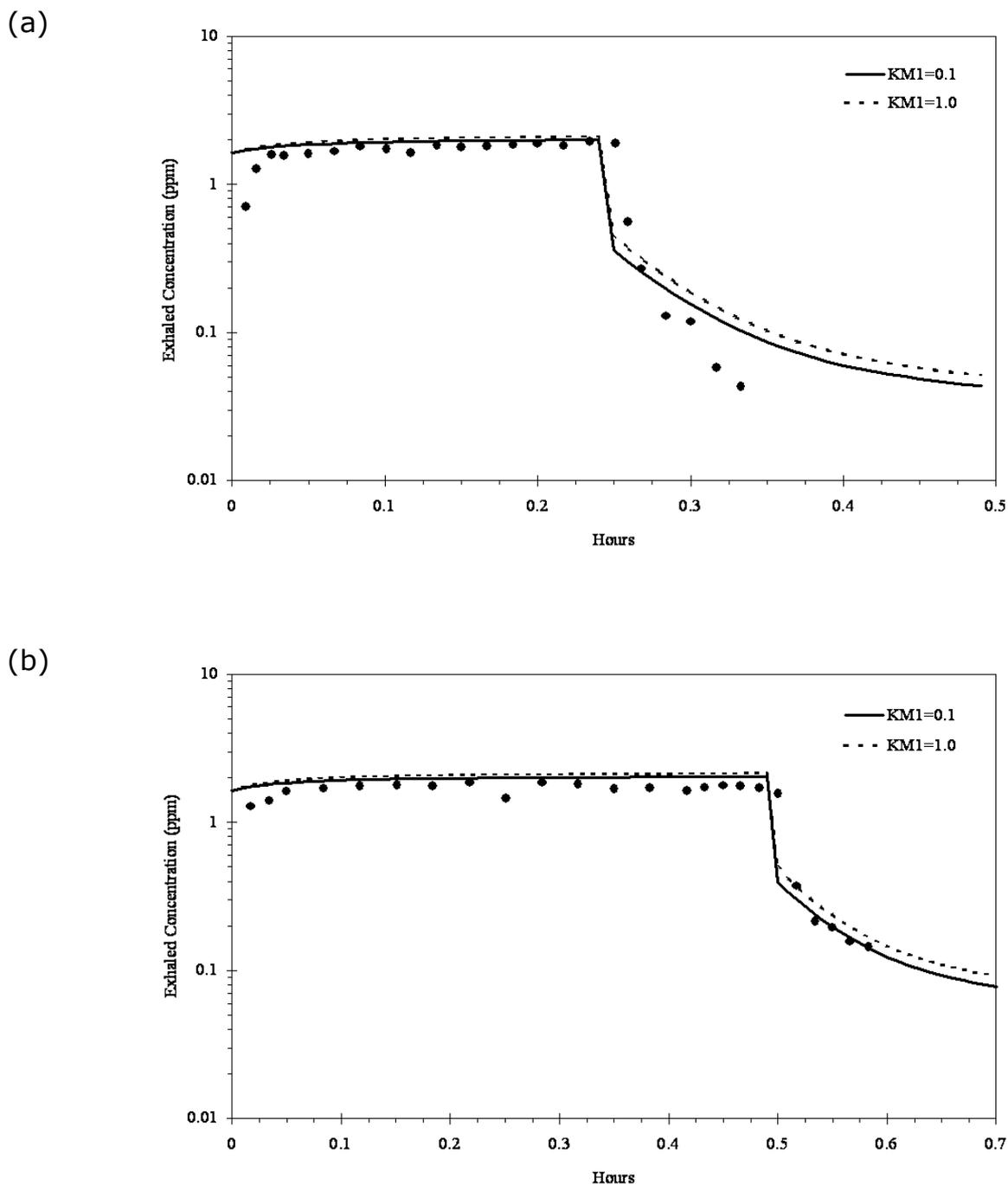


Figure 10. Model predictions (lines) and experimental data (symbols) for the exhaled air concentration during and following inhalation exposure of human subjects to a constant concentration of 2.5 ppm VC (Data taken from Buchter et al. 1978). (a) Subject A – the lines show the model predictions for (top to bottom) $KM1 = 1.0$ and 0.1 ; (b) Subject B – the lines show the model

predictions for (top to bottom) $KM1 = 1.0$ and 0.1 . The rest of the model parameters are those shown for the human in Tables 3 and 4.

In order to obtain separate estimates of V_{MAX1C} and $KM1$ in the human, higher exposure concentration closer to metabolic saturation would be required. Fortunately, cross-species scaling of CYP2E1 between rodents and humans appears to follow allometric expectations for metabolism very closely; that is, the metabolic capacity scales approximately according to body weight raised to the $3/4$ power (Andersen et al. 1987a). Support for the application of this principle to VC can be obtained from data on the metabolism of VC in non-human primates (Buchter et al. 1980). Based on data for the dose-dependent metabolic elimination of VC in the rhesus monkey, the maximum capacity for metabolism can be estimated to be about $50 \mu\text{mol/hr/kg}$. This equates to a V_{MAX1C} (the allometrically scaled constant used in the model) of approximately 4 mg/hr for a 1 kg animal, a value which is in the same range as those estimated for the rodents from the closed chamber exposure data. The similarity of V_{MAX1C} in humans and rats is also supported by an *in vitro* study which found the activity of human microsomes to be 84% of the activity of rat microsomes. Based on these comparisons, the human V_{MAX1C} was set to the primate value and $KM1$ was calculated using this value of V_{MAX1C} and the ratio of $V_{MAX1C}/KM1$ obtained from the closed chamber analysis. The ability of the resulting human model to reproduce constant concentration inhalation exposure data (Buchter et al. 1978) is shown in Figures 10a and 10b. From a comparison of the model predictions for $KM1 = 1.0$ and $KM1 = 0.1$, it can be seen that the reproduction of parent chemical concentrations in a constant concentration inhalation exposure is not a particularly useful test of the accuracy of the metabolism parameters in a PBPK model of a volatile compound. The discrepancies or agreement between the model and the data are primarily due to details of the physiological description of the individual, such as fat content, ventilation rate, blood/air partition, etc., rather than rate of metabolism.

Comparison of Risk Estimates for Human VC Inhalation

The model just described was used to calculate each of the pharmacokinetic dose metrics for angiosarcoma (RISK, RISKM, and RISKG) in the most informative of the animal bioassays (Maltoni et al. 1981, 1984, 1988, Feron et al. 1981), as well as for human inhalation exposure. The results of these calculations for the RISK dose metric (mg metabolized per kg liver) are shown in Table 3. The 95% upper confidence limits (UCLs) on the human risk estimates for lifetime exposure to 1 ppm VC were then calculated on the basis of each of the sets of animal bioassay data, using the LMS model. As shown in Table 4, the resulting risk estimates for lifetime human exposure to 1 ppm VC, based on the RISK dose metric, range from 1.52×10^{-3} to 15.7×10^{-3} . Because saturation of metabolism occurs well above the 1 ppm concentration in the human, estimates of risk below 1 ppm can be adequately estimated by assuming linearity (e.g., the risk estimates for lifetime human exposure to 1 ppb of VC would range from approximately 1.52×10^{-6} to 15.7×10^{-6}). It should be noted that although the animal studies represent both inhalation and oral exposure, the risk predictions in each case are for human inhalation exposure.

Table 3
Dose Metric Values for Angiosarcomas

					Daily Dose Metric	Lifetime Average Daily Dose
Study	Species	Duration	Dose	Incidence	RISK (mg/L)	RISK (mg/L)
Inhalation	Human	Continuous Exposure	1 ppm		1.614	1.614
Drinking Water			0.0286 mg/kg/d		0.603	0.603
Maltoni et al. 1988 (BT4) B Inhalation	Swiss Albino Mice (M)	4 hr/d, 5 d/wk for 30 of 104 wks	0 ppm	0/80		
			50 ppm	1/30	161.924	33.363
			250 ppm	9/30	775.615	159.811
			500 ppm	6/30	1245.220	256.570
			2500 ppm	6/29	1434.800	295.632
			6000 ppm	2/30	1479.270	304.795
			10000 ppm	1/26	1505.580	310.216
	Swiss Albino Mice (F)	4 hr/d, 5 d/wk for 30 of 104 wks	0 ppm	0/70		
			50 ppm	0/30	156.907	32.330
			250 ppm	9/30	673.015	138.671
			500 ppm	8/30	887.253	182.813
			2500 ppm	10/30	1197.670	246.773
			6000 ppm	11/30	1341.160	276.338
			10000 ppm	9/30	1405.330	289.560
Maltoni et al. 1981, 1984 (BT15 and BT1) - Inhalation	Sprague-Dawley Rats (M)	4 hrs/d, 5 d/wk for 52 of 147 wks	0 ppm	0/73		
			1 ppm	0/55	2.398	0.606
			5 ppm	0/47	11.985	3.028
			10 ppm	1/46	23.933	6.047
			25 ppm	4/40	59.552	15.047
		4 hrs/d, 5 d/wk for 52 of 135	50 ppm	1/29	117.989	32.463
			250 ppm	2/26	473.425	130.254

					Daily Dose Metric	Lifetime Average Daily Dose	
Study	Species	Duration wks	Dose	Incidence	RISK (mg/L)	RISK (mg/L)	
			500 ppm	6/28	593.928	163.409	
			2500 ppm	7/24	803.198	220.986	
			6000 ppm	10/25	911.248	250.714	
	Sprague-Dawley Rats (F)	4 hrs/d, 5 d/wk for 52 of 147 wks	0 ppm	0/45			
			1 ppm	0/48	2.343	0.592	
			5 ppm	0/43	11.698	2.956	
			10 ppm	0/42	23.332	5.895	
			25 ppm	1/41	57.838	14.614	
		4 hrs/d, 5 d/wk for 52 of 135 wks	50 ppm	0/26	113.653	31.270	
			250 ppm	1/28	375.989	103.447	
			500 ppm	0/22	425.029	116.939	
			2500 ppm	6/26	488.374	134.367	
			6000 ppm	3/17	522.359	143.718	
	Maltoni et al. 1981, 1984 (BT11) - Gavage	Sprague-Dawley Rats (M)	5 d/wk for 52 of 136 wks	0 mg/kg/d	0/60		
				0.021 mg/kg/d	0/15	0.488	0.133
0.214 mg/kg/d				0/15	4.962	1.355	
0.714 mg/kg/d				1/21	16.373	4.472	
2.38 mg/kg/d				0/34	50.390	13.762	
11.9 mg/kg/d				4/39	133.231	36.387	
35.7 mg/kg/d				8/36	203.079	55.463	
Sprague-Dawley Rats (F)				0 mg/kg/d	0/73		
				0.021 mg/kg/d	0/18	0.477	0.130
				0.214 mg/kg/d	1/19	4.835	1.321
				0.714 mg/kg/d	2/29	15.800	4.315

					Daily Dose Metric	Lifetime Average Daily Dose
Study	Species	Duration	Dose	Incidence	RISK (mg/L)	RISK (mg/L)
			2.38 mg/kg/d	0/37	45.330	12.380
			11.9 mg/kg/d	6/34	102.763	28.066
			35.7 mg/kg/d	9/35	143.866	39.291
Feron et al. 1981 - Diet	Wistar Rats (M)	135 wks 7 d/wk	0 mg/kg/d	0/55		
			1.7 mg/kg/d	0/58	39.539	39.539
			5.0 mg/kg/d	6/56	116.103	116.103
			14.1 mg/kg/d	27/59	325.845	325.845
	Wistar Rats (F)	144 wks 7 d/wk	0 mg/kg/d	0/57		
			1.7 mg/kg/d	0/58	38.611	38.611
			5.0 mg/kg/d	2/59	113.243	113.243
			14.1 mg/kg/d	9/57	316.628	316.628

Table 4
Risk Estimates Based on Angiosarcoma Incidence

95% UCL Risk / 1000 / ppm	RISK
Maltoni et al. (1988) BT4-Inhalation - male mice - female mice	1.52 3.27
Maltoni et al. (1981, 1984) BT14/BT1-Inhalation - male rats - female rats	5.17 2.24
Maltoni et al. (1981, 1984) BT11-Gavage - male rats - female rats	8.68 15.70
Feron et al. (1981) -Diet - male rats - female rats	3.05 1.10

Only the results for the RISK dose metric (mg metabolized per kg liver) are shown in Tables 3 and 4. The p-values for goodness of fit of the 1-stage model using the other alternative dose metrics (RISKM, and RISKG) were very similar; therefore, it is not possible to select one metric over another on the basis of agreement with the dose-response of the incidence data. Fortunately, the risks predicted for each of the studies by the various dose metrics were roughly similar. The RISKM metric, which is the most biologically plausible, resulted in lower risks than the RISK metric (ranging from 0.50×10^{-3} to 12.46×10^{-3} /ppm); while the RISKG metric, which is probably the least likely, resulted in risk estimates up to 30 percent higher than those shown in Table 4.

There are no consistent differences between risk estimates based on male and female animals, with the female-based risks being higher than the male-based risks in some studies, and lower in others, but generally agreeing within a factor of two to three. The risk estimates based on inhalation studies with mice (0.5×10^{-3} to 4.3×10^{-3} /ppm) agree very well with those based on inhalation studies with rats (1.46×10^{-3} to 5.94×10^{-3} /ppm), demonstrating the ability of pharmacokinetics to integrate dose-response information across species.

The risks estimated from the dietary administration of VC (0.94×10^{-3} to 3.13×10^{-3} /ppm) are also in good agreement with those obtained from the inhalation bioassays, showing good route-to-route correspondence of potency based on the pharmacokinetic dose metric. However, the estimates based on oral gavage of VC in vegetable oil (6.58×10^{-3} to 16.3×10^{-3} /ppm) are about 6-fold higher than either dietary or inhalation exposure. It has previously been noted in studies with chloroform that administration of the chemical in corn oil results in more marked hepatotoxic effects than observed when the same chemical is provided in an aqueous suspension (Bull et al. 1986). It has also been demonstrated that administration of corn oil alone leads to an increase in peroxisomal oxidative enzyme activity in rats (DeAngelo et al. 1989). The toxicity and oxidative environment created in the liver by continual dosing with large volumes of vegetable oil could serve to potentiate the effects of genotoxic carcinogens in the liver. In support of this suggestion, Newberne et al. (1979) found that incorporation of corn oil into the diet increased the yield of aflatoxin B₁-induced tumors in rats. A similar phenomenon could be responsible for the apparently higher potency of VC when administered by oil gavage compared to incorporation in the diet.

Epidemiological Analysis of Vinyl Chloride Carcinogenicity

In order to evaluate the plausibility of the risks predicted on the basis of the animal data, risk calculations were also performed on the basis of available epidemiological data. A linear relative risk dose-response model was used for analysis of the human data:

$$O = E(1 + \alpha*d),$$

where O is the observed number of liver tumors, E is the expected number of such tumors apart from any exposure, d is a cumulative dose metric (see discussion below) and a is a potency parameter that can be estimated by maximum likelihood techniques. Then it follows that the lifetime probability of liver cancer, $P(d)$, can be estimated by

$$P(d) = P_0(1 + a*d),$$

where P_0 is the background probability of liver cancer death. Actually, the lifetime risk should be estimated by a lifetable method, but the above approximation should be close enough for the purpose of these comparative potency estimates.

Now suppose that for a particular exposure scenario (e.g., a VC atmospheric concentration of 50 ppm, 8 hours a day, 5 days per week), the PBPK model predicts an average daily internal dose metric of X . Then the cumulative exposure that should be used in the dose-response model is $X*Y$, where Y is the number of years of such exposure. Note that to compute this PBPK-based cumulative dose, we must have an estimate of the "typical" workplace exposure concentration for each subcohort, separate from the number of years of exposure for the subcohort, rather than just a cumulative dose estimate. Only after the internal dose has been calculated with the PBPK model can the duration of exposure be applied to get a cumulative internal dose.

To obtain pharmacokinetic, human-based risk estimates, the PBPK model was run for the exposure scenario appropriate to each of the selected subcohorts from the studies discussed below. The resulting internal dose metrics were multiplied by the appropriate durations to obtain the cumulative internal doses, which were then input into the relative risk model along with the observed and expected liver cancer deaths for each subcohort to get an estimate of the maximum likelihood estimate and 95% confidence interval for a . Then, to determine the risk associated with a continuous lifetime exposure to 1 ppm for comparison with the animal results, the PBPK model was run for a 1 ppm continuous exposure, and the average daily value of the various internal dose metrics was calculated. Multiplying the dose metrics by 70 years gives the appropriate cumulative dose for the relative risk model. For P_0 sufficiently small (which it should be for liver cancer in humans), the extra risk for a lifetime exposure to 1 ppm VC will be approximately:

$$P_0*a*d_1$$

where d_1 = cumulative internal dose for 1 ppm continuous exposure. Using the 95% upper bound on the estimate for a provides a 95% upper confidence limit on the lifetime risk per ppm for comparison with the animal-based results obtained with the LMS model.

Three epidemiological studies that associated increased liver cancer with exposure to VC, and that provide sufficient information to support separate

exposure concentration and duration estimates (as opposed to just cumulative exposure estimates), were selected for this study: Fox and Collier (1977), Jones et al. (1988), and Simonato et al. (1991).

Fox and Collier (1977)

This study is probably the best with respect to providing information about duration of employment for different exposure-level groupings (see their Table 2). We have previously estimated the average exposure levels to be 12.5, 70, and 300 ppm for the low, medium, and high exposure groups, respectively (Clement, 1987); for comparison Chen and Blancato (1989) estimated averages of 11, 71, and 316 ppm. For the constant exposure groups, these concentrations were input into the human PBPK model, assuming 8 hrs/day and 5 days/week exposure, to get average daily internal dose metrics, which were then multiplied by the duration averages (assumed to be 5, 15, and 27 years) to get cumulative doses. For the intermittent exposure groups, exposure for 2 hrs/day, 5 days/week was assumed.

A weighted average was then performed of the cumulative doses within each exposure group (high, medium, and low), across duration of exposure categories and constant vs intermittent groups. This must be done because observed and expected numbers of liver cancers are reported only by exposure group (see their Table 9). The weighting was performed using the number of workers in the various subcohorts (their Table 2).

The resulting weighted dose estimates for each internal dose metric were then input into the relative risk model along with the observed and expected tumors:

<u>Cumulative Dose</u>	<u>Obs.</u>	<u>Exp.</u>
Average low cumulative dose	1	0.75
Average medium cum. Dose	1	0.77
Average high cum dose	2	0.13

The resulting risk estimates are shown in Table 5. The range of risk estimates reflects uncertainty in the appropriate value for P_0 , the background probability of death from liver cancer. The lower risk estimate was calculated using the value of P_0 derived in the Fox and Collier study, while the higher risk estimate was calculated using an estimate of the lifetime liver cancer mortality rate in the U.S. population (Chen and Blancato, 1989). An important factor to be aware of in interpreting these results is that the classification into exposure groups in this study was based on the maximum exposure level that a worker experienced. This leads to overestimation of cumulative exposure, particularly for the workers in the medium and high groups, and therefore a probable underestimation of risk when using the linear relative risk model.

Table 5
Human-Based Risk Estimates

95% UCL Risk / 1000 / ppm	
Fox & Collier (1977)	0.71 – 4.22
Jones et al. (1988)	0.97 – 3.60
Simonato et al. (1991)	0.40 – 0.79

Jones et al. (1988)

This study was an update of the cohort studied by Fox and Collier. Unfortunately, it does not provide as much information about duration of exposure, so the analysis must be limited to the autoclave workers. For those workers, four duration-of-employment categories are given (see their Table 4); in the present analysis estimated average durations of 1.5, 3, 7.5, and 15 years were used. Their Table 1 shows that the autoclave workers had exposures ranging between 150 and 800 ppm at various points in time. A value of 500 ppm was used in the PBPK model (8 hrs/day, 5 days/week) to get the average daily internal doses. The average daily internal doses were then multiplied by the 4 average durations of exposure to get four groups for analysis:

<u>Cumulative Dose</u>	<u>Obs.</u>	<u>Exp.</u>
Low	0	0.07
Mid 1	1	0.08
Mid 2	2	0.08
High	4	0.15

The resulting risk estimates are shown in Table 5. The lower risk estimate was calculated using the value of P_0 derived in the Jones et al. (1988) study, while the higher risk estimate was calculated using an estimate of the lifetime liver cancer mortality rate in the U.S. population (Chen and Blancato, 1989).

Simonato et al. (1991)

This study has the largest cohort and the most liver cancer deaths (24). Unfortunately, the exposure information may not be as accurate as in the other two studies discussed above, since it was collected from many different workplaces in several different countries, and since the original reporting of the exposure levels was relatively crude (ranges of <50, 50 -499, and \geq 500 ppm). As in the Fox and Collier study, the classification was based on the "highest level to which the workers were potentially exposed." Thus, as with the previous

studies, the estimates of risk from this cohort are probably underestimates of the true risk.

Another problem with the reporting of the results in this study is that the durations of exposure are not cross-classified according to exposure level as was done in the Fox and Collier report. In fact, there is very little information about duration of exposure that would allow estimation of an average value for the entire cohort, let alone the exposure groups. (Note that we can not use the cumulative exposure groupings, as discussed above, because we need to have exposure level separated from exposure duration.) The information in Simonato et al. (1991) Table 2 (person years of observation by duration of employment) was used to estimate an average duration under the following assumption: if the follow-up time does not depend on the duration of employment, then the differences in the person-years of follow-up is due to the numbers of individuals in each duration category. The weighted average (trying different averages for the ≥ 20 year group) gives an estimate of 9 years of employment. This duration was used with model-predicted daily dose metrics for average exposure level estimates of 25, 158, and 600 ppm. The cumulative internal doses were input into the relative risk model with the following observed and expected liver cancer deaths:

<u>Cumulative Dose</u>	<u>Obs.</u>	<u>Exp.</u>
Low cumulative dose	4	2.52
Medium cum. Dose	7	1.86
High cum. Dose	12	2.12

The resulting risk estimates are shown in Table 5. Again, the lower risk estimate was calculated using the value of P_0 derived in the Simonato et al. (1991) study, while the higher risk estimate was calculated using an estimate of the lifetime liver cancer mortality rate in the U.S. population (Chen and Blancato, 1989).

A comparison of the results of the analyses of the three sets of data, shown in Table 5, gives some indication of the consistency of the human results. The lifetime risk of liver cancer per ppm VC exposure estimated from the three studies only ranges over about one order of magnitude: from 0.4×10^{-3} to 4.2×10^{-3} . Very similar risk estimates were also obtained with the alternative dose metrics (RISKM and RISKG). Moreover, these estimates are in remarkable agreement with the estimates based on animal data shown in Table 4. However, any confidence produced by this agreement should be tempered by the likelihood, discussed above, that misclassification of exposure in the human studies may somewhat underestimate the true risk at lower doses. Nevertheless, the agreement of the pharmacokinetic animal-based risk estimates with the pharmacokinetic human-based risk estimates provides strong support for the assumption used in this study: that cross-species scaling of lifetime cancer risk can be performed on a direct basis of lifetime average daily dose (without applying a body surface area adjustment) when the risks are based on biologically appropriate dose metrics calculated with a validated PBPK model.

Pharmacokinetic Sensitivity / Uncertainty Analysis

Table 6 shows the normalized analytical sensitivities for the PBPK model used in this analysis. The normalized analytical sensitivity coefficient represents the fractional change in output associated with a fractional change in the input parameter. For example, if a 1% change in the input parameter results in a 2% change in the output, the sensitivity coefficient would be 2.0. In Table 6, the outputs are the dose metrics used in the analysis of angiosarcoma risk. The parameters in the table are defined in Tables 1 and 2. Sensitivity coefficients of less than 0.01 in absolute value were omitted from the table for clarity. None of the parameters display sensitivities significantly greater than 1.0, indicating that there is no amplification of error from the inputs to the outputs. This is, of course, a desirable trait in a model to be used for risk assessment.

It can be seen that of the 24 parameters in the VC model, 10 have essentially no impact on risk predictions based on any of the dose metrics, and only 8 have a significant impact on predictions based on RISK: the body weight (BW), alveolar ventilation (QPC), cardiac output (QCC), liver blood flow (QLC) and volume (VLC), blood/air partition coefficient (PB), the capacity (VMAX1C) and affinity (KM1) for metabolism by CYP2E1, and in the case of oral gavage, the oral uptake rate (KA). As discussed in the description of the PBPK model, all of these parameters could be reasonably well characterized from experimental data. However, the sensitivity of the risk predictions to the human values of these parameters implies that the risk from exposure to VC could vary considerably from individual to individual, depending on their specific physiology, level of activity, and metabolic capability.

The other dose metrics, RISKM and RISKG, are also sensitive to a number of the parameters in the model for the subsequent metabolism of the reactive metabolites, as well as for the GSH submodel (data not shown). Since these parameters could only be identified from data in rats, their values in the other species are uncertain. Given the sensitivity of RISKM and RISKG to these less certain parameters, and the general similarity of risks based on these two metrics to those based on the RISK metric, the use of the RISK metric would seem to be preferable for quantitative risk assessment.

Table 6
Normalized Parameter Sensitivity in Vinyl Chloride PBPK Model

	Rat Gavage (11.9 mg/kg)	Rat Inhalation (50 ppm,4hr)	Human Inhalation (1 ppm, continuous)	Human Drinking Water (1 ppm)
Dose Metric:	Risk	Risk	Risk	Risk
Parameter				
BW	-0.15	-0.26	-0.22	0.03
QPC	-0.08	0.32	0.11	-0.06
QCC	-0.06	0.65	0.44	-0.35
QFC	--- ¹	---	---	---
QLC	-0.06	0.64	0.44	-0.35
VFC	---	---	---	---
VLC	-1.03	-0.84	-0.66	-0.69
PB	0.09	0.73	0.88	0.09
PF	--	--	--	--
PS	--	--	--	--
PR	--	--	--	--
PL	--	--	--	--
VMAX1C	0.69	0.11	0.44	0.43
KM1	-0.07	-0.10	-0.44	-0.43
VMAX2C	0.06	--	--	--
KM2	-0.04	--	--	--
KA	0.29	--	--	--
KC02C	--	--	--	--
KGSMC	--	--	--	--
KFEEC	--	--	--	--
GSO	--	--	--	--
KBC	--	--	--	--
KS	--	--	--	--
KOC	--	--	--	--

¹ - less than 0.05 in absolute value

Monte Carlo Uncertainty / Variability Analysis

The sensitivity analysis described above does not consider the potential interactions between parameters; the parameters are tested individually. Also, sensitivity analysis does not adequately reflect the uncertainty associated with each parameter. The fact that the output is highly sensitive to a particular parameter is not important if the parameter is known exactly. To estimate the combined impact of uncertainty regarding the values of all the parameters, a Monte Carlo analysis can be performed. In a Monte Carlo analysis, the distributions of possible values for each of the input parameters are estimated. The Monte Carlo algorithm then randomly selects a value for each parameter from its distribution and runs the model. The random selection of parameter values and running of the model is repeated a large number of times (typically

hundreds to thousands), until the distribution of the output has been characterized.

To assess the impact of parameter uncertainty on risk predictions, a dose-response model must be selected. In this case the linearized multistage model was used, for the reasons discussed earlier. The actual analysis was performed with our software package, PBPk_SIM, which was developed for the Air Force specifically to perform such a Monte Carlo analysis on PBPk models. The PBPk_SIM program randomly selects a set of parameter values from the distributions for the bioassay animal and runs the PBPk model to obtain dose metric values for each of the bioassay dose groups. It then selects a set of parameter values from the distributions for the human and runs the PBPk model to obtain a dose metric value for a specified human exposure scenario. Finally, it runs the linearized multistage model (or other specified risk model) with the animal and human dose metric values to obtain the human risk estimate. This entire process is repeated a specified number of times until the desired distribution of risks has been obtained.

Tables 1 and 2 list the means (preferred values) and coefficients of variation (CV) used in a Monte Carlo uncertainty analysis of the PBPk model. Truncated normal distributions were used for all parameters except the kinetic parameters, which were assumed to be lognormally distributed. The CVs for the physiological parameters were estimated from data on the variability of published values (USEPA, 1988; Stan Lindstedt, 1992, personal communication), while the CVs for the partition coefficients were based on repeated determinations for two other chemicals, PERC (Gearhart *et al.*, 1993) and chloropentafluorobenzene (Clewell and Jarnot, 1994). The CVs for the metabolic and kinetic constants were estimated from a comparison of reported values in the literature and by exercising the model against the various data sets to determine the identifiability of the parameters which were estimated from pharmacokinetic data.

The results of the Monte Carlo analysis are shown in Table 7, which lists the estimated risks associated with lifetime exposure to 1 ppm VC in air or 1 mg/L VC in drinking water (assuming a drinking water ingestion rate of 2L/day). In all cases, the risk estimates represent the 95% UCL for risk, based on the LMS model. However, in order to characterize the impact of uncertainty in the pharmacokinetic parameters on the risk estimates, both the mean and the upper 95th percentile of the distribution of UCL risk estimates are shown. Thus the mean value represents the best estimate of the pharmacokinetically-based upper-bound risk for VC exposure, and the 95th percentile provides a reasonable value for the "highest probable" pharmacokinetic risk estimate, considering both pharmacokinetic uncertainty and uncertainty regarding the low-dose extrapolation. In general, the 95th percentiles of the distributions of risk estimates are within a factor of two of the means, indicating that

pharmacokinetic uncertainty/variability is a relatively small contributor to the overall uncertainty in a risk assessment for VC.

Table 7
Mean and 95th Percentile UCL Risk / 1000
For Angiosarcoma Based on the Pharmacokinetic Dose Metric*

95% UCL Risk / 1000		1 ppm Inhalation		1 mg/L Drinking Water	
Animal Route	Sex/Species	Mean/ UCL	95 th / UCL	Mean/ UCL	95 th / UCL
Inhalation	male mouse	1.89	3.38	0.67	1.18
	female mouse	3.89	6.95	1.39	2.33
Inhalation	male rat	6.80	14.31	2.45	5.60
	female rat	1.90	3.81	0.67	1.37
Oil gavage	male rat	9.45	17.22	3.36	5.72
	female rat	16.35	29.73	5.83	10.54
Diet	male rat	3.26	5.26	1.14	1.64
	female rat	1.15	1.87	0.41	0.60

* dose metric = lifetime-average total amount metabolized per day, divided by volume of liver

Discussion

Giving priority to the animal studies most closely approximating the human route of exposure, the animal-based estimate of the carcinogenic risk of angiosarcoma from lifetime exposure to 1 ppm VC in air is 2.75×10^{-3} , or $1.1 \times 10^{-6} (\mu\text{g}/\text{m}^3)^{-1}$, based on the geometric mean of the results shown in Table 4 for the inhalation studies with male and female rats and mice (Maltoni et al. 1981, 1984, 1988). This value is consistent with the range of risk estimates from the epidemiological studies of 0.4×10^{-3} to 4.2×10^{-3} risk per ppm VC, but is roughly a factor of 80 below the inhalation unit risk of $8.4 \times 10^{-5} (\mu\text{g}/\text{m}^3)^{-1}$ that has been used by the EPA since 1985 (Table 8). This difference is primarily attributable to the differences in cross-species scaling between the default approach and the pharmacokinetic approach. Similarly, the animal-based estimate of the carcinogenic risk of angiosarcoma from lifetime exposure to 1 $\mu\text{g}/\text{L}$ VC in drinking

water (assuming a drinking water ingestion rate of 2L/day) is $6.8 \times 10^{-7} (\mu\text{g/L})^{-1}$, or $0.024 (\text{mg/kg/day})^{-1}$, based on the geometric mean of the results for the dietary administration of VC in male and female rats (Feron et al. 1981). This value is also roughly a factor of 80 below the unit risk of $1.9 (\text{mg/kg/day})^{-1}$, or $5.4 \times 10^{-5} (\mu\text{g/L})^{-1}$ that has been in use by the EPA since 1985 (Table 8). However, in this case the difference is primarily due to the use of the incidence of liver angiosarcoma alone in this analysis, as opposed to the total incidence of all liver tumors (angiosarcoma, hepatocellular carcinoma, hepatocellular adenoma, and neoplastic lesions) plus lung angiosarcoma in the EPA risk assessment.

Table 8
Comparison of Cancer Risk Estimates for Vinyl Chloride

<u>Basis</u>	<u>Inhalation</u> (1 $\mu\text{g}/\text{m}^3$)	<u>Drinking water</u> (1 $\mu\text{g}/\text{L}$)
Current EPA HEAST Table (EPA 1999)	84.0×10^{-6}	54.0×10^{-6}
Pharmacokinetic Model	1.1×10^{-6}	0.7×10^{-6}
Human Epidemiology	$0.2 - 1.7 \times 10^{-6}$	

Although VC has often been cited as a chemical for which saturable metabolism should be considered in the risk assessment, saturation appears to become important only at very high exposure levels (greater than 250 ppm by inhalation or 25 mg/kg/day orally) compared to the lowest tumorigenic levels, and thus has little impact on the quantitative risk estimates. The important contribution of pharmacokinetic modeling is to provide a more biologically plausible estimate of the effective dose: total production of reactive metabolites in the target tissue. The ratio of this biologically effective dose to the administered dose is not uniform across routes and species. Therefore any estimate of administered dose is less adequate for performing route-to-route and interspecies extrapolation of risk.

In the pharmacokinetic risk calculations presented in this report, no BSA adjustment factor was applied to obtain the human risks. Although this may appear to represent a departure from previous EPA practice in a risk assessment for VC, this marks the first time a pharmacokinetic dose metric has been used. The dose metric was selected to be consistent with the position stated in the interagency pharmacokinetics group consensus report on cross-species extrapolation of cancer (USEPA 1992) that "...tissues experiencing equal average

concentrations of the carcinogenic moiety over a full lifetime should be presumed to have equal lifetime cancer risk." However, in the only pharmacokinetic risk assessment adopted by the EPA to this date, the pharmacokinetic dose metric used for methylene chloride was also a "virtual concentration" of an as yet unidentified reactive metabolite, and its use similarly appeared to be consistent with the EPA position. Nevertheless, in their actual application of the methylene chloride pharmacokinetic model, EPA chose to adjust the pharmacokinetic dose metric by a BSA scaling factor to consider potential species differences in pharmacodynamics (USEPA 1987).

The risk assessment performed in this study has focused on cancer risk from a continuous lifetime exposure, or at least an exposure over a large fraction of lifetime. Although there are certainly many uncertainties and unresolved issues regarding cross-species extrapolation of lifetime risks, there are even greater uncertainties regarding the extrapolation of partial-lifetime exposures. In particular, studies which have been performed with VC make it evident that extrapolation of partial lifetime exposure is not straightforward with this chemical. For example, in the comparative studies of partial lifetime exposure of rats to VC discussed earlier (Drew et al. 1983), while exposure from 0 to 6 months resulted in a similar tumor incidence to exposure from 6 to 12 months of life, exposure from 0 to 12 months produced a significantly different incidence than would be expected from the sum of the incidences for the two subintervals.

For angiosarcomas, on the one hand, exposure to VC from 0 to 6 months and from 6 to 12 months resulted in incidences of 5.3% and 3.8%, respectively, while exposure from 0 to 12 months resulted in a much higher incidence of 21.4%. For hepatocellular carcinomas, on the other hand, exposure to VC from 0 to 6 months and from 6 to 12 months resulted in incidences of 4.0% and 11.5%, respectively, while exposure from 0 to 12 months resulted in an incidence of only 7.1%. Thus this comparative bioassay does not provide support for a simple relationship of the observed incidence to the fraction of lifetime of the exposure. As discussed earlier, it seems reasonable to assume that newborns, with their higher rate of cell proliferation, should be at greater risk from genotoxic carcinogens, and some studies with VC support this assumption (Maltoni et al. 1981, Laib et al. 1989, Fedtke et al. 1990), although other well-conducted studies with VC do not (Drew et al. 1983). The issue of sensitive populations has never been seriously dealt with in quantitative carcinogenic risk assessment, but it would seem to be an appropriate consideration during risk management for specific potential exposures.

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Chapter 9

APPLYING MODE-OF-ACTION AND PHARMACOKINETIC CONSIDERATIONS IN CONTEMPORARY CANCER RISK ASSESSMENTS: AN EXAMPLE WITH TRICHLOROETHYLENE

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ABSTRACT

The guidelines for carcinogen risk assessment recently proposed by the U.S. Environmental Protection Agency (USEPA) provide an increased opportunity for the consideration of pharmacokinetic and mechanistic data in the risk assessment process. However, the greater flexibility of the new guidelines can also make their actual implementation for a particular chemical highly problematic. To illuminate the process of performing a cancer risk assessment under the new guidelines, the rationale for a state-of-the-science risk assessment for trichloroethylene (TCE) is presented. For TCE, there is evidence of increased cell proliferation due to receptor interaction or cytotoxicity in every instance in which tumors are observed, and most tumors represent an increase in the incidence of a commonly observed, species-specific lesion. A physiologically based pharmacokinetic (PBPK) model was applied to estimate target tissue doses for the three principal animal tumors associated with TCE exposure: liver, lung, and kidney. The lowest points of departure (lower bound estimates of the exposure associated with 10% tumor incidence) for lifetime human exposure to TCE were obtained for mouse liver tumors assuming a mode of action primarily involving the mitogenicity of the metabolite trichloroacetic acid (TCA). The associated linear unit risk estimates for mouse liver tumors are 1.5×10^{-6} for lifetime exposure to 1 microgram TCE per cubic meter in air and 0.4×10^{-6} for lifetime exposure to 1 microgram TCE per liter in drinking water. However, these risk estimates ignore the evidence that the human is likely to be much less responsive than the mouse to the carcinogenic effects of TCA in the liver, and that the carcinogenic effects of TCE are unlikely to occur at low environmental exposures. Based on consideration of the most plausible carcinogenic modes of action of TCE, a margin-of-exposure (MOE) approach would appear to be more appropriate. Applying an MOE of 1000, environmental exposures below 66 micrograms TCE per cubic meter in air and 265 micrograms TCE per liter in drinking water are considered unlikely to present a carcinogenic hazard to human health.

I. INTRODUCTION

Assessing the potential risk associated with human exposure to carcinogenic environmental contaminants represents an uncomfortable admixture of scientific evaluation and political policy, with the potential for enormous impact on both the public health and the economic well-being of the nation. A difficult challenge facing cancer risk assessors today is to realistically consider the implications of the chemical's mechanism(s) of carcinogenicity in developing a risk assessment approach for a particular carcinogenic effect. It has become increasingly difficult to justify the use of the same linear risk assessment approach both with chemicals that act through a purely radiomimetic, genotoxic mechanism, and with chemicals for which carcinogenicity is mediated by an inherently nonlinear process, such as increased cell proliferation secondary to cytotoxicity or receptor interaction. Mechanism-dependent risk assessment approaches are the only alternative for maintaining the credibility of cancer

potency estimates in the face of increasing sophistication in the understanding of the mechanisms of carcinogenicity.

The guidelines for carcinogen risk assessment recently proposed by the U.S. Environmental Protection Agency (USEPA) ¹ would appear to provide the flexibility necessary to move forward in this area.² Under the new guidelines, multiple options are available for performing a carcinogenic dose-response assessment, ranging from the use of a biologically based dose-response model to the use of linear or margin of exposure (MOE) default approaches. The selection of the dose-response approach to be used with a particular chemical is determined on the basis of the information available on the chemical, which considers both pharmacokinetic and mechanistic data. Significantly, the new guidelines depart from the usual definition of a default used in the past. Under the new guidelines, a default is defined as the "no-information" option, the use of which must be justified by the agency on the basis of the lack of sufficient information on a specific chemical to support a more preferred, chemical-specific approach. This definition stands in contrast to past practice, in which the default position was treated as the preferred approach, and justification was required for departing from it on the basis of chemical-specific information.

Risk assessments for chemical carcinogens must necessarily be iterative in nature. It is in the nature of scientific inquiry that understanding develops slowly, as experimental information accumulates and theories can be tested and refined. Risk assessments, however, cannot be postponed indefinitely until an adequate understanding of the carcinogenicity of a particular chemical has been achieved. Therefore, it is necessary to attempt to perform the most scientifically defensible assessment possible, given the information available at that time, and to be ready to revise the estimate, periodically, whenever important new information is developed. In the last few years there has been a significant improvement in the level of understanding regarding chemical carcinogenesis in general and the mechanisms of carcinogenicity of trichloroethylene (TCE) in particular. The purpose of the study reported here was to attempt to perform a state-of-the-science risk assessment for TCE in the spirit of the new USEPA cancer guidelines, using to as great an extent as possible the information currently available on pharmacokinetics and carcinogenic mode of action. An attempt will be made to provide a fairly thorough discussion of the available data and the decision criteria associated with each step in the risk assessment process for TCE, in the hope of illuminating some of the issues and considerations that must be addressed in the implementation of the new USEPA cancer guidelines for any chemical.

A. Consideration of Pharmacokinetics and Metabolism

As will be described later, the primary target tissue for the carcinogenicity of TCE identified in animal studies are the liver, lung, and kidney. For each of these target tissues there is evidence that the carcinogenicity of TCE may be associated with one or more of its metabolites: trichloroacetic acid (TCA) and dichloroacetic acid (DCA) in the liver,^{3,4} chloral (CHL) in the lung,⁵ and 1,2-dichlorovinylcysteine (1,2-DCVC) in the kidney.⁶ Thus a comprehensive cancer

risk assessment for TCE should be based on an analysis of tissue dosimetry for all three target tissues, including a description of the kinetics of the metabolites imputed to play a role in the carcinogenic activity.

A powerful technique for performing tissue dosimetry is physiologically-based pharmacokinetic (PBPK) modeling. Briefly, PBPK modeling attempts to describe the relationship between external measures of applied dose (e.g., amount administered or concentration in food, water, or air) and internal measures of biologically effective dose (e.g., amount metabolized or concentration in the tissue displaying the toxic response), using as realistic a description of mammalian physiology and biochemistry as is necessary and feasible.⁷⁻¹⁰ Thus nonlinear biochemical processes can be incorporated into the model and the behavior of the animal-chemical system can be predicted for different routes of exposure over a wide range of exposure conditions.

The ability of PBPK modeling to support cross-species dosimetry is of particular importance for risk assessment. The physiological and biochemical parameters in the model can be changed from those for the test species to those that are appropriate for humans to provide a biologically meaningful animal-to-human extrapolation. The ultimate aim of using PBPK modeling in risk assessment is to provide a measure of dose that better represents the "biologically effective dose"; that is, the dose that causally relates to the toxic outcome. The improved dose metric can then be used in place of traditional dose metrics (such as total amount absorbed) in the appropriate dose-response model to provide a more accurate extrapolation to human exposure conditions. Advantages of applying PBPK modeling in risk assessment have been discussed for both cancer¹¹⁻¹⁵ and noncancer endpoints.¹⁶⁻¹⁸ In addition, the use of PBPK modeling has been recommended to improve route-to-route extrapolation^{19, 20} and the estimation of risk for chemical mixtures.²¹

Simple pharmacokinetic dosimetry approaches have previously been used by regulatory agencies in cancer risk assessment; for example, the USEPA made use of estimates of total metabolized dose in its cancer risk assessments for TCE.^{22, 23} However, the first case where an agency used a full PBPK approach in a published chemical risk assessment was in the USEPA's latest revision of its inhalation risk assessment for methylene chloride.²⁴ In 1989, after a detailed multi-agency evaluation of the available PBPK information and a review by the USEPA Scientific Advisory Board, the USEPA revised the inhalation unit risk and risk-specific air concentrations for methylene chloride in its Integrated Risk Information System (IRIS) database,²⁵ citing the PBPK model of Andersen *et al.*²⁶ The resulting risk estimates were lower than those obtained by the default approach by nearly a factor of ten. Application of the PBPK model for methylene chloride in a cancer risk assessment for occupational exposure has also been described,²⁷⁻²⁹ and a PBPK model was used by the Occupational Safety and Health Administration (OSHA) in their rulemaking for methylene chloride.³⁰ More recently, the EPA has used a PBPK model for vinyl chloride³¹ in its risk assessment for this chemical.³²

Cancer risk assessments using PBPK models have also been proposed for many other chemicals, including TCE.³³⁻³⁶ However, methylene chloride and vinyl chloride represent the only cases to date in which cancer risk assessments conducted by federal regulatory agency have used a PBPK model for estimating cancer risks. Part of the reason for the slow progress of incorporating PBPK modeling in cancer risk assessment is the concern of regulatory agency risk assessors about uncertainties in its implementation.³⁰ These concerns are not without basis; the potential impact of uncertainty in PBPK-based risk assessment has been a subject of some controversy in the literature.^{27-29, 37-42} Nevertheless, the new USEPA cancer guidelines strongly encourage the use of pharmacokinetic and tissue-dose information in both the qualitative hazard identification step and in the quantitative dose-response evaluation for a chemical.

B. Consideration of Carcinogenic Mode of Action

Clearly, pharmacokinetic information describing the relationship between the environmental exposure to a chemical and the target-tissue exposure to a primary carcinogenic metabolite can be an important factor in conducting a chemical-specific risk assessment. However, the incorporation of pharmacokinetic information alone may not provide a more accurate assessment of human risk if mode-of-action information is ignored. This problem can be illustrated by a comparison of inhalation risk estimates for liver cancer from TCE with similar estimates for the known human carcinogen, vinyl chloride. In the case of vinyl chloride, there is strong epidemiological evidence from several occupational cohorts demonstrating that vinyl chloride is a potent human liver carcinogen. Indeed, it was possible to use the results of these epidemiological studies to estimate the cancer potency of vinyl chloride directly, resulting in risk estimates that were very close to those derived from animal bioassays.³¹ In the case of TCE, on the other hand, although there have been a large number of well-conducted studies of chronic occupational exposure to TCE at concentrations similar to or even higher than the exposures to vinyl chloride, none of these studies has provided unequivocal evidence of carcinogenicity, as will be discussed later.

Despite this strong contrast in the human occupational evidence regarding carcinogenic risk, the comparison of pharmacokinetically based risk estimates for TCE and vinyl chloride provided in Table 1 demonstrates the implausible result of very similar risk estimates for these two chemicals when low-dose-linear approaches are used with animal bioassay data. As mentioned above, the most recently published USEPA risk assessments for TCE^{22, 23} make use of pharmacokinetic information. Specifically, estimates of total metabolism from pharmacokinetic studies in rodents and humans, scaled by body surface area, were used as the basis for the potency calculations. The resulting USEPA estimates, based on rodent bioassays, of the human cancer risk from lifetime exposure to 1 $\mu\text{g}/\text{m}^3$ of TCE in air are on the order of 1.3 - 1.7 per million.^{22, 23} A more recent pharmacokinetic risk assessment using a PBPK model arrived at a similar estimate using model-predicted area under the concentration-time curve (AUC) in the blood for the metabolite TCA as the basis for the potency

calculations.³⁵ The current USEPA risk assessment for vinyl chloride also considers pharmacokinetic information, and the potency calculations are based on metabolism estimates obtained with a PBPK model.³¹ The risks of vinyl chloride exposure predicted with this model from rodent inhalation bioassays are shown in Table 1 for comparison with the pharmacokinetically-based risk estimates for TCE.

Table 1: Comparison of Human Liver Cancer Risk Estimates for Inhalation of TCE and Vinyl Chloride Using Animal Bioassay Data and Pharmacokinetic Dose Metrics^a

<u>Source</u>	<u>Basis</u>	<u>Risk per μg/m³ (per million)</u>
TCE:		
USEPA ^{22, 23}	mg metabolized /kg body weight (scaled by body surface area)	1.3 - 1.7
Fisher and Allen ³⁵	AUC ^b for TCA	1.9
Vinyl Chloride:		
Clewell et al. (2001) ³¹	mg metabolized / kg liver weight	0.6 - 2.0

^a Risk of liver cancer in humans for lifetime continuous exposure to 1 microgram per cubic meter (μg/m³) in air, based on animal bioassay data, using pharmacokinetic dose metrics in the linearized multistage model to estimate the 95% upper confidence limit on risk per million individuals

^b Area under the concentration curve

As can be seen in Table 1, the pharmacokinetically-based risk estimates for vinyl chloride are very similar to those calculated for TCE. However, this similarity of the pharmacokinetically-based low-dose-linear risk estimates for liver cancer from these two chemicals stands in stark contrast to the marked difference in human evidence for liver carcinogenicity from occupational exposures. Thus it would appear that pharmacokinetics alone is inadequate to provide a reasonable comparison of the human risk for liver cancer from these two chemicals.

Just as the pharmacokinetics and metabolism of a chemical must be considered in order to obtain a realistic measure of internal tissue exposure to the active form of the chemical, the pharmacodynamics and mechanism of action of the chemical-induced carcinogenic process must also be considered in order to obtain a realistic measure of the expected response to that exposure. 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. In the USEPA's Draft Final Guidelines for Carcinogen Risk Assessment,¹ 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 draft 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, as well as a conceptual framework for mode-of-action evaluation that has also been adopted by IPCS.⁴³ Indeed, one of the more striking features of the proposed USEPA cancer guidelines is the emphasis they place on the consideration, throughout the risk assessment, of a chemical's carcinogenic mode of action.

While mode of action 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 mode of action. 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 -microglobulin 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.⁴⁴ The qualitative implications of mode-of-action information have also been discussed for other carcinogenic processes such as rodent forestomach tumors (for which there is no corresponding organ in the human), in the case of butylated hydroxyanisole⁴⁵, and bladder tumors resulting from irritation by crystalline deposits, in the case of saccharin⁴⁶. An example of an agency 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.⁴⁷ Mode-of-action information has also been used by the USEPA's Risk Assessment Forum to modify the quantitative portion 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 in the case of thyroid follicular cell carcinogenesis.⁴⁸ The quantitative use of chemical-specific pharmacokinetic and mode-of-action information to provide a basis for departing from the default linear dose-response approach has also been suggested for a number of chemicals where carcinogenicity was believed to be secondary to toxicity, including ethylene dichloride,⁴⁹ ethyl acrylate,⁵⁰ and chloroform.⁵¹ The USEPA's recent risk assessment for chloroform⁵² represents the first case of a cancer risk assessment that departs from the standard low-dose-linear dose-response paradigm on the basis of chemical-specific mode-of-action information.

C. Risk Assessment Approach

Figure 1 illustrates the process involved in performing a risk assessment for TCE that considers both pharmacokinetics and mode of action. The process, of course, begins on the right side of the diagram, with the hazard identification studies that provide evidence of the carcinogenicity of TCE and identify the tumors and target tissues of concern. Mechanistic information specific to each of the tumors of concern must then be evaluated to develop a hypothesis for the mode of action in each target tissue. The role of the mode of action is to provide the basis for linking target tissue chemical exposure with the biological or biochemical effects in the target tissue that lead to the observed cancer response. In parallel, the information shown on the left side of the diagram must also be gathered: data on the pharmacokinetics and metabolism of TCE and its metabolites, including each of its putative carcinogenic metabolites (CHL, TCA, DCA, and 1,2-DCVC). The actual risk assessment then proceeds from left to right in Figure 1. The pharmacokinetic and metabolism data are used to build a pharmacokinetic description, such as a PBPK model, in order to provide predictions of the concentration profiles for TCE and its active metabolites in each of the target tissues for cancer, whether associated with exposure to TCE in the animal bioassays or in potential human exposure scenarios. The specific mode of action associated with the production of a particular tumor provides the basis for identifying the dose metric to be used in these calculations. The mode of action plays a fundamental role in driving expectations regarding both the dose-response for tumor incidence within a species and the nature of cross-species scaling. These expectations, in turn, drive decisions concerning the most appropriate risk assessment approach and the assumptions to be made where chemical-specific data are lacking.

TCE Risk Assessment Factors

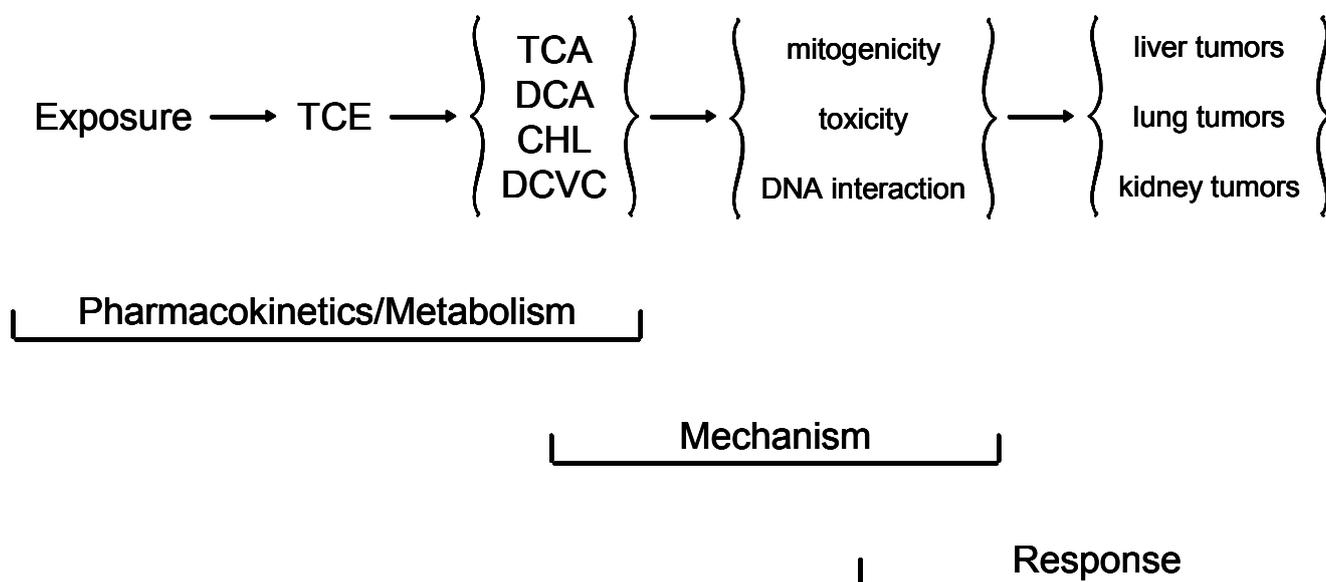


FIGURE 1. Diagram of the factors which must be considered in a cancer risk assessment for trichloroethylene (TCE). Information on the pharmacokinetics of TCE and its active metabolites, trichloroacetic acid (TCA), dichloroacetic acid (DCA), chloral (CHL), and dichlorovinylcysteine (DCVC) is necessary to support target tissue dosimetry. Information on the carcinogenic mode of action in each target tissue is then necessary to determine the appropriate measure of tissue dose, as well as to suggest the nature of the relationship (linear, nonlinear, threshold) between tissue dose and tumor response.

II. EVIDENCE FOR THE CARCINOGENICITY OF TRICHLOROETHYLENE

The first step in the cancer risk assessment for a chemical is the hazard identification, in which the evidence for carcinogenicity, and its relevance to potential human environmental exposures, is evaluated. Under the new USEPA guidelines, not only chronic epidemiological and animal bioassay data are evaluated, but also genotoxicity, pharmacokinetic and mechanistic data. The possibility that the chemical may only be carcinogenic under certain conditions of exposure (route, dose, etc.) can also be considered. Only tumor data for TCE will be examined in this section; pharmacokinetic, genotoxicity and mechanistic data will be examined in subsequent sections.

Although a large number of studies have demonstrated tumors in animals following exposure to TCE,⁵³ the relevance of these animal results to the question of the human carcinogenicity of TCE has frequently been questioned.⁵⁴⁻⁵⁶ The American Conference of Government Industrial Hygienists now classifies TCE into Group A5, not suspected as a human carcinogen, based on a well-conducted,

negative epidemiological study performed in an aircraft maintenance facility at Hill Air Force Base.^{57, 58} The International Agency for Research on Cancer (IARC), on the other hand, assigned TCE to Group 2A, probably carcinogenic to humans, based on their assessment of sufficient data in animals and limited data in humans.⁵⁹ The human evidence considered significant by IARC was the consistency of an association of TCE exposure with slightly increased incidences of liver/biliary tract tumors and non-Hodgkins lymphoma in studies of three cohorts in the U.S.,⁵⁷ Sweden,⁶⁰ and Finland,⁶¹ despite the fact that all three studies were characterized as negative by the original investigators because the increases were not statistically significant. Since that time, several additional well-conducted studies of occupational exposure to TCE in the U.S.⁶²⁻⁶⁴ and Denmark^{65, 66} have been reported; these studies were also characterized by the authors as failing to support an association between TCE exposure and increased cancer mortality.

In contrast to these essentially negative results, a study in what was formerly East Germany⁶⁷ found that workers repeatedly exposed to very high airborne concentrations of TCE (estimated to have sometimes been on the order of 1000 ppm) showed a highly significant incidence (7/169) and mortality (2/169) from kidney cancer as compared to controls (0/190 and 0/190). Moreover, the tumors seen in these workers were reported to be similar, both in locality and in histology, to the tumors observed in rats exposed to TCE. However, this study has been criticized because it was based on a previously recognized cluster of kidney cancer cases.⁶⁸ Nevertheless, two subsequent case control studies of renal cell cancer patients have found an association with a history of occupational TCE exposure.^{69, 70} Further evidence for the association of TCE with the occurrence of these kidney lesions is the observation of detoxification products of a potentially reactive and mutagenic TCE metabolite in the urine of human workers exposed to TCE by inhalation. Because the development of the renal cell carcinoma observed in these subjects has been associated with mutations in the von-Hippel-Lindau (VHL) tumor suppressor gene, two independent studies were performed to investigate whether TCE exposure could result in specific mutations of the VHL gene. Unfortunately, the results of these two studies are contradictory, with one study in Germany⁷¹ finding a unique mutation pattern, involving multiple mutations and loss of heterozygosity, associated with TCE exposure, while the other study in Switzerland⁷² revealed no unique mutation pattern. Despite the equivocal nature of the human evidence, the correspondence between rodents and humans for these rare kidney tumors, similar to the case of liver angiosarcoma from vinyl chloride, justifies increased concern for kidney lesions in a revised cancer risk assessment for TCE.

The animal cancer bioassays that have been conducted for TCE are summarized in Table 2 (at end of chapter). By far the most common carcinogenic outcomes associated with TCE exposure in animal studies are increased liver and lung tumors in several strains and both sexes of mice.⁷³⁻⁷⁸ Statistically increased tumor outcomes observed in only a single study include malignant lymphoma in HAN:NMRI mice exposed by inhalation,⁷⁹ renal tubular cell adenoma and carcinoma in male F344 rats exposed by oral gavage,⁷⁴ and

benign testicular (Leydig cell) tumors in Sprague-Dawley rats exposed by inhalation.^{77, 78} Of these less commonly found outcomes, the kidney tumors have raised the greatest concern since they are not observed in control animals. Bioassay results for metabolites of TCE are also shown in Table 2. Hepatocellular carcinoma is induced by several of the oxidative metabolites of TCE: CHL, TCA, and DCA.^{3, 80-84}

With regard to conducting a quantitative risk assessment for the carcinogenicity of TCE, none of the human studies described above provide the necessary combination of a statistically significant association and an adequate characterization of exposure. Therefore, the animal bioassays provide the only reliable basis for a quantitative risk estimate. The USEPA's published quantitative cancer risk estimates for TCE have been based on animal bioassays, specifically liver and lung tumors in mice. In 1983, the USEPA calculated unit risks for TCE of $4.1 \times 10^{-6} (\mu\text{g}/\text{m}^3)^{-1}$ for inhalation and $0.54 \times 10^{-6} (\mu\text{g}/\text{L})^{-1}$ for drinking water using data on the incidence of liver tumors in male B6C3F1 mice given TCE in an oil vehicle by gavage;^{73, 85} the linearized multistage model⁸⁶ was used with a calculation of absorbed TCE dose scaled by body surface area to obtain these estimates.⁸⁷ In 1985, lower unit risks of $1.3 \times 10^{-6} (\mu\text{g}/\text{m}^3)^{-1}$ for inhalation and $0.32 \times 10^{-6} (\mu\text{g}/\text{L})^{-1}$ for drinking water were recalculated on the basis of the same oral gavage bioassays using the results of pharmacokinetic studies^{54, 88, 89} to calculate total metabolized dose in both animals and humans, rather than absorbed dose, although the body surface area adjustment was still applied to obtain the human equivalent dose.²² In 1987, USEPA calculated a new inhalation unit risk of $1.7 \times 10^{-6} (\mu\text{g}/\text{m}^3)^{-1}$ based on the incidence of mouse lung and liver tumors in inhalation bioassays,⁷⁶⁻⁷⁸ again using a calculation of metabolized dose and the body surface area adjustment.²³

III. PHARMACOKINETICS AND METABOLISM

For ease of presentation, information on the pharmacokinetics and metabolism of TCE and its metabolites will be described before discussing mode-of-action considerations. However, it should be noted that the two areas must actually be considered together. Pharmacokinetic and metabolic data provide insights into the possible carcinogenic modes of action and the potential roles of parent chemical, metabolites, and the metabolic processes themselves. Mechanistic data, on the other hand, may identify minor metabolites that are crucial to the risk assessment, that might otherwise have been overlooked due to their low production relative to other metabolites. The following discussion summarizes the experimental evidence for the pharmacokinetics and metabolism of TCE and its major metabolites, TCA and trichloroethanol (TCOH), as well as the minor metabolites, DCA, CHL, and 1,2-DCVC, that have been suggested to play an important role in the carcinogenicity of TCE.

TCE is a volatile, lipophilic chemical that distributes readily throughout all tissues, including the brain, but partitions preferentially into fat tissue. In contrast, its major metabolite TCA is a water-soluble chemical that preferentially distributes into the plasma and richly-perfused organs, and is found only in

relatively lower concentrations in the muscle and fat. The properties of TCOH are somewhat intermediate between the other two compounds.⁹⁰ Clearance of TCE occurs both by exhalation and by metabolism.⁹¹ A schematic of the metabolic pathways for TCE is shown in Figure 2, with the major oxidative pathway, which takes place primarily (but not exclusively) in the liver, shown to the right of the diagram and the minor glutathione-dependent pathway, which involves several locations including the liver and kidney, shown to the left.

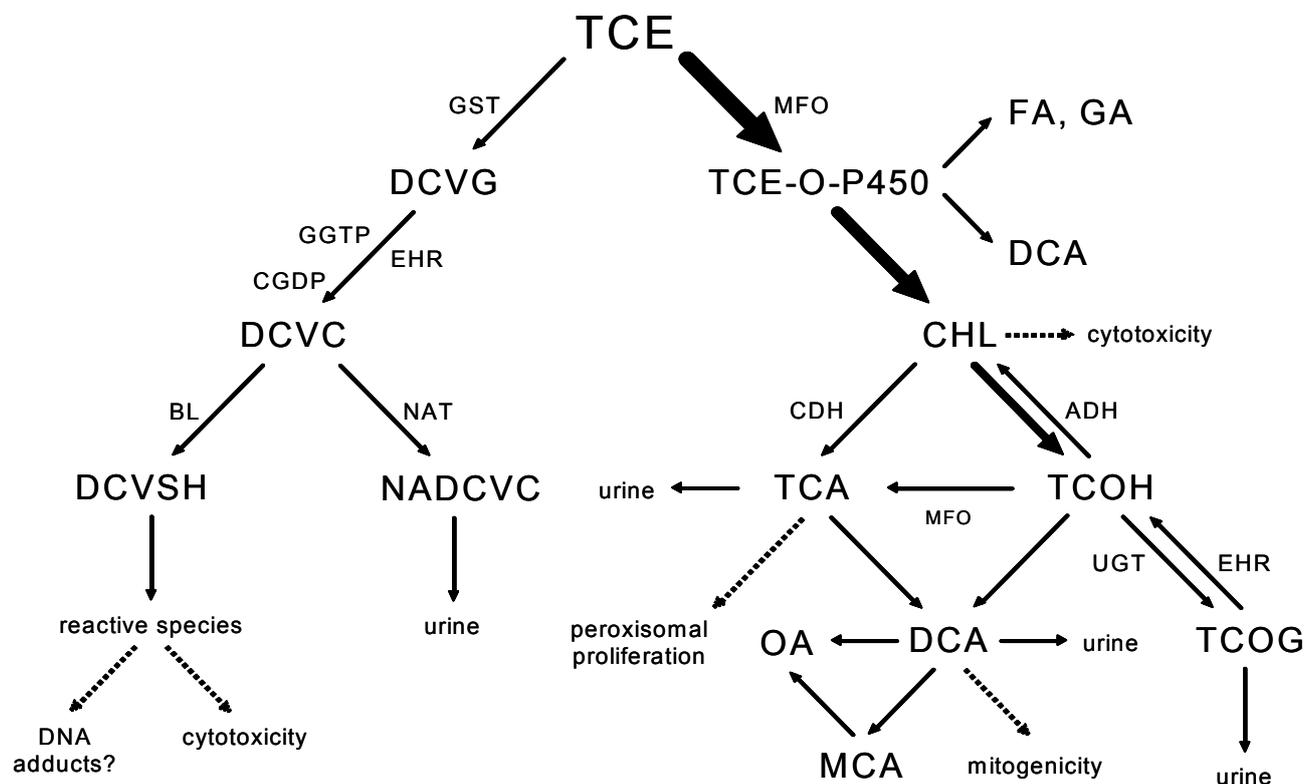


FIGURE 2. Metabolism of TCE. Abbreviations not given in text: (right pathway) CDH: chloral dehydrogenase (aldehyde oxidase); EHR: enterohepatic recirculation; FA: formic acid; GA: glyoxylic acid; OA: oxalic acid; TCE-O-P450: oxygenated TCE-Cytochrome P450 transition state complex; TCOG: TCOH glucuronide; UGT: UDP glucuronosyl transferase; (left pathway) BL: cysteine conjugate β -lyase; CGDP: cysteinyl-glycine dipeptidase; DCVG: dichlorovinyl glutathione; DCVSH: dichlorovinyl mercaptan; GGTP: γ -glutamyl transpeptidase; NADCVC: N-acetyl dichlorovinylcysteine; NAT: N-acetyl transferase.

A. Oxidative Metabolism

TCE is much more extensively metabolized in the mouse than in the rat, whether TCE is administered orally⁵⁴ or by inhalation.⁹² The primary route of metabolism for TCE, shown on the right side of the diagram in Figure 2, is oxidation via the microsomal mixed function oxidase (MFO) system, now referred to as Cytochrome P450, or CYP.⁹³⁻⁹⁹ A minor pathway for TCE metabolism, involving conjugation with glutathione (GSH) by glutathione transferase (GST),

has also been observed;¹⁰⁰ this pathway, which is shown on the left side of the diagram, will be described in the next section.

The principal oxidative metabolite formed *in vitro* is CHL (in its hydrated form, chloral hydrate),^{93, 94, 97} which is subsequently reduced to TCOH in the cytosol or oxidized to TCA in either the cytosol or mitochondria.⁹³ CHL is not stable *in vivo*, and circulating concentrations are relatively low compared to its breakdown products, TCA and TCOH.⁹⁰ Within a few hours of the administration of 50 mg/kg chloral hydrate to a child, the rapid initial clearance of CHL was essentially complete, with subsequent blood concentrations paralleling the time-course for TCOH but approximately an order of magnitude lower, suggesting a continuing production of CHL from TCOH.¹⁰¹

The principle circulating metabolite of TCE in the blood is TCA, which accumulates in the body due to protein binding¹⁰² and slow excretion,¹⁰³ while TCOH is readily excreted.^{104, 105} TCA appears to be derived both directly from CHL, and indirectly, from TCOH.¹⁰⁴⁻¹⁰⁶

Based on both *in vitro* and *in vivo* studies, the metabolism of TCE has been suggested to consist of oxidation of TCE to CHL by the MFO system, followed by either oxidation of CHL to TCA by an aldehyde oxidase, also known as chloral dehydrogenase (CDH), or reduction to TCOH by alcohol dehydrogenase (ADH) with subsequent glucuronidation. Oxidation of TCOH to TCA by the MFO system has also been proposed. {Muller, 1975 #291}

Four different isozymes of Cytochrome P450 have been found to play a role in the oxidative metabolism of TCE in rodents: 1A1/2, 2B1/2, 2C11/6, and 2E1; CYP 2E1 appears to have the highest affinity for TCE, although the other isozymes can become important at higher TCE concentrations.⁹⁷ In humans, CYP 2E1 was found to account for more than 60% of TCE metabolism *in vitro*, with smaller contributions from 1A1, 1A2, and 3A4; variation in CYP 2E1 activity across tissues from 23 humans was less than 10-fold¹⁰⁷. Sex, pregnancy, and age related differences in metabolism can also result from normal variations in CYP 2E1 content.⁹⁸ Moreover, increased metabolic capacity can result from the induction CYP 1A1/2 (e.g., by aromatics), 2B1 (e.g., by phenobarbital), or 2E1 (e.g., by ethanol).^{6, 96} However, due to the high affinity of CYP 2E1 metabolism, the clearance of TCE is typically flow-limited at low TCE concentrations; therefore, variations in enzyme activity may not result in significant changes in TCE metabolism for human exposure conditions.¹⁰⁸

Inhalation exposures of human volunteers to TCE concentrations from 27 to 201 ppm showed no evidence of metabolic saturation or of a change in the proportion of TCA to TCOH.¹⁰⁹ Saturation of TCE metabolism has been observed in mice, rats, and dogs.^{110, 111} The relative proportion of the major metabolites does not appear to be a strong function of dose; however, repeated dosing does appear to increase the production of TCA at the expense of TCOH,¹¹² and the relative production of CO₂ increases with increasing dose in mice.⁶ The production of TCA in humans appears to be highly variable, and generally

somewhat higher than in other animals. For example, in one study the production of TCA from chloral hydrate in different individuals varied from 5 to 47%.¹⁰⁴

Human *in vivo* studies with TCE⁹⁸ have identified the major urinary metabolites to be TCOH (50% of the administered TCE dose), primarily as the glucuronide, and TCA (19%); monochloroacetic acid (MCA) was also identified as a minor metabolite (4%) in these studies. Another minor metabolite, N-(hydroxyacetyl)-aminoethanol, has also been identified in human (and rodent) urine following TCE exposure, and TCE-derived oxalic acid has been detected in the rodent.¹¹³ A study of TCE metabolism in nonhuman primates¹¹⁴ found that TCA was partially excreted as the glucuronide, particularly at longer times after dosing; the authors suggest that since the detection of TCA glucuronide had not been reported previously, TCA excretion may have been under-reported in earlier studies (such as the human study cited above). The glucuronidation of TCA is supported by the observation that TCA is excreted in the bile of rats and mice.¹¹² Urinary excretion represents the major route of elimination of the metabolites, with fecal excretion, in the form of TCOH glucuronide, accounting for less than 5% of the total.¹¹⁴ The low fecal excretion is apparently associated to some extent with enterohepatic recirculation of TCOH (i.e., biliary excretion of the glucuronide, followed by hydrolysis and reabsorption of TCOH), which has also been suggested to occur in rats.¹¹⁵

DCA has been reported to be a minor urinary metabolite of TCE (less than 1%) in both rats and mice,^{6, 112, 113, 116} while MCA has been reported to be present at less than 0.1%.¹¹² DCA has also been reported in the urine of mice dosed with TCA.¹¹⁷ Both MCA and DCA were detected in the urine of an acute intoxication patient who had ingested approximately 70 mL of TCE,¹¹⁸ and DCA was reported to reach maximum concentrations on the order of 0.1 μM in subjects exposed to 100 ppm TCE for 4 hours.¹¹⁹ A recent *in vitro* study with mouse and rat liver tissues concluded that unlike most other chlorinated compounds, which are metabolized primarily by the microsomal enzymes of the MFO system, DCA degradation appears to occur primarily in the cytosol in a process that requires GSH.¹²⁰ The enzyme responsible for this process has been identified as glutathione transferase zeta, and the principal product is glyoxylic acid.¹²¹ The kinetics of DCA have been extensively studied in the human due to its clinical use.¹²²⁻¹²⁵ The peak concentration and AUC of DCA in the plasma after intravenous administration are linear up to approximately 20 mg/kg, but above 20 to 30 mg/kg some individuals display evidence of saturation of metabolism.¹²³ The apparent volume of distribution and half-life for DCA are 0.3 L/kg (range: 0.09 to 0.60) and 1.05 hr (range: 0.25 to 1.87), respectively.¹²⁴ Significantly, the clearance in humans appears to be much more rapid than would be expected from allometric scaling of animal data; in a comparative study,¹²² the half-lives in rats, dogs, and humans were 2.1B4.4 hrs, 17.1B24.6 hrs, and 0.33B0.6 hrs, respectively.

It has recently been reported that under some conditions DCA can be artificially produced from TCA in blood samples (but not serum, plasma, or urine

samples) as an artifact of the analysis. Specifically, if acidification of the blood sample is performed rapidly after collection of the sample, before the iron in hemoglobin has been oxidized by exposure to air, DCA can be generated from TCA present in the sample.¹²⁶ Subsequent to this discovery, a study¹²⁷ identified additional sources of artifactual formation of DCA from TCA during analysis. Using analytical methods that avoided artifactual DCA formation, these investigators were unable to identify DCA in blood (with a 1.9 μM detection limit) after either intravenous dosing with 100 mg/kg TCE or oral dosing with 1000 mg/kg TCE. Thus this artifact may have severely compromised the data discussed in this section on DCA blood concentrations following administration of TCA or TCE.^{110, 128, 129} The artifactual formation of DCA from TCA *ex vivo* calls into serious question the evidence, discussed in the next paragraph, that DCA can be produced *in vivo* from TCA, but does not necessarily invalidate the conclusion that DCA may be produced *in vivo* from the metabolism of TCE.

Studies in rats have suggested that DCA may be produced from TCA.¹²⁸ It has also been reported that DCA was produced in a roughly linear fashion from perchloroethylene,^{130, 131} at levels consistent with production from TCA, the principle metabolite of perchloroethylene. However, an analysis of data on the dose-response and elimination kinetics of DCA formed from TCE lead to the conclusion that another source of DCA was required in addition to TCA, but that the data were inconsistent with the second source being the initial oxidation step;¹²⁹ instead, the production of DCA from TCOH was hypothesized. A metabolic study of TCA and DCA in rats and mice¹²⁸ found that DCA was more rapidly metabolized than TCA: more than 50% of the administered TCA from an oral dose was excreted unchanged in the urine as compared to only 2% of administered DCA. Plasma concentration-time curves for TCA were similar in mice and rats, while those for DCA were greater in rats than in mice, both when DCA was administered and when it was derived from TCA.¹²⁸

There is evidence that exposure to high concentrations of DCA inhibits its own metabolism by glutathione transferase zeta¹³². In studies with human volunteers,^{124, 125} the excretion half-life for DCA increased 2- to 6-fold following repeated intravenous doses of DCA on the order of 10 to 50 mg/kg. Inhibition was only slowly reversible, taking from one week to greater than three months to resolve. In the rat and mouse, exposure to 0.2 or 2 g/L of DCA in drinking water for 14 days caused a significant increase in the blood concentration-time profiles of a subsequent dose of DCA^{133, 134}. The half-maximal inhibition concentration in mouse, rat and human are similar, on the order of 40 μM .¹³² Therefore, while DCA self-inhibition can be observed at high doses of DCA, it is not of concern for human environmental exposures to DCA or its chemical progenitors.

B. Metabolism in the lung

As with most chemicals, the preponderance of the metabolic clearance of TCE appears to take place in the liver. It has been demonstrated, however, in studies with an isolated ventilated perfused lung preparation,¹³⁵ that the male

F344 rat lung also possesses a limited oxidative metabolic capability for TCE. While the affinity (K_m) for the lung metabolism observed in that study was similar to the affinity observed in the liver, the capacity (V_{max}) of the lung metabolism was less than 1% of the capacity of the liver. These results suggest that lung metabolism is not an important contributor to total *in vivo* metabolism in the rat, and that the rat lung does not possess a significant first-pass (pre-systemic) clearance capability for inhaled TCE. However, these results do not rule out the possibility that metabolism in the lung could produce sufficient local exposure to metabolites to produce toxicity and/or carcinogenicity.

C. Conjugative Metabolism

In mouse, rat, and human, a small proportion of TCE appears to be metabolized by enzymatic conjugation with GSH, principally by GST in the liver,^{136, 137} followed by further metabolism in the kidney to the cysteine conjugate, 1,2-DCVC.¹³⁸ The GST metabolic pathway is shown on the left side of Figure 2. Delivery of 1,2-DCVC to the kidney may also be mediated by enterohepatic recirculation, in which glutathione conjugate excreted in the bile is converted by gut bacteria to the cysteine conjugate, which is then reabsorbed.¹³⁹ The GSH conjugate has been identified both *in vitro*, with rat liver microsomes, and in the bile of rats given 2.2 g/kg TCE in corn oil.¹⁰⁰ The TCE glutathione conjugate has also been observed in the blood of human subjects exposed to TCE at 100 ppm for 4 hours.¹⁴⁰ The cysteine conjugate has also been identified in the urine of animals dosed with TCE.¹⁴¹

The bioactivation of 1,2-DCVC to a reactive and mutagenic thioacetylating intermediate is performed by cysteine conjugate β -lyase in the kidney.¹⁴² Although similar β -lyase activity has been measured in the kidney and liver, the two enzymes are distinct.¹⁴³ Detoxification and clearance of 1,2-DCVC takes place by urinary excretion of the N-acetyl derivative.^{100, 144} In a study with perchloroethylene,¹⁴⁵ it was determined that the excretion of the N-acetyl derivative was dose-related (a higher fraction of N-acetyl derivative was excreted at doses where the oxidative pathway was saturated), and was significantly greater in the rat than in the mouse. However, measurements of acid-labile protein adducts associated with 1,2-DCVC suggest that the activation of 1,2-DCVC in the kidney may be as much as 12-fold greater in mice than in rats, and that the kidney tissue exposure to 1,2-DCVC-derived reactive species from oral dosing with TCE may be twice as great in the mouse as in the rat.^{146, 147}

The activity of β -lyase has been measured in the liver and kidney of both humans and rats. One research group has reported a specific activity in human kidney on the order of 2.0 to 3.6 nmol/min/mg cytosol,^{148, 149} as compared to 6.45 to 7.6 nmol/min/mg cytosol in the rat.¹⁴³ Another research group, however, has reported a maximum velocity (V_{max}) of only 0.8 nmol/min/mg cytosol in the human, with an affinity (K_m) of 0.29 mM, compared to V_{max} = 7.5 nmol/min/mg cytosol and K_m = 1.6 mM in rat kidney cytosol in the same study.¹⁵⁰ Data for perchloroethylene on the relative activity of liver cytosolic glutathione transferase

and kidney cytosolic cysteine conjugate β -lyase suggest that the human activity of both enzymes is roughly 10-fold lower than the rat.¹⁴⁵ On the other hand, the specific activity of N-acetyl-transferase in kidney cytosol appears to be very similar across species: 0.41 nmol/min/mg cytosol in the human, as compared to 0.35-0.61 in the rat and 0.94 in the mouse.¹⁵¹

The fact that N-acetyl-DCVC has been identified in the urine of humans exposed to TCE both occupationally¹⁵¹ and in controlled exposures,¹⁵² indicates that exposure of the kidney to DCVC does occur in the human. In the occupational study,¹⁵¹ the concentrations of total N-acetyl-DCVC (both the 1,2 and 2,2 isomers) in the workers' urine was about one third of that measured in rats dosed orally with 50 mg/kg TCE. The ratio of total N-acetyl-DCVC to TCA in the workers' urine ranged from 0.03 to 0.3, while in rats it ranged from 0.025 to 0.045, and in mice from 0.014 to 0.065. However, more recent data,¹⁵² obtained in controlled inhalation studies with both rats and human subjects, suggest that relative urinary excretion of total N-acetyl-DCVC metabolites is actually somewhat lower in humans than in rats.

IV. MODE OF ACTION

The discussion in this section will focus on the three animal target tissues of greatest concern: liver, lung, and kidney. In each tissue, evidence for the possible mode (or modes) of action of TCE will be explored in order to lay the basis for (1) the identification of elements that must be included in the pharmacokinetic model, (2) the selection of the most appropriate dose metrics for exposure of that tissue, and (3) the determination of the approach to be used for the dose-response assessment for that tissue. In particular, the selection of the mode of action plays a major role in determining the basis for cross-species extrapolation and the method to be used for low-dose extrapolation (linear or MOE).

In general, much more information has been collected relevant to the liver carcinogenicity of TCE than for the lung and kidney tumors, but uncertainties remain in all three cases. As mentioned earlier, risk assessments must be conducted in the face of such uncertainty. Science continually progresses and risk assessors can only hope to provide an interim best estimate based on a snapshot of the state of the science at that point in time. It will always be true that the use of ongoing research when it became available could improve and perhaps radically change that risk assessment. To the extent possible, the risk assessor must therefore attempt to estimate the potential impact of existing uncertainties on the current assessment.

A. Liver

As understanding of carcinogenic processes in the rodent liver and of the effects of TCE has evolved, a number of possible mechanisms for the liver carcinogenicity of TCE have been suggested. These alternative mechanisms can be loosely characterized as involving genotoxicity, cytotoxicity, and promotion.

More recently, as evidence became available that several of the metabolites of TCE also induce liver tumors (CHL, TCA and DCA), attention has focused on the possible role of these metabolites in the induction of liver cancer by TCE. In particular, it has been suggested that the production of TCA and DCA from TCE under bioassay exposure conditions is adequate to account for the incidence of liver tumors in the TCE bioassays.^{4, 110}

1. Genotoxicity

At one time, covalent binding to DNA was considered to be a common feature of all chemical carcinogenicity, and the mutagenicity, and hence the carcinogenicity, of chlorinated ethylenes was expected to correlate with the reactivity of their epoxide intermediates, which was taken as a predictor of the potential for DNA adduct formation.¹⁵³ In the case of TCE, it was believed that rapid conversion of the presumed epoxide intermediate to chloral was consistent with a low mutagenic and carcinogenic activity.¹⁵³ Investigators have repeatedly emphasized the lack of mutagenic or DNA-binding evidence for a direct genotoxic effect of TCE in the liver.^{88, 154} Nevertheless, in an *in vivo* study, a low rate of covalent binding to DNA was taken as an indication that TCE is possibly a weak initiator, and the production of adducts was imputed to the GST pathway.¹⁵⁵

There is a similar sparsity of direct evidence for genotoxic potential for the major circulating metabolites of TCE. CHL has been found to be genotoxic in a number of *in vitro* studies,¹⁵⁶ but is present only at relatively low concentrations, on the order of 1 mg/L or less, after TCE doses associated with liver tumors in bioassays.⁵⁴ The generation of free radicals from CYP2E1 oxidation of CHL, TCA, and TCOH *in vitro* has been shown to produce lipid peroxidation to carcinogenic species;¹⁵⁶ the relative production of free radicals from the three compounds, CHL \square TCA \square TCOH is consistent with the source of the free radicals being the oxidation of TCA in every case. However, *ras* mutational frequencies in TCA-induced tumors are essentially identical to those in spontaneous tumors, suggesting that TCA treatment does not lead to genotoxicity *in vivo*.¹⁵⁷ Evidence of possible genotoxicity in the form of single strand breaks in DNA,^{158, 159} and increased DNA synthesis as evidenced by thymidine incorporation,¹⁶⁰ has been observed for TCA and DCA. However, other investigators have been unable to reproduce the reported DNA strand break effects,^{161, 162} and the thymidine incorporation data are susceptible to a variety of explanations, including mitogenicity, cytotoxicity,⁹¹ and the potential for direct effects of DCA and TCA on the thymidine pool.¹⁶⁰ No increases in oxidative DNA damage were seen with TCA or DCA at carcinogenic drinking water concentrations in mice, whereas brominated analogues administered at the same concentrations produced measurable, dose-related increases in 8-hydroxydeoxyguanosine.¹⁶³

Two studies of *in vivo ras* mutation spectral data for DCA^{157, 164} reported a shift in the frequency with which specific *ras* mutations were detected in DCA-induced tumors compared to spontaneous tumors. These data were interpreted as evidence of either initiation activity or selective promotion of specific cell subpopulations. There has also been a report of evidence for the mutagenicity of

DCA,¹⁶⁵ although a more recent *in vitro* mutagenicity study on DCA was entirely negative,¹⁶⁶ and an *in vivo* genotoxicity study¹⁶⁷ suggested that DCA might be an extremely weak inducer of genetic damage in mice at a drinking water concentration of 3.5 g/L, but not at lower concentrations. Since DCA has been shown to produce tumors in mice at a drinking water concentration of 0.5 g/L,⁸¹ it is unlikely genotoxicity is the primary mechanism of DCA carcinogenicity.

Overall, the evidence relating to the genotoxicity of TCE and its metabolites is inadequate to support a primarily genotoxic mechanism for the observed liver carcinogenicity of TCE, but it does raise some concerns relevant to the question of residual low-dose risks below the threshold for other potential modes of action.

2. Cytotoxicity

As an explanation for the observed carcinogenicity of TCE in the mouse liver, but not in the rat liver, an epigenetic mechanism was proposed⁸⁸ in which the higher rates of metabolism observed in the mouse would produce recurrent toxicity, and a consequent reparative hyperplasia, resulting from the binding of an unspecified reactive intermediate with cellular macromolecules; consistent with this mechanism, toxicity was observed at high doses in mice but not rats, and there was little evidence of DNA binding. A subsequent pharmacokinetic study¹⁶⁸ demonstrated that the relationship between the acute hepatotoxicity of TCE and the total production of urinary metabolites was linear, and it was suggested that this result was consistent with the hypothesis that the toxicity was produced by a reactive metabolite. Citing the proposed cytotoxic mechanism for the carcinogenicity of TCE,⁸⁸ it was recommended that total metabolism be used as a pharmacokinetic dose measure for the carcinogenicity of TCE in place of the administered dose. Largely in response to this study, the EPA recalculated the carcinogenic potency of TCE, based on the NCI⁷³ and NTP⁸⁵ gavage studies, using pharmacokinetic data on total metabolism of TCE in the mouse and human;²² the pharmacokinetically derived potencies were almost identical to the potencies calculated previously using administered dose.⁸⁷ Total metabolism was also used as the basis for the later derivation of a revised inhalation carcinogenic potency of TCE based on inhalation bioassay data.²³

A cytotoxic mechanism has recently been proposed as the basis for the liver carcinogenicity of TCE.¹⁶⁹ Recurrent toxicity, leading to reparative hyperplasia, has also been suggested to be a likely contributing factor to the carcinogenicity of other halogenated hydrocarbons,^{170, 171} particularly in the case of chloroform, where toxicity was believed to result from binding of reactive metabolites to critical cellular macromolecules.^{17, 51, 172} However, studies on other chemicals that are similar to TCE do not support the possibility of a carcinogenic mechanism based solely on binding to cellular macromolecules. For example, the binding in mice from two noncarcinogens, 1,1-dichloroethane and 1,1,1-trichloroethane, was 2- to 18-fold greater than from two closely-related carcinogens, 1,2-dichloroethane and 1,1,2-trichloroethane.¹⁷³

There is a considerable body of evidence supporting the production of unidentified reactive species in the initial metabolism of TCE,^{95, 174-177} similar to that observed with ethanol.¹⁷⁸ Moreover, repeated exposure to TCE has been associated with cytotoxicity in mice, but not rats, when the TCE was administered in a corn oil gavage,^{88, 179} although not when an aqueous vehicle was used.¹⁷⁹ However, there has been no evidence demonstrating a link between the liver toxicity of TCE and a sustained, generalized reparative hyperplasia under bioassay conditions that could plausibly lead to increased tumor incidence, such as has been shown with chloroform.¹⁸⁰⁻¹⁸³ Indeed, there is molecular biological evidence that chloroform and TCE have quite different modes of action. An evaluation of *in vivo ras* mutation frequency data for several chemicals¹⁸⁴ concluded that the presence of H-*ras* proto-oncogene activation in only a small fraction (21%) of chloroform-induced hepatocellular neoplasms, as opposed to its activation in more than 60% of control animal tumors, was supportive of a nongenotoxic mode of action. In contrast, H-*ras* activation in TCE- and DCA-induced tumors were on the order of 60%, similar to spontaneous tumors.

Glycogen accumulation, vacuolization, focal necrosis and reparative hyperplasia have been reported at drinking-water bioassay concentrations with DCA, but not TCA, in mice.^{3, 83, 160} However, a recent study with rats demonstrated similar tumor potency for DCA in the rat compared to the mouse, but with no evidence of necrosis or increased cell proliferation,¹⁸⁵ effectively ruling out a cytotoxic mode of action for this chemical. Thus while cytotoxicity may contribute to the incidence of liver tumors in some TCE bioassays, particularly those performed by gavage in corn oil, it appears highly unlikely that cytotoxicity and reparative hyperplasia are the principal mode of action underlying the production of liver tumors from TCE.

3. Promotion

TCE administered by gavage at 1000 mg/kg daily for 7 weeks acted as a weak promoter of rat liver altered foci in one initiation/promotion study using diethylnitrosamine as the initiator.¹⁸⁶ The TCE metabolites, TCA and DCA have also been shown to promote foci and tumors initiated by N-methyl-N-nitrosourea.¹⁸⁷ A promotional mechanism has sometimes been proposed as an explanation for the fact that the liver carcinogenicity of TCE has been observed in mice but not rats, based on the observation that a much greater peroxisomal proliferation response is observed in mice, as compared to rats, exposed to TCE.^{54, 154, 188-190} With regard to the major circulating metabolites of TCE, peroxisome proliferation is also produced by TCA and, with less potency, by DCA; the mouse again being the more sensitive species.¹⁹¹ In fact, based on structure-activity considerations, it is likely that the metabolite TCA is responsible for the peroxisomal activity of TCE: most of the known peroxisome proliferators are acids or are metabolized to acids.^{192, 193} This conclusion is supported by *in vitro* studies showing that TCA, but not TCE, activated cloned peroxisome proliferators activated receptor alpha (PPAR- α).¹⁹⁴

A number of peroxisome proliferators, including DCA,¹⁹⁵ have been used successfully in humans for the treatment of hyperlipidemia, but their use has been criticized due to the evidence that they induce liver cancer in rodents.¹⁹⁶ In general, the carcinogenic peroxisomal proliferators have not been found to be mutagenic or directly genotoxic,⁵⁵ and it is widely believed that they act through one or more epigenetic mechanisms: (a) oxidative stress, leading to oxidative damage of DNA,¹⁹⁶ (b) generalized cell proliferation,¹⁹⁷ (c) focal cell proliferation,¹⁹⁸ and (d) interaction with steroid hormones.¹⁹⁹ That these effects are secondary to activation of PPAR- α has been demonstrated by the lack of liver carcinogenicity and related early events in PPAR- α knockout mice following exposures to a peroxisome proliferator.^{200, 201}

It has been observed that some peroxisomal proliferators are much more potent carcinogens than others, even though they produce similar levels of peroxisomal proliferation and enzyme induction.²⁰² The key difference affecting potency appears to lie in the ability to induce sustained cell proliferation in a subpopulation of cells.^{197, 198, 203} A rationale for the appearance of focal, as opposed to general, hyperplasia from exposure to peroxisomal proliferators¹⁹⁸ is that normal cells apparently cease to respond to the chemical's mitogenic signal after a short period of time, but that some altered cells (i.e., the preneoplastic cells) are able to maintain a sustained proliferative response leading eventually to tumor formation. A study of proto-oncogene activation in tumors produced by TCE, perchloroethylene, and DCA¹⁶⁴ concluded that exposure to these chemicals provides a selective growth advantage to spontaneously occurring mutations. Interestingly, studies in PPAR- α knockout mice demonstrated a lack of altered cell cycling for treatment with TCE or TCA, whereas DCA induced similar alterations in both the control and knockout mice.²⁰⁴

An attractive hypothesis developed to explain the promotional effects of phenobarbital,^{205, 206} but which is consistent with the observed effects of many peroxisome proliferators, suggests that the mitogenic signal from a chemical leads to an initial burst of organ-wide proliferation that in turn engenders the production of an opposing cytostatic signal (e.g., TGF- β) from the surrounding epithelial tissues and down-regulation of the cellular receptors for growth factors. The prolonged maintenance of this dynamically unstable situation by chronic exposure to the mitogen provides a selective pressure that favors the development of a subpopulation of altered cells that have lost the ability to respond to the cytostatic signal. These altered cells have therefore escaped from the inhibition and can respond to the mitogenic signal, greatly increasing their rate of growth as compared to normal cells, thus enhancing the probability of cell division errors leading to cell transformation. A suppression escape mechanism has been proposed for the liver carcinogenicity of dioxin.^{207, 208} This mechanism is consistent with the observed promotion of basophilic foci by peroxisome proliferators, including TCA.⁸³

Although there is still considerable uncertainty regarding the mechanism of carcinogenicity of peroxisomal proliferators,²⁰⁹ the conclusion often reached by those who have evaluated this mechanism has been that the liver tumorigenicity

is essentially irrelevant to humans.^{192, 193} This conclusion is based primarily on the observation that humans (and nonhuman primates) appear to be much less sensitive to hepatic peroxisomal proliferation than rodents.²¹⁰⁻²¹³ However, attempting a quantitative comparison between the rodent and the human on this basis is problematic, since it assumes that the cross-species sensitivity for the peroxisomal response can be assumed to apply to the carcinogenic response as well. The adequacy of this assumption depends on the mechanism of tumorigenicity and its relationship to the peroxisomal response. If, for example, the tumorigenicity were thought to be produced by oxidative stress secondary to increased peroxisomal oxidation, it might seem reasonable that the much lower human peroxisomal response implies a much lower relative cancer risk compared to the rodent. However, a recent study has demonstrated that oxidative damage from several haloacetates was independent of peroxisome proliferation.¹⁶³

Similarly, if mitogenesis is the underlying mechanism of carcinogenicity, the relationship of the tumor responses across species cannot be based on the peroxisomal response unless it can be demonstrated that the dose-response for the two effects, peroxisomal and mitogenic, is directly related. In fact, there does appear to be some interrelationship between the peroxisomal proliferation and mitogenicity produced by these chemicals. Avidity of binding to a cytoplasmic transport protein, liver fatty acid binding protein (L-FABP), has been shown to correlate with the potency of peroxisome proliferators, and its function is required for the production of the mitogenic response to these chemicals *in vitro*. This protein functions as an intracellular carrier of fatty acids in hepatocytes, and binds such endogenous substrates as linoleic acid, arachidonic acid, and prostaglandin E₁, as well as a number of growth modulatory compounds.²¹⁴ However, the relationship between the dose responses for peroxisome proliferation and mitogenesis varies from strain to strain in the mouse: in the BALB/c mouse the threshold for the induction of peroxisomal oxidation and mitogenesis occurs at similar exposure levels, while in the C57BL/6N mouse mitogenesis occurs at exposure levels at least one order of magnitude lower than the threshold for increased peroxisomal oxidation.²¹⁵ This lack of correspondence clearly argues against the use of peroxisomal proliferation as a surrogate for the variation of the mitogenic response across species.

In the case of DCA, the onset of neoplasia in the rat from chronic treatment occurs at concentrations (0.5 g/L) well below the onset of increased peroxisomal activity (1.6 g/L), indicating that the peroxisomal response is not a requirement for the tumorigenicity of this chemical.¹⁸⁵ However, DCA has been shown to alter hepatic glucocorticoid receptor binding activity, and the dose-response for the receptor interaction correlated with that for DCA liver tumorigenicity.²¹⁶ Glucocorticoids exert a significant effect on cellular metabolism, differentiated function, and proliferation. DCA also stimulates glycogen accumulation in normal hepatocytes through an insulin-dependent mechanism,²¹⁷ but DCA-induced tumors possess significantly different insulin receptor levels than marginal tissues and do not accumulate glycogen.²¹⁸ In general, data on the effects of DCA exposure are consistent with a mitogenic

mode of action in which a subpopulation of cells is promoted by selective pressure. Although transient increases in organ-wide cell proliferation have been reported,⁸³ repeated exposure of mice to DCA in drinking water eventually results in generalized inhibition of both mitosis²¹⁹ and apoptosis,²²⁰ while continued treatment results in the formation of focal hyperplastic lesions.^{3, 221, 222} It has been suggested that the tumors produced by DCA arise from these areas of focal hyperplasia.²²² Indeed, it has been demonstrated that the hyperplastic nodules produced by DCA include clusters of cells expressing the same tumor markers as the adenomas and hepatocarcinomas, supporting their preneoplastic role.^{220, 221} Significantly, no such clusters were observed outside of the hyperplastic nodules, and very few of the commonly observed altered hepatic foci were found, suggesting that the hyperplastic nodules are the only significant preneoplastic lesion in DCA-induced hepatocarcinogenesis.^{221, 222}

Marked differences have been noted in the phenotype and cell replicative behavior of tumors induced by DCA and TCA.^{223, 224} The altered cells induced by DCA exposure, which are predominantly eosinophilic,^{83, 187} appear to be significantly different from the altered foci affected by TCA and other classic peroxisome proliferators, which are basophilic.^{83, 187, 225} *Ras* mutational spectra data also support a distinction between the effects of DCA and TCA: while both DCA- and TCA-induced tumors in mice show the same overall *ras* mutational frequency and spectrum as spontaneous liver tumors, DCA-induced tumors showed a significant shift in the frequency with which the different specific mutations were detected.¹⁵⁷ Interestingly, another study of *ras* mutational spectra that included TCE and DCA, but not TCA,¹⁶⁴ found similar shifts for both DCA and TCE.

Regardless of the specifics of the mechanisms by which DCA and TCA induce tumors in rodents, if a mitogenic mechanism is primarily responsible for the liver carcinogenicity of TCE, the species differences in susceptibility to tumors will largely depend on the extent of qualitative and quantitative differences in the human and rodent response to a sustained mitogenic signal. Of particular importance in this regard is the recent discovery that IGF2R, one of the growth-factor receptors necessary for the response to TGF- β , is maternally imprinted in the mouse (that is, only one allele is functional), but not in the human;²²⁶ the fact that the human has two functional copies of this gene as compared to a single copy in the mouse suggests that mice would be much more susceptible than humans to liver carcinogenesis from mitogens.²⁰⁸

4. Combination of Effects

To summarize the discussion on the carcinogenic mode of action of TCE in the liver, there is no single compelling mechanism that can reliably be used as the sole basis for estimating the dose-response for human liver cancer. However, there is mounting evidence that the primary carcinogenic insult in the liver associated with TCE exposure is the stimulation of increased cell proliferation in altered cells, probably due to the mitogenic activity of the metabolites, TCA and DCA. While neither genotoxicity nor cytotoxicity appear to play a dominant role

in the production of liver tumors from TCE, it is not possible to unequivocally rule out their contribution to the observed dose-response in some animal studies. In particular, even if the modes of action for TCA and DCA appear to be primarily mitogenic, cytotoxicity due to the metabolism of TCE may exacerbate the tumorigenic effects of DCA and TCA, particularly in the corn oil gavage bioassays.¹⁷⁹ Nor is it possible to discount the possibility that low-level genotoxicity from one or more of the mutagenic TCE metabolites, such as CHL, could result in a small but finite cancer risk at human exposure concentrations well below the thresholds for the nonlinear, cell-proliferation dependent processes that may dominate at bioassay concentrations.

In spite of the potential for multiple mechanisms of carcinogenicity involving several chemical species, it is important to attempt to determine what process and chemical species, if any, may dominate in the production of liver tumors associated with exposures to TCE at bioassay concentrations. In the case of TCE liver tumors, it seems reasonable to focus on the metabolites of TCE that have been shown to produce hepatocellular carcinomas: CHL, TCA, and DCA. All three of these compounds are hepatocarcinogenic in the mouse when administered in drinking water at concentrations on the order of 1 g/L.^{82, 83} It might be hoped that evidence on the nature of the tumors induced by each compound, as well as the conditions under which they are produced, could be used to determine which metabolites play the most important role in the liver carcinogenicity of TCE.

One of the key observations regarding the liver carcinogenicity of TCE that has repeatedly been emphasized is that liver tumors are seen in mice, but not in rats. A comparison of the species dependence for the carcinogenic metabolites would therefore seem appropriate. TCA reaches concentrations of several hundred mg/L in the plasma of mice, as opposed to 5- to 10-fold lower concentrations in rats, following oral dosing with 1000 mg/kg TCE.^{54, 110} Moreover, TCA itself appears to be more potent in mice than in rats: TCA was carcinogenic in mice exposed to drinking water concentrations as low as 1 g/L (140 mg/kg/day) for 82 weeks,⁶⁵ but not in rats exposed to 5 g/L (378 mg/kg/day) for 104 weeks.²²⁷ Thus the potency of TCA across species, as well as the observed species dependence of TCE liver carcinogenicity, is consistent with species differences in its pharmacokinetics. DCA, on the other hand, produced tumors at 0.5 g/L drinking water concentrations in both mice (93 mg/kg/day) and rats (139 mg/kg/day) in 2-year exposures.^{82, 185} However, while DCA has been identified as a urinary metabolite of TCE in both rodent species,⁶ detectable plasma concentrations of DCA (on the order of 2B6 mg/L) were observed in mice, but not rats, following similar oral doses of TCE.¹¹⁰ Therefore, if DCA were responsible for the carcinogenicity of TCE, lower circulating concentrations of DCA in the rat might account for the lack of TCE carcinogenicity in that species. CHL, which is present at similar concentrations (on the order of 1 mg/L) in the blood of both rodent species after oral dosing with 1000 mg/kg TCE,⁵⁴ is carcinogenic in the mouse, but not in the rat.^{82, 228} However, the carcinogenicity of CHL would be of interest in a risk assessment for TCE only if there were evidence that CHL produced tumors through a mode of

action (e.g., genotoxic or cytotoxic) not mediated by its metabolites, TCA and DCA.

Another comparison that can be made is the relationship between the internal exposure to each of the stable carcinogenic metabolites at which tumors are observed, and the internal exposure to that metabolite produced under TCE bioassay conditions associated with liver tumors. Based on the available pharmacokinetic studies,¹²⁸ the internal exposure, as measured by the average daily AUC in the plasma, resulting in carcinogenicity is much lower for DCA than for TCA. The average daily AUC for DCA at a low carcinogenic dose (0.5 g/L) in mice and rats can be estimated to be about 4 and 2 mg-hrs/L, respectively. It must be remembered, however, that DCA may inhibit its own metabolism at this dose; therefore the pharmacokinetic studies may severely underestimate the concentrations achieved during the DCA bioassay. In contrast, the average daily AUC for TCA at its lowest carcinogenic dose in the mouse (1 g/L) is on the order of 1600 mg-hrs/L. Although, as mentioned earlier, most of the data on DCA production from TCA and TCE were compromised by a sampling artifact that may have increased apparent DCA concentrations, these data can still be used to provide a rough comparison of the average daily AUCs for DCA and TCA under TCE bioassay conditions. Based on pharmacokinetic data,^{35, 54, 92, 110, 129} the average daily AUCs at the lowest carcinogenic exposures in a TCE mouse bioassay (1169 mg/kg/day or an exposure concentration of 100 ppm) would be on the order of 800B1000 mg-hrs/L for TCA, and 4-7 mg-hrs/L for DCA, where the AUC for DCA may be greatly overestimated. Thus this comparison would suggest that both metabolites could be present at carcinogenic levels under TCE bioassay conditions associated with the induction of liver tumors. On the other hand, an analysis based on more recent artifact-free data on DCA concentrations resulting from TCE dosing¹²⁷ came to the conclusion that there was insufficient DCA present to account for liver tumors observed in TCE exposed mice²²⁹. However, the most recent bioassay with DCA²³⁰ has observed tumorigenic effects (increased multiplicity) at a dose (0.05g/L) where DCA blood concentrations may be below the reported detection limit of 1.9 μ M.¹²⁷ Thus the potential for DCA to contribute to the carcinogenic processes resulting in tumors in mice from TCE exposure cannot be ruled out.

B. Lung

As mentioned earlier, tumors have been observed in the lungs of mice exposed to TCE by inhalation.⁷⁶⁻⁷⁸ The mechanism in this case appears to be entirely different from those just described for the liver. In a well-designed experimental effort,^{5, 231} investigators combined *in vivo* and *in vitro* experiments to elucidate the mechanism of TCE carcinogenicity in the mouse lung. In the *in vivo* studies,⁵ female mice and rats were exposed to TCE at a range of inhaled concentrations at and below the concentrations at which lung tumors had been observed in mice, and the effects of TCE in the lung were determined. A specific lesion, characterized by vacuolization of lung Clara cells, was observed in mice, but not rats. There was evidence of a threshold for the Clara cell effects at about 20 ppm: the majority of Clara cells were unaffected at 20 ppm, and all enzyme

markers were normal, while at 200 ppm most of the Clara cells showed marked vacuolization, accompanied by marked loss of Cytochrome P450 activity. Although the toxicity appeared to resolve by the end of five days of repeated exposure, it reoccurred when exposure was subsequently repeated following a 2-day recovery period. Presumably the resolution of toxicity could be secondary to the loss of Cytochrome P450 activity (assuming metabolism is responsible for the toxicity), which would also explain its return after a period of recovery. In a more recent study,²³¹ mice were exposed to 450 ppm TCE 6-hrs/day for two 5-day periods, and *in vivo* rates of lung cell replication were measured. At the end of the fifth exposure cell replication rates were 10 times normal. Following a 2-day recovery period, cell proliferation rates had returned to normal, but after the tenth exposure were again increased about 6-fold.

Mice exposed to 100 ppm CHL by inhalation displayed Clara cell lesions similar to those observed with 1000 ppm TCE. In contrast to these results, only mild effects were observed with TCOH inhaled at 100 ppm, and none were observed with 500 mg/kg TCA given intraperitoneally (the effects had been observed with intraperitoneally administered TCE at 2000 mg/kg). These results suggested that CHL was responsible for the toxicity. *In vitro*,⁵ mouse lung Clara cells were shown to metabolize TCE to CHL, TCOH, and TCA, with CHL being the major metabolite. Significantly, no TCOH glucuronide was detected. In comparison with mouse Clara cells, mouse hepatocytes were shown to produce primarily TCOH and its glucuronide. In both cell preparations, a steady state concentration of CHL was achieved. Separate *in vitro* studies demonstrated that mouse Clara cells possess a relatively low activity for the glucuronidation of TCOH as compared either to the glucuronidation of other substrates in the lung or to the glucuronidation of TCOH in the liver.

On the basis of this evidence, the investigators concluded that the observed acute toxicity in the lung was a result of accumulation of CHL in Clara cells resulting from a limitation in the formation of TCOH and its glucuronide. The specificity of this lesion for the Clara cells can be rationalized in terms of their relatively high Cytochrome P450 activity, coupled with a limited ADH and UDP glucuronosyl transferase (UGT) activities.²³¹ The implications of these results for the lung tumorigenicity of TCE are 2-fold. First, the accumulation of CHL, if it does occur *in vivo*, has clear carcinogenic implications, since CHL has been shown to be genotoxic in a number of studies.¹⁵⁶ Secondly, the recurrent cytotoxicity and increased cell proliferation observed with intermittent exposure could either exacerbate any genotoxic effect or promote a spontaneous lesion. The fact that the lung tumors were generally benign is significant in this respect; the production of an increased incidence of spontaneous benign tumors is more consistent with a nongenotoxic, cell-proliferative mechanism. Ideally, studies of the labeling index in the lung following subchronic exposure could provide confirmation of the importance of hyperplasia in the lung carcinogenicity of TCE.

C. Kidney

A variety of mechanisms have been identified for the kidney effects of halogenated hydrocarbons.²³² The fact that tumors are observed only in the rat might suggest that they are associated with the male rat nephropathy described for many hydrocarbons, in which the accumulation of a male rat-specific α_2 -u-globulin in proximal tubular cells leads to hyaline droplet accumulation, necrosis, increased cell proliferation, and cancer.²³³ However, a study designed specifically to evaluate this possibility showed evidence of the hyaline droplet accumulation and increased cell replication with perchloroethylene but not with TCE.²³³ It was also felt that the oxidative metabolism of TCE was unlikely to explain the kidney carcinogenicity in rats, since the rate of metabolism in the liver greatly exceeds that in the kidney, and no liver tumors are seen in the rat.¹⁴¹ An alternative mechanism was proposed, in which direct conjugation of TCE with glutathione (GSH) in the liver was followed by further metabolism in the kidney to a cysteine conjugate that could then be cleaved to a reactive intermediate in the kidney tubular cells.¹³⁸ The cysteine conjugate formed from TCE, 1,2-DCVC, has been shown to be highly nephrotoxic²³⁴ and mutagenic in the Ames test.⁶

As with the lung carcinogenicity of TCE, more than one mechanism may play a role in the kidney tumors. The tumors produced in the kidney by TCE are very rare tumors in control animals, and do not appear to be associated with the exacerbation of spontaneous processes, suggesting that a genotoxic mechanism may be responsible. On the other hand, in the only bioassay that reported a significant increase in kidney tumors from TCE,²³⁵ cytotoxicity was observed in the kidney at both the low and high doses, while tumors were observed only at the high dose. Kidney cytotoxicity was also reported in association with a nonstatistically-significant incidence of kidney tumors in another study.^{77, 78} Urinary markers of renal toxicity have also been detected in human renal cell cancer patients,²³⁶ as well as in a patient suffering from acute TCE intoxication.¹¹⁸

However, dosing of mice with 1,2-DCVC in drinking water for 46 weeks produced clear evidence of toxicity at 87 weeks, but no evidence of tumors.²³⁷ The significance of this result is enhanced by the observation that activation of 1,2-DCVC in the kidney appears to be much greater in the mouse than in the rat, and the mouse also appears to be more responsive to the induction of cell proliferation by 1,2-DCVC than the rat.¹⁴⁶ Moreover, measurements of acid-labile protein adducts in the kidney associated with 1,2-DCVC suggest that the production of 1,2-DCVC-derived reactive species in the kidney resulting from an oral dose of 1000 mg/kg TCE may actually be greater in mice than in rats,^{146, 147} and mice but not rats showed increased cell proliferation in the kidney in response to treatment with TCE at 1000 mg/kg. Other studies in the rat also fail to support the suggestion that significant hyperplasia is produced in the kidney from exposure of rats to TCE.²³³ Thus, whether a genotoxic or cytotoxic mechanism involving 1,2-DCVC is proposed, it is difficult to explain either the negative results of the 1,2-DCVC bioassay in the mouse, or the greater

sensitivity of the rat compared to the mouse with regard to kidney tumors from TCE. As a result, some investigators have questioned the role of 1,2-DCVC in the renal toxicity and subsequent tumor development in rats.²³⁸ Nevertheless, a mode of action for TCE in the kidney involving mutagenicity and cytotoxicity from 1,2-DCVC is the most supportable choice at present, especially since no viable suggestion for an alternative source of the observed tumorigenicity has been provided in any of the studies just described.

V. SELECTION OF DOSE METRICS

One of the most crucial steps in a pharmacokinetic risk assessment for a chemical is the selection of the metric to be used to describe target tissue dose. The pharmacokinetic dose metrics most commonly applied to characterize the exposure of a tissue to a chemical are either the peak concentration or the AUC of the active form of the chemical in that tissue or the production of a reactive species in the tissue. As mentioned earlier, the USEPA used a pharmacokinetic dose metric, mg metabolized/kg/day scaled by body surface area, in their published risk assessments for TCE.^{22, 23} At the time of the risk assessments it was believed that the carcinogenicity of TCE was related to the production of reactive intermediates during its metabolism, so total mg TCE metabolized per kg body weight (as a surrogate for liver weight) per day would be an appropriate pharmacokinetic dose metric. Body surface area scaling was performed to consider possible differences in sensitivity (pharmacodynamics) between humans and mice. However, used in this way, body surface area scaling could also be interpreted as accounting for the typical allometric scaling of clearance across species; thus it could be considered to be a pharmacokinetic conversion from a comparison on the basis of equal dose to one on the basis of equal AUCs, in this case from metabolized doses to AUCs for a circulating metabolite such as TCA. Thus the published USEPA risk assessments could also be considered estimates of the risk for cancer assuming a mode of action involving TCA, without an assumption of greater human pharmacodynamic sensitivity. In fact, this interpretation would be consistent with the most recent agency position on cross-species extrapolation for cancer.²³⁹ The following sections provide rationales for the selection of pharmacokinetic dose metrics in each target tissue that are consistent with the metabolism and mode-of-action information described above, and discuss alternative dose metrics that could be considered.

A. Liver

If, as was once thought, reactive species produced during the metabolism of TCE were responsible for its liver carcinogenicity, an appropriate dose metric would be average daily amount of metabolism divided by the volume of the liver, as was used in the pharmacokinetic risk assessments for methylene chloride²⁶ and vinyl chloride.³¹ However, current information suggests that one or both of two stable metabolites, TCA and DCA, are primarily responsible for the liver tumor incidence observed in mice dosed with TCE.^{4, 110, 128} The commonly accepted form of the dose metric for the chronic interaction of a stable metabolite with a tissue is the AUC in the tissue. This mathematical form

implicitly assumes that the cumulative effect of the metabolite on the tissue is linear over both concentration and time. In this case, the most appropriate dose metric would reflect liver tissue exposure (average daily AUC) to both TCA and DCA.^{4, 110, 128} If it is assumed that both DCA and TCA contribute to the carcinogenicity of TCE in the liver, the proportion of the observed tumor risk to assign to each metabolite could be based on their relative potencies when dosed directly. However, as mentioned earlier, data on the AUCs for DCA resulting from exposures to TCA were compromised by a sampling artifact that could lead to overestimates of DCA concentrations in the presence of TCA,¹²⁶ making it impossible to estimate the separate contributions of TCA and DCA. Moreover, data on DCA concentrations following human exposure to TCE are extremely limited. As a simplifying assumption, all of the tumorigenicity of TCE can simply be ascribed to TCA, as was assumed by Fisher and Allen.³⁵

Strictly speaking, the AUC for TCA should actually be calculated for the concentration in the liver; however, the use of the AUC in the plasma provides a surrogate for the liver AUC that can more readily be validated against experimental data. Since risk estimates are based on the ratio of the animal and human dose metrics, this effectively amounts to an assumption that the ratios of the plasma concentrations of the acids to their concentrations in the liver are constant across species. In fact, data on the binding of TCA in the plasma of rats and humans,¹¹¹ suggest that TCA in plasma is bound to a much greater extent in the human (~80%) than in the rat (~50%). More recent data²⁴⁰ confirm these results and show that the fraction bound in the mouse is even lower (~20%). Assuming binding of TCA in liver cells is similar across species, it can be estimated that the liver to plasma TCA concentration ratio in the human would be about 25% of the ratio in the mouse. Moreover, the observed dose-response for the binding is such that at lower human exposures the binding would be greater than 80%, while at higher mouse exposures the binding would be lower than 20%. Thus using the average daily AUC of TCA in the plasma as the dose metric would overestimate the liver exposure to TCA in the human by at least 4-fold, and possibly more. A pragmatic resolution of this problem is to simply adjust the model-predicted human dose metrics for AUC TCA by the ratio of the mouse and human free TCA fractions in plasma; that is, the human dose metrics should be divided by a factor of four.

It is possible that although average daily AUC is generally considered a reasonable metric for tissue exposure, other forms of the dose metrics for DCA and TCA might be more appropriate for their modes of action. If the tumorigenic effects of these chemicals are related to some aspect of their interaction with a receptor, peak concentrations (C_{MAX}) might actually be more appropriate than average daily AUCs. It has been suggested¹⁶⁹ that the use of peak concentration of TCA in plasma as a dose metric for increased liver carcinoma incidence in B6C3F1 mice provides good correspondence between 2-year exposures to TCE by inhalation and TCA in drinking water. There are other possible forms for dose metrics that might be useful for describing such a nonlinear process. For example, "time above a critical concentration" has been suggested as an appropriate dose metric for the effects of methotrexate, whose toxicity

demonstrates a strong dependence on dose-rate.²⁴¹ Another nonlinear dose metric that has recently been discussed for receptor mediated effects is based on average receptor occupancy.²⁰⁷ Unfortunately, the more an attempt is made to include pharmacodynamic events into a dose metric, the more difficult it becomes to collect the data necessary for its use. In the case of TCE, there are currently no experimental data available to evaluate the use of such alternative pharmacodynamic dose metric approaches. Of the possible dose metrics, only average daily AUC, C_{MAX} , and time above a critical concentration can be estimated from the data currently available.

B. Lung and Kidney

Based on the metabolism and mode-of-action information discussed above, it would appear that the dose metric for the lung should be based on achieved CHL concentrations or average daily AUC in the tracheobronchial region. Similarly, at present the most appropriate metric for the kidney target tissue is the production of reactive species from 1,2-DCVC in the kidney. If the kidney and lung carcinogenicity of TCE is considered to result primarily from enhanced cell proliferation secondary to recurrent toxicity, possible dosimetry approaches could include measures of cytotoxicity, cell death, or cell division, as has been proposed for the liver carcinogenicity of chloroform.¹⁷² In the case of chloroform, fairly complicated dosimetry approaches involving the instantaneous rates of metabolism and distributions of cellular sensitivity have been suggested.^{17, 51, 242} To apply these approaches to the kidney and lung carcinogenicity of TCE, would require extensive studies similar to those that have been conducted with chloroform at the CIIT Centers for Health Research (formerly the Chemical Industry Institute of Toxicology).¹⁸⁰⁻¹⁸³ Of particular note, the dose response for cytotoxicity in these subchronic studies with chloroform is markedly different from that observed in acute and *in vitro* studies.⁵¹ It would appear that caution must be used when the dose-response for a surrogate measure of tissue response is derived from *in vitro* or short-term *in vivo* experiments. If genotoxicity is also considered to be quantitatively important in the lung and kidney target tissues, an even more complicated dose metric would be required. The use of a dose metric based on the product of DNA-protein crosslinks and cell labeling index has been suggested for evaluating the incidence of nasal tumors from formaldehyde exposure, assuming a carcinogenic mode of action involving both genotoxicity and cytotoxicity.^{243, 244} Again, extensive studies paralleling those conducted with formaldehyde at the CIIT Centers for Health Research would be required to apply this approach to TCE.

VI. DESCRIPTION OF PBPK MODEL

For each of these three target tissues of concern for the risk assessment, liver, lung, and kidney, there is evidence that the carcinogenicity of TCE may be associated with one or more of its metabolites: TCA and DCA in the liver,^{4, 110} CHL in the lung,⁵ and 1,2-DCVC in the kidney.¹⁴¹ Therefore target tissue dosimetry information is needed for each of these metabolites in both the affected animal species and in the human. Available pharmacokinetic data could

be used directly to estimate target tissue dosimetry, such as was done by the USEPA in their published risk assessments for TCE.^{22, 23} Direct use of pharmacokinetic data was also performed to support the TCE metabolite potency comparison in the liver mode-of-action section, above. However, incorporation of the same pharmacokinetic data into a comprehensive PBPK description provides a number of advantages: (1) the consistency of diverse pharmacokinetic data sets involving different routes of exposure and different species can be tested, (2) the ability of the model to accurately extrapolate pharmacokinetic behavior across concentration, route, and species can be evaluated, and (3) model can be used to predict pharmacokinetic behavior under conditions for which experimental data are unavailable. To be useful in a comprehensive cancer risk assessment for TCE, a PBPK model should provide a description of TCE pharmacokinetics and metabolism, along with a description of the kinetics of the metabolites suggested to play a role in the carcinogenic activity in each of the three target tissues: liver, lung, and kidney.

A large number of PBPK models have been developed for TCE. However, many are only parent chemical models; that is, they provide a pharmacokinetic description of TCE itself, but do not include an explicit description of the pharmacokinetics of any of the metabolites.^{33, 34, 245-251} Two of these parent chemical models have been employed to calculate total metabolized dose in support of a cancer risk assessment for TCE.^{33, 34} However, these parent chemical models cannot be used for predicting tissue exposure to specific metabolites. Models of both TCE and its metabolites have also been developed. In a series of publications, Sato and coworkers have described the use of a simple PBPK model to study the kinetics of TCE and its metabolites in humans,²⁵² to evaluate the impact of changes in physiological factors²⁵³ and environmental factors²⁵⁴ on the kinetics of TCE in the human, and to predict the effects of interactions with ethanol consumption on TCE kinetics.²⁵⁵ However, the structure of these models would not support the animal-to-human extrapolation of pharmacokinetic dose metrics needed for risk assessment.

Fisher and coworkers developed a PBPK model for TCE and its principal metabolite, TCA, in the pregnant²⁵⁶ and lactating²⁵⁷ rat, as well as in the mouse.⁹² These rodent models, together with a similar model of TCE and TCA in the human,²⁵⁸ served as the basis for a PBPK-based risk assessment for TCE liver carcinogenicity³⁵ based on either average daily total metabolism or average daily AUC for TCA. These models provided the first successful cross-species pharmacokinetic description for a metabolite of TCE. The model used in the present analysis²⁵⁹ built on the work of Fisher and Allen³⁵ by adding limited descriptions of additional metabolites (TCOH, DCA, CHL, 1,2-DCVC) and target tissues (lung and kidney). Fisher and colleagues have also continued to elaborate and refine their PBPK models for TCE, focusing on the metabolites of interest for liver carcinogenicity. Published models include (1) a model of the kinetics of TCE, CHL, TCA, DCA, and TCOH in the B6C3F1 mouse based on data from corn oil gavage exposures,²⁶⁰ (2) a model of TCE, TCA, and TCOH in the human based on data from controlled human inhalation exposures,¹¹⁹ (3) a model of TCE, TCA, and TCOH kinetics in the rat that considers enterohepatic

recirculation of TCA and TCOH following oral or intravenous exposure to TCE,²⁶¹ and (4) a model of inhaled TCE and its oxidative metabolites in the B6C3F1 mouse.²⁶² Together, these models provide a capability for estimating dose metrics in the mouse, rat, and human in support of a risk assessment for TCE liver carcinogenicity, in a similar fashion to the approach described in the present analysis. A potential advantage of these mouse PBPK models^{260, 262} is that their calibration includes data on TCA concentrations in the liver. However, since there was no human data on liver concentrations, the human model¹¹⁹ could not be similarly calibrated. Therefore, the relationship of liver and blood TCA dosimetry must still be inferred from data on plasma binding of TCA, as discussed earlier.

A. PBPK Model Structure

A diagram of the PBPK model for TCE and its metabolites that was used in this analysis is shown in Figures 3 and 4. A full description of this model has been published previously,²⁵⁹ but a fairly detailed summary of its features will be presented here since it provides the basis for all of the risk calculations in this analysis. The model was written in the Advanced Continuous Simulation Language (ACSL, The AEGIS Technologies Group, Inc., Austin, Texas). The parent chemical portion of the model (Figure 3) includes individual tissue compartments for the liver, gut tissue, fat, and tracheo-bronchial region of the lungs. All other tissues are lumped into rapidly perfused (kidney, brain, alveolar region of lungs, etc) and slowly perfused (muscle, skin, etc) compartments. The model includes both inhalation and oral routes of exposure. Oral gavage is modeled using a two-compartment description of the gastrointestinal tract,²⁶³ rather than the single compartment description used by Fisher and Allen,³⁵ in order to better simulate the timecourse for the uptake of TCE from corn oil gavage. Allometric scaling is used throughout the model (volumes scaled by body weight, flows and metabolic capacities scaled by body weight to the three-quarters power, rate constants scaled by body weight to the negative one-quarter power) to simplify intraspecies and interspecies extrapolation. Parent chemical dose metrics provided in the model include the concentration of TCE in blood and tissues, as well as the AUC for TCE in the blood.

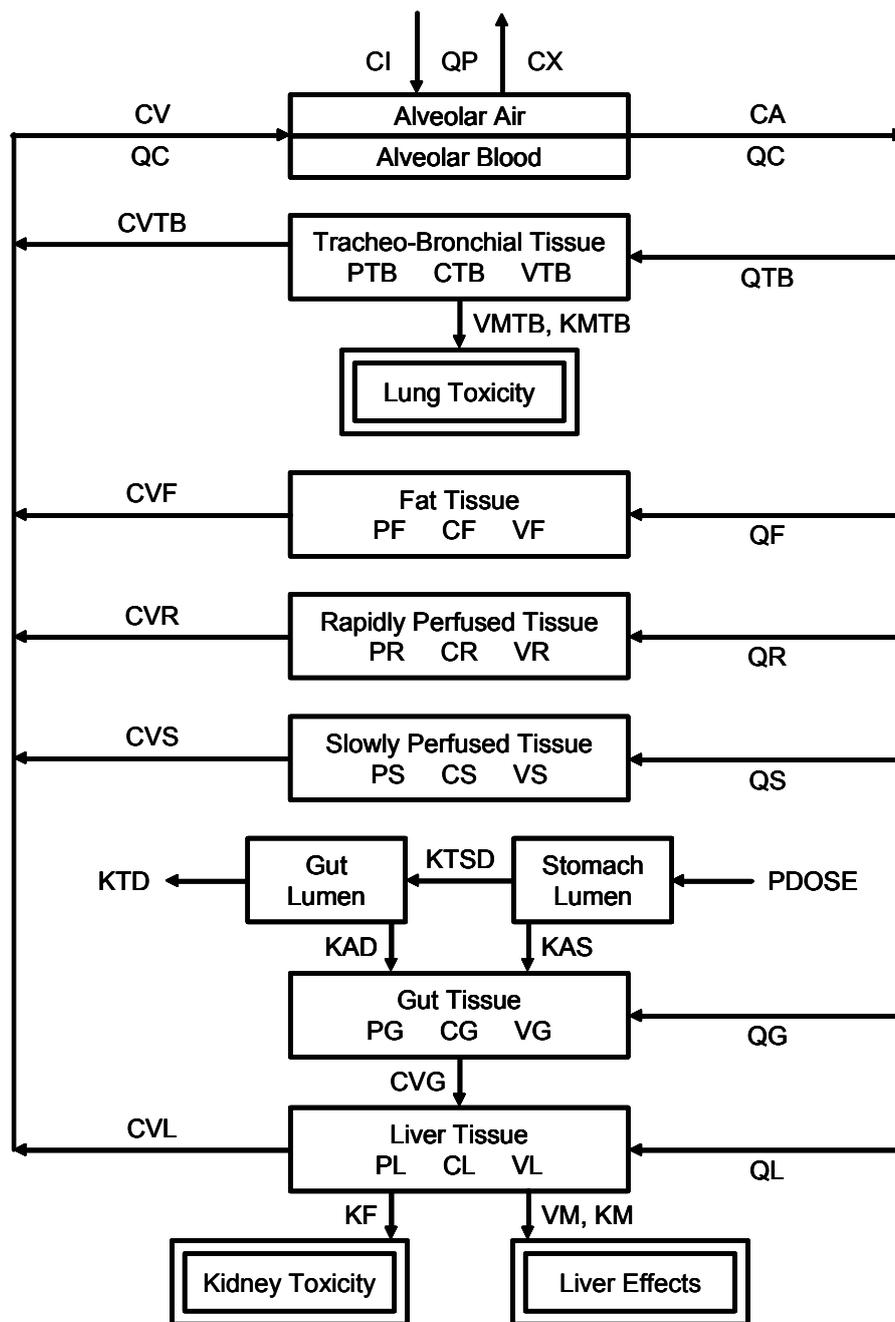


FIGURE 3. PBPK model for TCE: parent chemical model. Abbreviations not listed in Table 1: CA: concentration in arterial blood; CI: concentration in inhaled air; CV: concentration in venous blood; CX: concentration in exhaled air; C&: concentration in compartment &, where & = F for fat, G for gut tissue, L for liver, R for richly perfused tissues, S for slowly perfused tissues, and TB for tracheo-bronchial tissue; CV&: concentration in venous blood leaving compartment &.

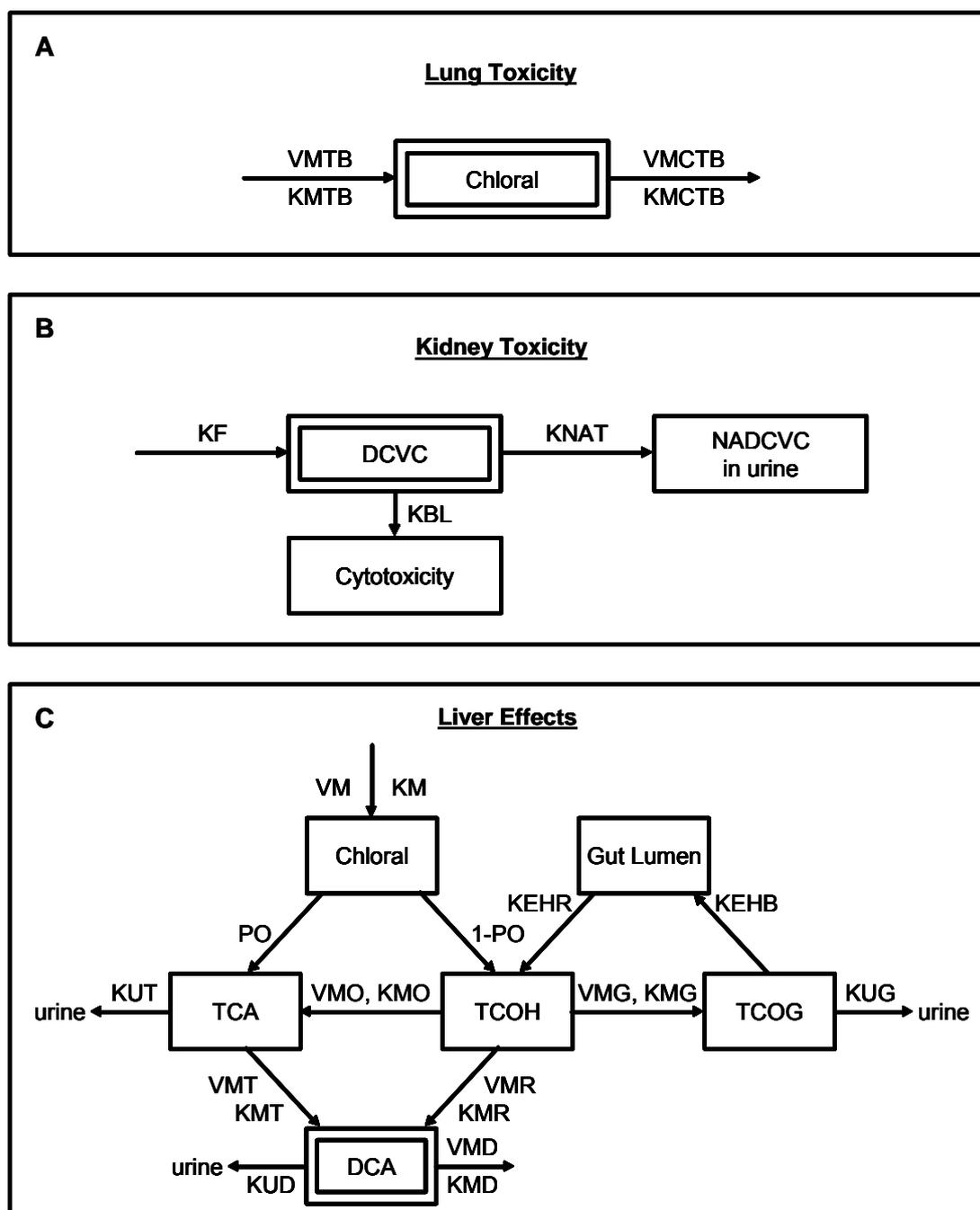


FIGURE 4. PBPK model for TCE: metabolism submodels for lung (A), kidney (B), and liver (C) target tissues.

1. Lung Submodel

The model includes three target tissue submodels in which metabolism takes place: lung, kidney, and liver (Figure 4). Michaelis-Menten kinetics are assumed for all metabolic processes. The tracheo-bronchial region of the lungs, which receives its own arterial blood supply, is described separately to support the modeling of *in situ* metabolism in this region by the Clara cells. This approach for describing metabolism in the cells lining the airways of the lung was

felt to be more biologically accurate than the sequential gas exchange and lung tissue compartments used in the methylene chloride model.²⁶ However, as long as metabolism in the lung is unimportant for presystemic clearance, as is the case for TCE and methylene chloride, the two descriptions should yield similar results. The dose metrics provided for the lung are the instantaneous concentration and AUC for CHL in the tracheo-bronchial region, which is assumed to be produced by saturable production and clearance of CHL in Clara cells. No systemic circulation of CHL is considered in the model.

2. Oxidative Metabolism

Apart from the limited metabolism occurring in the lung, the model assumes that all oxidative metabolism takes place in the liver. The dose metric provided to describe metabolism (for comparison with the USEPA estimates) is the total amount of TCE metabolized divided by the body weight. The model does not actually calculate the formation and metabolism of CHL in the liver, but instead assumes that TCA and TCOH are formed in a fixed yield from the oxidative metabolism of TCE. In the model, TCOH can subsequently be oxidized to TCA, conjugated with glucuronic acid, or reduced to DCA. DCA can also be produced from the reduction of TCA. Biliary excretion of TCOH glucuronide and enterohepatic recirculation of free TCOH is described, with only the glucuronide being excreted in the urine. Dose metrics for use with the liver target tissue include the concentrations and AUC for DCA and TCA in the plasma.

3. Conjugative Metabolism

The model also includes a linear metabolic pathway representing conjugation of TCE by GST. The model implicitly assumes that all glutathione conjugation of TCE in the liver leads eventually to the appearance of 1,2-DCVC in the kidney. Clearance of 1,2-DCVC by N-acetyl-transferase into the urine is also modeled. The dose metric provided in the model for the kidney (KTOX) is the total production of a thioacetylating intermediate from 1,2-DCVC, divided by the volume of the kidney.

B. PBPK Model Parameters

The parameters for the model are listed in Table A-1 (at end of chapter), with the parameters for the parent chemical portion of the model listed first, followed by the parameters for each of the metabolites in turn. This table also provides definitions of each of the parameters.

1. Parameters for the Parent Chemical

The physiological parameters, with two exceptions, were obtained from the final report of the ILSI Risk Science Institute Working Group on Physiological Parameters, which was funded by the EPA and the American Industrial Hygiene Council.²⁶⁴ The exceptions were the cardiac output (QCC) in the mouse, which was taken from the recommendations of Arms and Travis,²⁶⁵ and the alveolar

ventilation (QPC) in the human, which was obtained from Astrand and Rodahl.²⁶⁶ In the model, the tissue volumes and blood flows for the gut, liver, and tracheo-bronchial region are subtracted from the values shown for "all rapidly perfused tissues" to obtain the parameters for the rapidly perfused tissue compartment shown in Figure 3, and those for the fat are subtracted from the values shown for "all slowly perfused tissues" to obtain the parameters for the slowly perfused tissue compartment. The kidney volume shown in the table is used only in calculations for the kidney dose-surrogate; as shown in Figure 3, the kidney is not described separately in the parent chemical model.

The partition coefficients for TCE were obtained from the work of Fisher and Allen;^{35, 92, 258} the partition coefficients for the gut and tracheo-bronchial tissues were assumed to be the same as those reported for the richly perfused tissues. The oral uptake parameters were estimated from data on the appearance of TCE and its metabolites in the blood following oral gavage in mice and rats. Different oral uptake parameter values were required for aqueous^{110, 111, 129} and corn oil⁵⁴ vehicles. For some parameters, values chosen for calculating risk assessment dose metrics were different from those chosen to reproduce pharmacokinetic data. For example, human dose metrics were calculated using a value for QPC that corresponds to the EPA's standard assumption of a total ventilation rate of 20 m³/day; the corresponding value for QCC was estimated from Astrand and Rodahl.²⁶⁶ Similarly, animals used in pharmacokinetic studies tend to have lower average body weights than animals used in cancer bioassays, so body weights appropriate to each case were used in the model.

2. Parameters for Oxidative Metabolism

Initial values for the metabolic parameters for TCE were obtained from the work of Fisher and Allen;^{35, 92, 258} however, the metabolic and clearance parameters for TCA, TCOH, and DCA were derived primarily on the basis of fitting pharmacokinetic data in the rodent^{54, 110, 111, 129} and human^{89, 106, 267} such as those depicted in Figures 5 through 8. When possible, parameters were also estimated from independent studies; for example, data from rodents and humans dosed with TCA were used to estimate the volumes of distribution and urinary excretion rate for TCA.^{92, 106} Since the model contains a large number of metabolic and clearance parameters, many of which are highly correlated, the parameter values estimated by this process (i.e., the kinetic parameters for TCA, TCOH, and DCA) cannot be considered to be unequivocally identified.

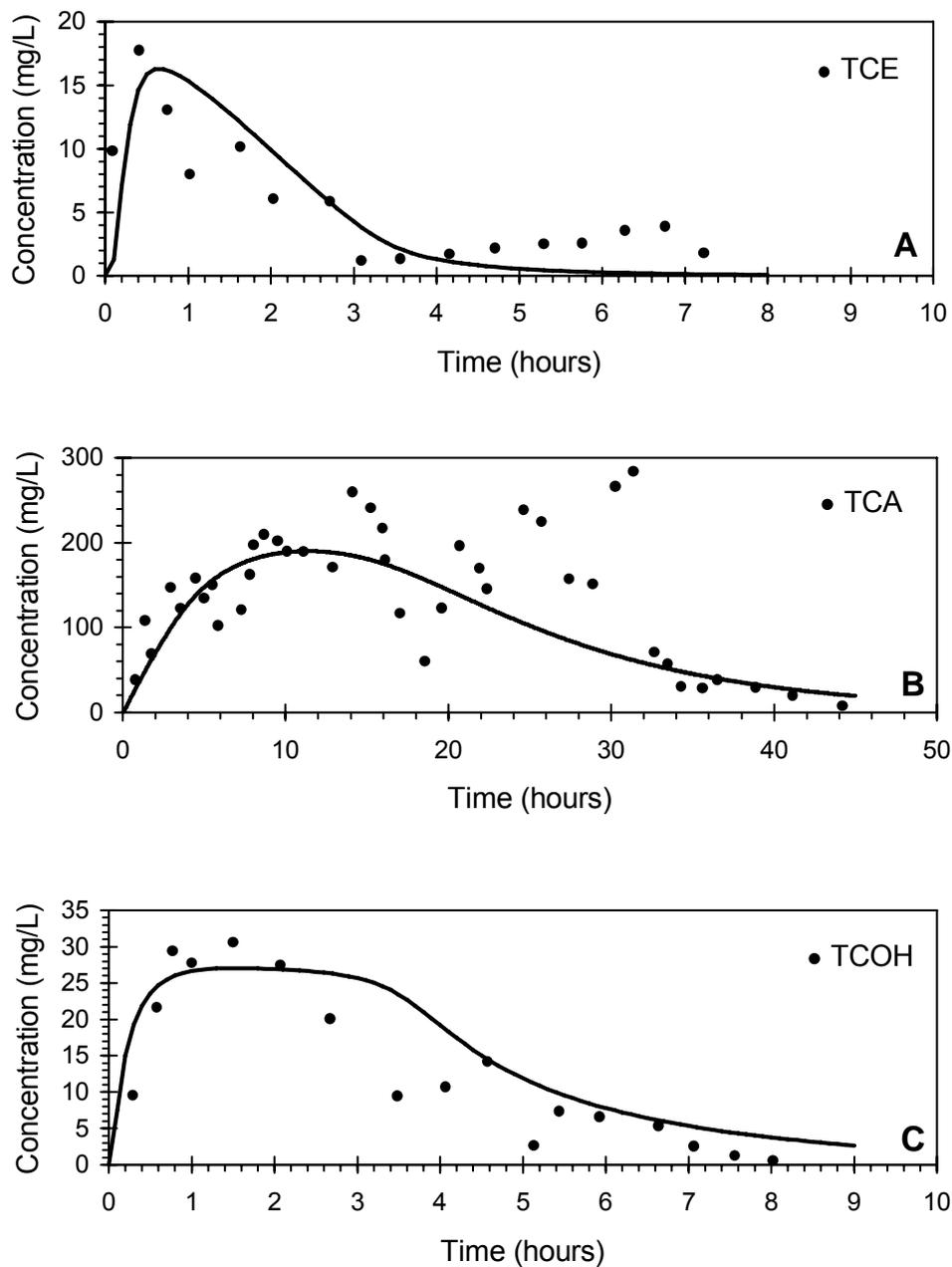


FIGURE 5. Mean observed⁵⁴ and predicted blood concentrations of TCE (A), TCA (B), and free TCOH (C) following corn oil gavage with 1000 mg/kg TCE in mice. Simulation of corn oil gavage was conducted with a value of 0.27/hr for KAD.

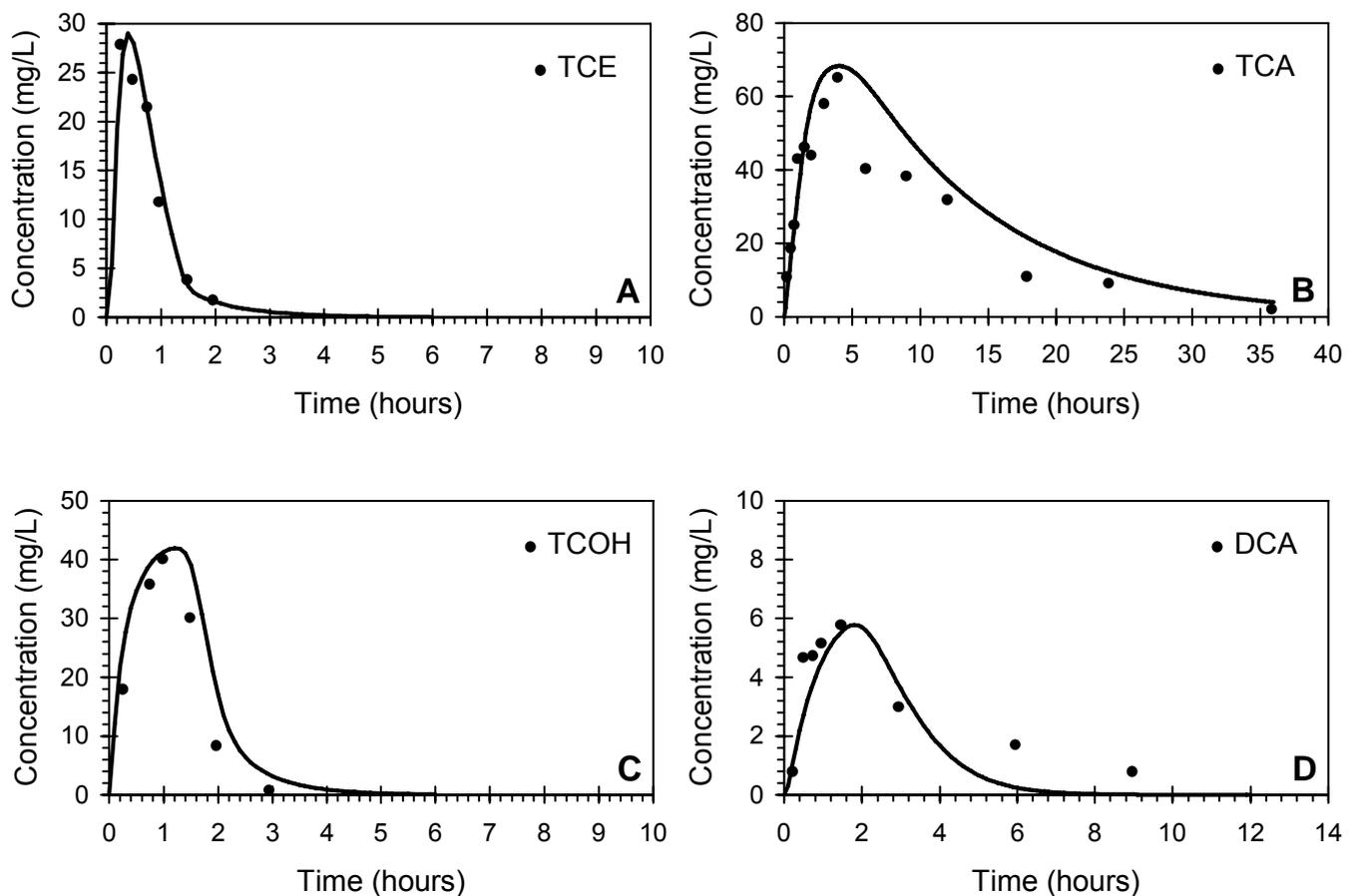


FIGURE 6. Mean observed¹²⁹ and predicted blood concentrations of TCE (A) and metabolites TCA (B), TCOH (C), and DCA (D) following aqueous gavage with 499 mg/kg TCE in B6C3F1 mice. Simulation of aqueous gavage was conducted with a value of 1/hr for KAD.

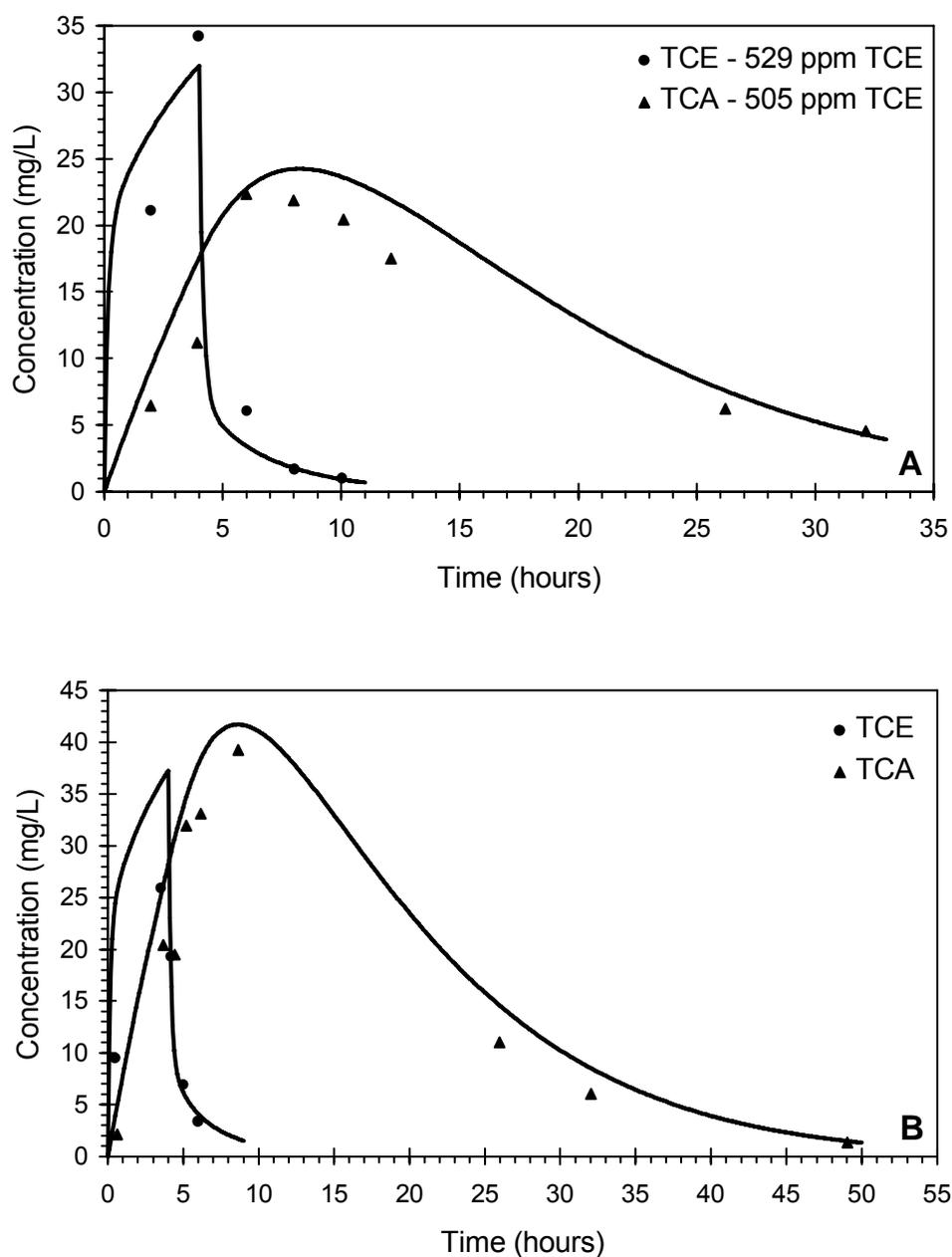


FIGURE 7. Mean observed⁹² and predicted concentrations of TCE in blood and TCA in plasma in F-344 rats exposed to TCE by inhalation. The figures show (A) TCE blood concentrations in male rats exposed for 4 hr to 529 ppm TCE vapors and TCA plasma concentrations in male rats exposed for 4 hr to 505 ppm TCE vapors; (B) TCE blood and TCA plasma concentrations in female rats exposed for 4 hr to 600 ppm TCE vapors.

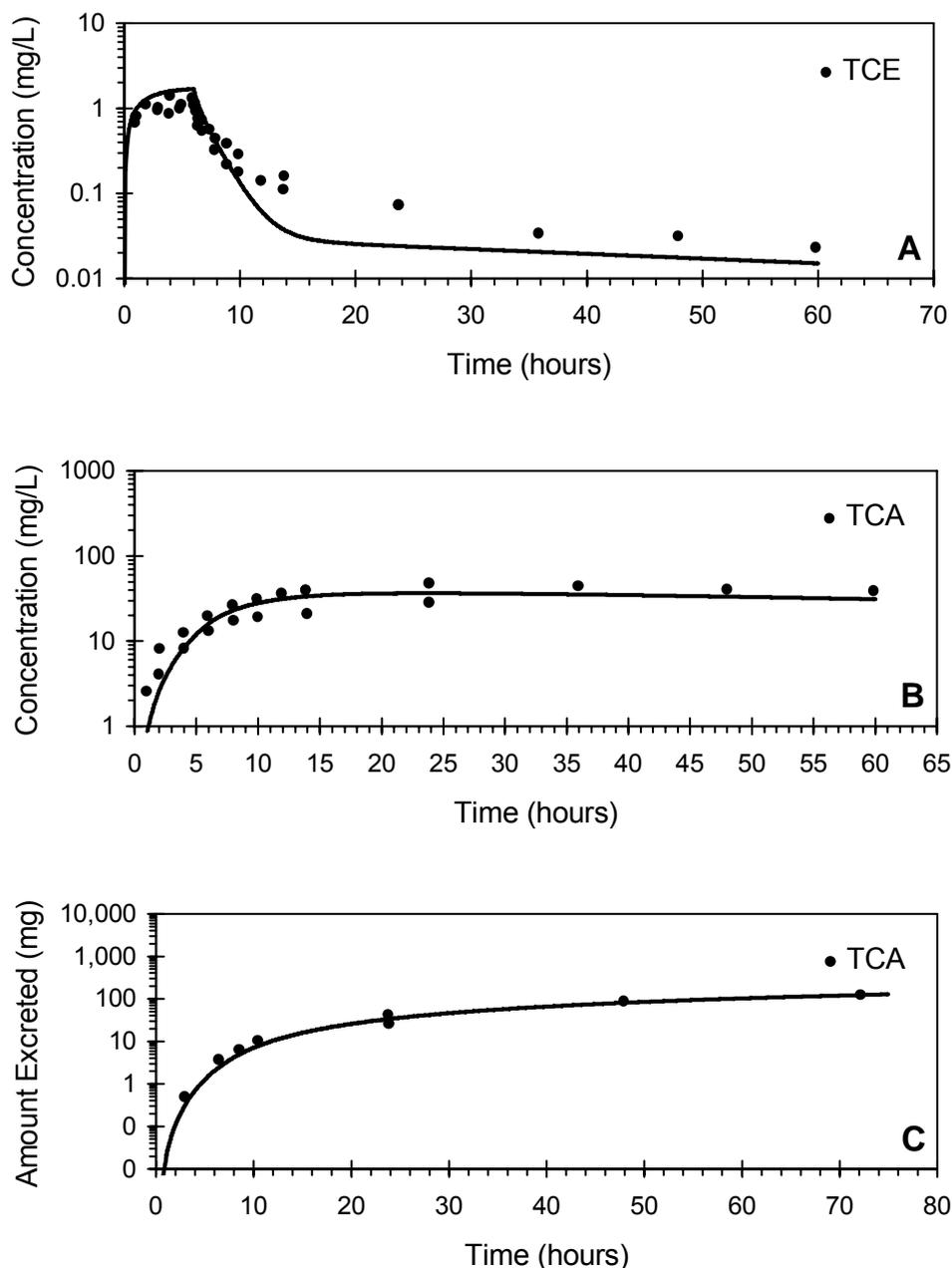


FIGURE 8. Mean observed^{106, 267} and predicted kinetics of TCE and its metabolites during and after a single 6-hr exposure of human subjects to 100 ppm TCE. The figures show (A) TCE blood concentrations (mg/L); (B) TCA plasma concentrations (mg/L); (C) cumulative urinary TCA excretion (mg); (D) total TCOH plasma concentrations (mg/L); (E) cumulative urinary TCOH excretion (mg).

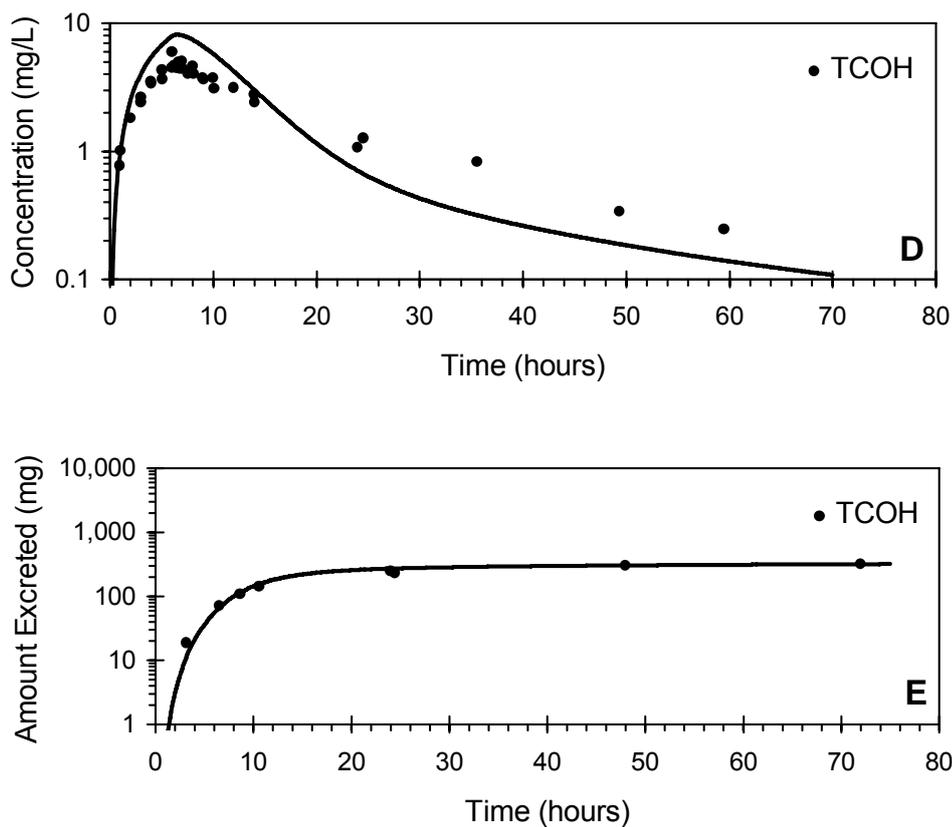


FIGURE 8. (Continued) Mean observed^{106, 267} and predicted kinetics of TCE and its metabolites during and after a single 6-hr exposure of human subjects to 100 ppm TCE. The figures show (A) TCE blood concentrations (mg/L); (B) TCA plasma concentrations (mg/L); (C) cumulative urinary TCA excretion (mg); (D) total TCOH plasma concentrations (mg/L); (E) cumulative urinary TCOH excretion (mg).

Fortunately, the constraint of holding all parameters relatively constant across exposure scenarios within a given species, and (to the extent justified by the data) across species, results in relatively little freedom to obtain alternative parameterizations that demonstrate equivalent success. One of the constraints on the parameterization not obvious from the figures is the fact that of the total TCOH extractable from the blood, roughly 80% is present as free TCOH in the human,²⁶⁷ while roughly 70-85% is present as the glucuronide in the rodent.^{111, 129} In the figures in this paper, the model concentrations shown represent either free TCOH (in rodents) or total (in humans), corresponding to the experimental data provided.

It is informative to note the departures from simple allometric expectations that were required on the basis of the experimental data across species. As with most other xenobiotics, the mouse shows a relatively greater, and more variable, capacity (VMC) for oxidative metabolism of TCE than the rat and human. Also in keeping with evidence from other P450 substrates, the affinity for oxidative metabolism of TCE in the human is roughly an order of magnitude less (i.e., the value of K_M is larger) than in the rodents. A striking difference between humans and rodents, which was clearly demanded by the experimental data, was that the oxidation of TCOH to TCA appears to be high affinity (small value of K_{MO}), low capacity (small value of $VMOC$) in the rodent but low affinity, high capacity in the human. It may be that this disparity reflects the involvement of different enzymes (e.g., MFO in the rodent vs. ADH in the human). The result of this species difference is that although the model uses a similar value across species for the stoichiometric split (PO) of TCA and TCOH from CHL, the apparent ratio of TCA to TCOH predicted (and observed) over the entire time-frame of an exposure to TCE is much higher in the human than in the rodent. The capacity (VMGC) for glucuronidation of TCOH in the human, on the other hand, is much lower than in the rodent, as reflected in the greatly different ratios of free TCOH to glucuronide in the blood, mentioned above. The prolonged timecourses of TCOH in the human provide clear evidence of biliary excretion (KEHBC) and enterohepatic recirculation (KEHRC). Evidence for enterohepatic recirculation was equivocal in the rodents, however, with recirculation being required to reproduce some data, but being contradicted by other data in the same species.

The least well characterized portion of the oxidative metabolism pathway is the description of the kinetics of DCA. The only species in which DCA has been reproducibly detected in the plasma following TCE exposure is the mouse, and these data were used to obtain values for the production (VMRC and K_{MR}) and clearance (VMDC and K_{MD}) in the mouse. (The artifactual production of DCA from TCA in blood samples, noted earlier in this report, may have compromised some of the data on DCA plasma concentrations used to parameterize the production and clearance of DCA in the mouse.) Assuming that the affinities (K_{MR} and K_{MD}) are constant across species, the capacities in the other species were estimated (for VMDC) from the reported half-lives of DCA across species,¹²² or (for VMRC in the human) from data on peak DCA concentrations in human subjects exposed to TCE by inhalation.¹¹⁹ Since the clearance of DCA in the rat is actually slower than in the mouse,¹²⁸ the capacity for production of DCA

(VMRC) in the rat was set to the lower human value rather than that of the mouse in order to be consistent with the failure of investigators to observe DCA in the plasma of the rat following administration of TCE.¹¹⁰ The renal clearance of DCA (KUDC) was assumed to be the same as that observed for TCA (KUTC) in the same species. As mentioned earlier, the most striking departure from allometric expectations for the kinetics of DCA is the extremely high clearance in the human compared to the other species.

3. Parameters for Lung Metabolism

The parameters in the PBPK model for predicting the lung dose metric are the capacity and affinity for the production of CHL (VMTBC and KMTB) and the capacity and affinity for its clearance (VMCTBC and KMCTB). In the model, the production of CHL in the tracheobronchial region was assumed to be associated with the P450 activity in that tissue. This is the assumption that was made in the pharmacokinetic risk assessment for methylene chloride.²⁶ The approach used in that risk assessment was also used to obtain the parameters in this case: the affinity in the lung was assumed to be the same as in the liver for the same species, and the relative capacity of the lung compared to the liver was determined on the basis of P450 activity measured with standard substrates.²⁶ This approach produced estimates of relative lung activity across species that were consistent with the limited observations available for TCE.²³¹ Clearance of CHL was assumed to be performed by a low affinity, high capacity enzyme system such as alcohol dehydrogenase; it was further assumed to scale across species according to allometric expectations (i.e., by body weight to the 3/4 power). Again, these assumptions were consistent with the limited data available on the clearance of CHL in the lung across species.²³¹ The parameters in the PBPK model were chosen such that the concentrations of CHL in the lung of the mouse predicted by the model were consistent with those observed in recent studies.²⁶⁰

4. Parameters for Conjugative Metabolism

The parameters in the PBPK model for predicting the kidney dose metric are the production of 1,2-DCVC by the GST pathway (KFC), its activation by β -lyase (KBLC), and its clearance by N-acetyl-transferase (KNATC). First-order rate constants are used because the production of metabolites by the GST pathway is quite low, and saturation of enzyme capacity is unlikely. As discussed earlier, the capacity and affinity of β -lyase in the kidney have been measured in both rats and humans.¹⁵⁰ These data were used to estimate the apparent first-order rate constants (KBLC) used in the model. No data were available on the activity of β -lyase in the mouse, so the relationships between β -lyase metabolic parameters in mice and rats reported for trichlorovinylcysteine derived from perchloroethylene¹⁴⁵ were assumed to apply for 1,2-DCVC as well. For N-acetyl-transferase, only specific activity data across species were available.¹⁵¹ These data were converted to the corresponding rate constants (KNATC) by assuming the affinity of N-acetyl-transferase for 1,2-DCVC was the same as that measured for β -lyase in the same species. This assumption is supported by the similarity

of the affinities of N-acetyl-transferase and β -lyase for DCVC in the rat: 3.3mM and 1.6 mM, respectively.^{150, 268}

Finally, measurements of oxidative and conjugative metabolites in the urine following TCE exposure¹⁵² were used to obtain estimates of the GST pathway rate constant (KFC). The oxidative pathway was represented by total excretion of TCA plus TCOH, while the conjugative pathway was represented by excretion of 1,2-DCVC. Data from the same study on excretion of 2,2-DCVC were not used. Unlike 1,2-DCVC, there was no evidence of a dose-response for 2,2-DCVC as a function of TCE exposure in humans or rodents; similar amounts of 2,2-DCVC were excreted for TCE exposures ranging from 40 to 160 ppm. Ignoring 2,2-DCVC is unlikely to significantly effect the risk assessment for TCE, since 1,2-DCVC is clearly the more toxic and mutagenic of the two isomers.²⁶⁹ It was found²⁵⁹ that the model could be made to agree quite well with the urinary data when allometric scaling was assumed for conjugative metabolism (i.e., using the same value of the scaled parameter, KFC, in rat and human). This result is consistent with the observed allometric scaling of the GST pathway for methylene chloride.²⁷⁰

Additional data on urinary metabolite concentrations following oral gavage of rats with 50 mg/kg TCE,¹⁵¹ although not suitable for comparing with the model, were consistent with the inhalation data, suggesting that there is not an effect due to route of exposure. Therefore, the value estimated from inhalation was used to obtain the kidney dose metrics for the rat for both inhalation and oral exposures.

C. PBPK Model Validation

In the strictest sense of the word, validation of a PBPK model would require testing the predictions of the model against data not used in the development and parameterization of the model.¹⁰ Ideally, each of the model parameters would have been estimated from separate experiments and the performance of the model could then be tested against pharmacokinetic data such as those shown in Figures 5 through 8. In practice, there are simply not enough experimental data to separately identify all of the parameters in a model as complex as the PBPK model for TCE described in this paper. Moreover, as in this case, there are often no data available with which to validate important components of the model. Therefore, the validity of the model for its intended purpose must be evaluated on the basis of the comprehensiveness of its predictive power and the reasonableness of the parameters used to fit the various data sets. The parameterization of the PBPK model for TCE has already been discussed. Examples of the ability of the model described to reproduce data on TCE and TCA kinetics in the mouse, rat, and human, for both inhalation exposure and oral gavage, are shown in Figures 5 through 8. The comparisons presented in these figures represent only a small portion of the data sets used in developing and validating the PBPK model.²⁵⁹ Given the wealth of data on TCE, TCA, and TCOH concentration timecourses in both rodents and humans, there can be relatively high confidence in the predictions of the PBPK model for these

chemicals. Similarly, model predictions for the total amount of TCE metabolized per kg body weight are generally within a factor of 2 of data on inhalation and oral exposures of mice, rat, and humans.^{54, 88, 89, 112, 113} Unfortunately, there is a lack of similar data to provide confidence in the model predictions for 1,2-DCVC in the kidney, CHL in the lung, and DCA in the liver, so these predictions must be viewed as less certain.

D. PBPK Model Parameter Sensitivity

Parameter sensitivity analysis was performed for the parameters associated with TCE and TCA in the PBPK model described above.²⁵⁹ The resulting normalized analytical sensitivity coefficients represent the fractional change in model output associated with a fractional change in the input parameter. For example, if a 1% change in the input parameter results in a 2% change in the output, the sensitivity coefficient would be 2.0. In this analysis, the outputs chosen were the dose metrics for the average daily total amount of TCE metabolized per kg body weight (AMET) and the average daily AUC for TCA. Of the 29 parameters in the TCE/TCA portion of the model, 12 have essentially no impact on risk predictions based on the two dose metrics, and only 5 have a significant impact: the alveolar ventilation (QPC), the capacity for metabolism of TCE (VM) and the fraction of TCA produced from the metabolism of TCE (PO), and the volume of distribution (VDTAC) and rate of excretion (KUTC) of TCA. None of the parameters display sensitivities greater than 1.0, indicating that there is no amplification of error from the inputs to the outputs. This is, of course, a desirable trait in a model to be used for risk assessment. Sensitivities for the other metabolites in the model were similar to those for TCA; that is, none of the parameters demonstrated sensitivities greater than one in absolute value, and (except for QPC) only the parameters directly related to the production and clearance of a metabolite showed significant sensitivities (close to one) for that metabolite.

E. PBPK Model Uncertainty

There are a number of ways of characterizing the uncertainty associated with the use of a PBPK model. The best approach to be used depends on the level of uncertainty. In the case of the TCE model, the level of uncertainty varies considerably from one portion of the model to another. Some parameters in the model, such as those for TCE, TCA, and TCOH are relatively well established by data, and the uncertainties can be addressed fairly quantitatively. Under these conditions the preferred method for characterizing the overall model uncertainty is to perform Monte Carlo analysis, as discussed below. On the other hand, other parameters in the model, such as those associated with the production and clearance of 1,2-DCVC in the kidney and CHL in the lung, are based on inadequate and often conflicting data and the uncertainties cannot be adequately quantified to support such a rigorous analysis. For these aspects of the model, an appropriate method for characterizing uncertainty is to simply calculate the range of dose metrics that could reasonably be expected, given the existing data.

1. Liver Dose Metric

To estimate the combined impact of uncertainty regarding the values of the parameters, a Monte Carlo analysis was performed for the principal liver dose metric, the lifetime average daily AUC for TCA.²⁵⁹ In support of the Monte Carlo analysis, the distributions of possible values for each of the input parameters were estimated.^{18, 29, 48, 130} The Monte Carlo algorithm randomly selects a set of parameter values from the distributions for the bioassay animals and runs the PBPK model to obtain dose metric values for each of the bioassay dose groups. This process is repeated the large number of times until a distribution of dose metrics has been obtained that is stable. A similar process is performed using parameter values from the distributions for the human to obtain a dose metric distribution for a specified human exposure scenario.

The results of the Monte Carlo analysis²⁵⁹ indicated that the 5th and 95th percentiles of the dose metric distributions for the average daily AUC for TCA were generally within a factor of 2 to 3 of the mean in both mouse and human, and all of the 90% confidence intervals for the dose metrics ranged over somewhat less than an order of magnitude. These results are similar to those reported for a PBPK model of methylene chloride.²⁹ However, this Monte Carlo analysis was conducted with a preliminary version of the model, based on the description of Fisher and Allen,³⁵ that assumed a fixed stoichiometric yield of TCA from TCE. An uncertainty analysis of the penultimate version of the model was also conducted,²⁷¹ using the Markov Chain Monte Carlo (MCMC) technique. The version subjected to the MCMC analysis differed from the final version described here in that the final version of the model used newly published data on DCVC excretion,¹⁵² which resulted in a decrease in the parameter for the linear metabolism pathway (KFC), and newly available data on DCA in the human,¹¹⁹ which resulted in a reduction of the parameter for the production of DCA (VMRC).

In the MCMC analysis,²⁷¹ the model parameters were re-estimated on the basis of the resulting goodness of fit of model predictions to the same kinetic data sets used to develop the model.²⁵⁹ The changes in parameters resulting from the MCMC analysis were minor; the most striking exception was a 4-fold increase in the metabolic capacity (VMC), and a 3-fold decrease in the affinity (KM) in the human. These changes would actually have little impact on the predictions of the model for the metabolite of interest for the liver risk assessment (TCA), since nearly all of the TCE reaching the liver is metabolized using either set of parameters. However, they would result in a 10-fold decrease in the dose metric prediction for CHL in the lung for drinking water exposures. This result demonstrates one of the problems with MCMC analysis. The sensitivity of the model to the parameters in the case of the data sets used in the MCMC analysis (in this case, inhalation data for TCE, TCA, and TCOH), may be very low, yielding an uncertain parameter estimate, while the sensitivity of the model to the same parameters for the exposure conditions and dose metrics used in the risk assessment (e.g., kidney and lung dose metrics for drinking water exposures) may be very high, yielding an inaccurate risk estimate.

The estimated uncertainty distribution for the principal dose metric for the liver risk assessment, the AUC for TCA, was somewhat broader than that estimated for the earlier version of the model: the 5th and 95th percentiles of were approximately a factor of 6 from the geometric mean in the human, and a factor of 3 to 4 in the mouse. The overall uncertainty in PBPK-based liver risk estimates suggested by the MCMC analysis was roughly a factor of 7 between the 5th and 95th percentiles and the mean.²⁷¹

2. Lung Dose Metric

The greatest source of uncertainty regarding the calculation of the lung dose metric is the paucity of data on the metabolic production and clearance of CHL in the lung across species. Of course, there are also other sources of parameter uncertainty associated with the lung dose metric. Additional experimental data are needed before a quantitative estimate of the overall uncertainty associated with this metric could be confidently attempted. Table 3 shows the predicted lung dose metrics for the principal inhalation bioassays providing a dose-response for lung tumors,⁷⁶⁻⁷⁸ for the highest oral exposure of the mouse, for the highest rat exposures, and for several human exposure scenarios. The different exposures have been ordered according to the predicted value of the lifetime average daily dose (LADD) based on the AUC for CHL. The much lower dose metric values in the human compared to the rodent result primarily from the assumption that the clearance of CHL in the lung scales according to allometric expectations, while measured P450 activity in the lung across species is used directly. The measured lung P450 activity falls off much more rapidly than allometric expectations. These assumptions appear to yield dose metrics across species consistent with the observations of Odum and coworkers⁵ that toxicity occurred in the lungs of mice, but not rats, at similar exposure concentrations. If the scaling of CHL clearance in the lung were assumed to be similar to the observed scaling of lung P450, the dose metric in the rat would be 10-fold greater, suggesting that the lung concentrations of CHL in these studies were higher in the rats than in the mice. The uncertainty in the lung risk estimates resulting from this data gap is significant. If this more conservative scaling assumption were used, the human dose metrics would increase by as much as a factor of 500.

Table 3: Lung Tumor Dose Metrics²⁵⁹

	Chloral in Lung	
	AUC LADD ^a	C _{MAX} ^b
mouse*** - 600 ppm ^c	9.4	2.6
mouse* - 450 ppm ^d	7.9	1.6
mouse - 1000 mg/kg ^e	5.9	3.4
mouse** - 300 ppm ^c	3.7	1.0
rat - 600 ppm ^c	2.8	0.3
mouse* - 100 ppm ^d	1.6	0.3
mouse - 100 ppm ^c	1.2	0.3
mouse - 50 ppm ^d	0.8	0.2
human - 100 ppm ^f	0.016	0.003
human - 50 ppm ^g	0.01	0.002
human - 1 ppm ^h	0.002	--
human - 1 mg/L ⁱ	2x10 ⁻⁵	--

*** Significantly increased lung tumors in more than one study

** Significantly increased tumors in at least one study

* Increased tumors in at least one study (not statistically significant)

^a Lifetime average daily area under the chloral concentration curve in the tracheo-bronchial region (mg-hrs/L)

^b Maximum concentration achieved in the tracheo-bronchial region (mg/L)

^c Inhalation, 7 hrs/day, 5 days/week, 78/104 weeks (Maltoni^{77, 78})

^d Inhalation, 7 hrs/day, 5 days/week, 104 weeks (Fukuda⁷⁶)

^e Oil gavage, 5 days/week, 103 weeks (NTP 1990⁷⁴)

^f Occupational exposure (8 hrs/day, 5 days/week, 45 years) - current PEL

^g Occupational exposure - current TLV-TWA

^h Continuous inhalation over a lifetime

ⁱ Drinking water - lifetime continuous

3. Kidney Dose Metric

The overriding source of uncertainty regarding the calculation of the kidney dose metric is the inadequate and often conflicting data in the literature for the conjugative pathway. Specific data gaps include the affinity of kidney N-acetyl transferase in the human and the activities of the glutathione transferase pathway in rat and human. Table 4 shows the predicted kidney dose metrics for the principal bioassays providing a dose-response for kidney tumors,^{74, 77, 78} for the highest oral exposure of the mouse, and for several human exposure scenarios. The different exposures have been ordered according to the predicted

value of the LADD based on the production of the toxic thiol per gram of kidney tissue (KTOX). It is notable that using best estimates of the metabolic parameters for the various steps in the conjugative pathway, the model-predicted dose metrics are higher for the rat exposures associated with tumors than for the highest dose in mice. However, as with the lung target tissue, it will not be possible to provide an accurate assessment of the overall uncertainty in the kidney dose metric until consistent, reproducible data are collected.

Table 4: Kidney Tumor Dose Metrics²⁵⁹

	Reactive Thiol in Kidney LADD (KTOX) ^a
rat** - 1000 mg/kg ^b	73.6
rat - 500 mg/kg ^b	32.0
rat* - 600 ppm ^c	19.6
mouse - 1000 mg/kg ^b	13.5
rat - 300 ppm ^c	6.3
rat - 100 ppm ^c	0.23
human - 100 ppm ^d	0.23
human - 50 ppm ^e	0.09
human - 1 ppm ^f	0.008
human - 1 mg/L ^g	0.0004

** Significantly increased tumors in at least one study

* Increased tumors in at least one study (not statistically significant)

^a Lifetime average daily amount (mg) reactive metabolite generated per gram of kidney

^b Oil gavage, 5 days/week, 103 weeks (NTP, 1990⁷⁴)

^c Inhalation, 7 hrs/day, 5 days/week, 78/104 weeks (Maltoni^{77, 78})

^d Occupational inhalation (8 hrs/day, 5 days/week, 45 years) current PEL

^e Occupational inhalation - current TLV-TWA

^f Continuous inhalation over a lifetime

^g Drinking water - lifetime continuous

VII. QUANTITATIVE RISK ASSESSMENT

The dose metric calculations performed with the PBPK model can now be used to characterize acceptable human exposure to TCE. In addition to serving as the basis for extrapolation from high to low dose and across routes of exposure, the dose metrics predicted with the PBPK model also serve as the basis for extrapolation across species, on the assumption that an equal LADD in the target tissue of rodent or human results in the same lifetime cancer risk.²³⁹ Clearly, this aspect of the role of the target tissue dose metric must be carefully

considered during the selection of the dose metric to be used in the risk assessment calculations.

If available, a biologically based dose-response (BBDR) model would be preferred for performing the quantitative risk assessment.¹ Such a BBDR model would take the tissue dosimetry from the PBPK model as input and predict the resulting tumor incidence over time in rodents or humans. However, as defined by the USEPA,¹ a BBDR model cannot be simply empirical; that is, it cannot merely represent a statistical fit to bioassay tumor incidence data. Instead, the parameters in the model must have direct biological correspondence, similar to the requirements for the parameters in the PBPK model, and must have been determined on the basis of experiments other than the animal bioassays themselves. Unfortunately, no such BBDR models as yet exist, so one of the default procedures (linear or MOE) must be used instead, as will be described below.

A. Dose-Response Approaches

For each target tissue a decision must be made, on the basis of the assumed mode of action, regarding the most appropriate approach for using the dose metrics; that is, which dose-response procedure should be applied to characterize the acceptability of human exposures. Following the two-step, default dose-response assessment process described in the new USEPA guidelines, target tissue dosimetry from the PBPK model is used as input to an empirical dose-response model, such as the benchmark dose (BMD) model,²⁷² for extrapolation within the experimental range; the mode of action is then used as the basis for selecting the most appropriate default procedure to be used to extrapolate below the experimental range.

The two principal alternatives for low-dose extrapolation of animal data are the linear and nonlinear (MOE) approaches. In both of these approaches dose metrics from the PBPK model are used in the empirical dose-response model to estimate a "point of departure" for low-dose extrapolation, usually the dose associated with a 10% increase in tumor incidence in the animal bioassays (the ED₁₀), or the 95% lower bound on the ED₁₀ (the LED₁₀). In the linear option, a straight line is drawn from the point of departure to the origin (zero risk at zero dose), and the risk is estimated from the dose metric at the human exposure of interest. Under the new guidelines, this simple methodology replaces the historical use by the USEPA of the linearized multistage model⁸⁶ to perform low-dose-linear extrapolation of carcinogenic risk. In the nonlinear option, the margin between the point of departure and a human exposure of interest is calculated and the resulting MOE is evaluated in terms of its acceptability given the assumed mode of action of the chemical, as well as other considerations.

The specifics of the MOE approach have varied from one draft of the proposed guidelines to the next, and may change again before they are finalized. The most recent draft¹ indicates that the MOE should be defined as the ratio between the point of departure in the cancer dose-response analysis and the

non-cancer reference dose (RfD) or reference concentration (RfC) for the chemical, and the acceptability of this MOE should then be evaluated. Regardless of the details, however, a difficulty with the MOE approach is that, unlike the linear approach, it does not provide an estimate of risk from a given exposure. By its very nature, use of the MOE approach involves the assumption that there are inadequate data to provide an estimate of the risk below the point of departure. It is only assumed that the risk falls off much more than linearly with dose (i.e., that the overall dose-response below the point of departure is sublinear). At the same time, since there are not adequate data to estimate risk below the point of departure, the evaluation of what constitutes an adequate MOE is problematic. Various drafts of the USEPA guidelines have provided suggestions regarding issues that should be considered in the evaluation of an MOE. These considerations include human interindividual variability, uncertainty in animal to human extrapolation, severity (e.g., use of tumor data vs. preneoplastic lesions), steepness of the dose-response, and persistence. In the evaluation of adequate MOEs for TCE in this analysis, the following factors were considered:

Severity of endpoint used. Apart from any other considerations, if the LED₁₀ is based on tumor incidence a 10% incidence of cancer would not seem to be a prudent basis for defining acceptable exposure. Use of a severity factor of 10 would presumably reduce cancer incidence well below 1% given the assumed sublinear dose-response. Severity factors lower than 10 could be used for an LED₁₀ based on preneoplastic or non-neoplastic precursor data.

Uncertainty in calculation of target tissue dose. Even when a PBPK model has been used to estimate target tissue dose, there is some uncertainty regarding the accuracy of the estimates, particularly in target tissues where data are limited and model predictions are not well validated. A factor of up to 10 could be applied for the use of highly uncertain dose metrics.

Uncertainty in animal to human extrapolation of response. In general, empirical evidence suggests that, when pharmacokinetically appropriate dose metrics are used, animals and humans exposed to the same lifetime average daily dose of a chemical will sustain the same lifetime risk of cancer.²³⁹ Nevertheless, data on a specific chemical may suggest that humans may be more or less susceptible to its carcinogenic effects. In such cases a factor of 0.1 to 10 could be applied.

Human interindividual variability. The new USEPA guidelines recommend the use of a factor of 10 to assure protection of sensitive individuals.

It should be noted there is no discussion in the new guidelines of similar considerations in the use of the linear default; only cross-species adjustment of dosimetry is addressed. Presumably, many of these same issues should be evaluated in defining the acceptable level of risk for a chemical calculated by the linear method. Nevertheless, historical practice in the use of low-dose-linear cancer risks has essentially ignored the issue of human variability and has used

the default body surface area scaling as both a pharmacokinetic adjustment and an animal-to-human extrapolation uncertainty factor. Hopefully this inconsistency will be resolved by the time the proposed new guidelines are finalized by the USEPA.

B. Dose Metric Evaluation

There are two main criteria for evaluating the suitability of alternative dose metrics for use in the risk assessment: biological plausibility and consistency with tumor dose-response. The biological plausibility of the various TCE dose metrics has already been discussed. In this section, consistency of the dose metrics with the tumor dose-response will be evaluated across dose groups, exposure routes, and species, using the metric as the basis for comparison. Presumably, higher values of the dose metric should be associated with positive outcomes, while lower values should be associated with negative outcomes.

1. Liver Dose Metric

Table 5 shows the predicted liver dose metrics for the principal animal bioassays providing a dose-response for liver tumors,^{73-75, 77, 78} for the highest rat exposures in these same studies, and for several human exposure scenarios. The different exposures have been ordered according to the predicted value of the LADD based on the AUC for TCA. Bioassay exposures associated with LADDs for AUC TCA of greater than 1150 were uniformly positive, while bioassay exposures with LADDs less than 700 were uniformly negative. The highest exposures of rats produced LADDs considerably less than those producing tumors in the mouse, consistent with the negative results in the rat bioassays.

The most striking feature of the results for this target tissue compared to the lung and kidney is that two of the three highest dose metrics were obtained for the human occupational exposure scenarios. The relatively high LADDs for AUC TCA in the human reflect the much slower clearance of TCA compared to the rodent.

Table 5: Liver Tumor Dose Metrics²⁵⁹

	TCA	
	AUC ^a	C _{MAX} ^b
human - 100 ppm ^c	5490	413
human - 50 ppm ^d	2854	215
mouse*** - 600 ppm ^e	1748	157
mouse*** - 600 ppm ^f	1488	175
mouse** - 300 ppm ^e	1322	123
mouse** - 2339 mg/kg ^g	1270	134
mouse** - 1739 mg/kg ^g	1184	126
mouse** - 1000 mg/kg ^h	1184	111
mouse - 300 ppm ^f	1135	138
mouse** - 1169 mg/kg ^g	1069	115
mouse - 869 mg/kg ^g	986	107
mouse** - 100 ppm ^e	798	76
mouse - 100 ppm ^f	687	86
rat - 1000 mg/kg ^h	331	23
rat - 600 ppm ^f	249	23
human - 1 ppm ⁱ	303	13
human - 1 mg/L ^j	14	0.6

*** Significantly increased lung tumors in more than one study

** Significantly increased tumors in at least one study

^a Lifetime average daily area under the plasma concentration curve (mg-hrs/L)

^b Maximum concentration achieved during exposure (mg/L)

^c Occupational inhalation (8 hrs/day, 5 days/week, 45 years) - current PEL

^d Occupational inhalation - current TLV-TWA

^e Inhalation, 6 hrs/day, 5 days/week, 104 week (Bell⁷⁵)

^f Inhalation, 7 hrs/day, 5 days/week, 78/104 week (Maltoni^{77, 78})

^g Oil gavage, 5 days/week, 78/90 week (NCI 1976⁷³)

^h Oil gavage, 5 days/week, 103 week (NTP 1990⁷⁴)

ⁱ Inhalation - lifetime continuous

^j Drinking water - lifetime continuous

2. Lung Dose Metric

The lung dose metric calculations shown in Table 3 can be used to evaluate the consistency of the CHL dose metrics with the bioassay results for the lung. As mentioned previously, the entries in the table are arranged in decreasing order of LADD for AUC CHL. Bioassay exposures associated with higher LADDs for AUC CHL tended to be positive, while bioassay exposures with lower AUC CHL LADDs were negative. Neither metric is consistent with the fact that the oral mouse bioassays were negative for lung tumors, suggesting that direct uptake of inhaled TCE into the respiratory tract epithelium could increase lung tissue exposure compared to oral dosing.

3. Kidney Dose Metric

The kidney dose metric calculations shown in Table 4 can be used to evaluate the consistency of the KTOX dose metric with the bioassay results. Bioassay exposures associated with higher KTOX LADDs tended to be positive, while bioassay exposures with lower KTOX LADDs were negative. The mouse dose metric is well below that of the rat, consistent with bioassay results. The lower dose metric values in the mouse result from the higher ratio of clearance (KNATC) to intoxication (KBLC) in the mouse as compared to the rat.

C. Point of Departure Calculations

Using the dose metrics just discussed, an ED₁₀ and LED₁₀ were calculated for each of the positive bioassays for TCE. The calculations were performed with a multistage (polynomial) dose-response model,²⁷² using the LADDs for the animal bioassays from Tables 3-5 as input. The results, shown in Table 6, are presented in terms of the internal target tissue dose metrics, in the units listed for the corresponding tissues in Tables 3-5. It can be seen that similar LED₁₀ estimates were obtained from different bioassays in the case of the lung and kidney, but individual LED₁₀ estimates in the liver ranged over more than an order of magnitude. The greater variation in the liver is partly due to an apparently higher potency of TCE when administered in corn oil gavage than when administered by inhalation, although there also appear to be sex and strain differences.

Table 6: Calculation of EDs and LEDs in Each Target Tissue^a

Study	Species	Sex	ED ₁₀ ^b	LED ₁₀ ^c
Lung (AUC CHL in lung):				
Fukuda ⁷⁶	ICR mice	F	5.5	2.4
Maltoni ^{77, 78}	Swiss mice	M	8.2	4.7
	B6C3F1 mice	F	3.6	2.4
Geometric Mean:			5.5	3.0
Kidney (KTOX):				
Maltoni ^{77, 78}	Sprague-Dawley rats	M	29.6	23.3
NTP 1990 ⁷⁴	F344 rats	M	106	55.0
Geometric Mean:			56	36
Liver (AUC TCA in blood):				
Bell ⁷⁵	B6C3F1 mice	M	<u>551</u> ^d	<u>296</u>
		F	<u>1784</u>	<u>1348</u>
Maltoni ^{77, 78}	Swiss mice	M	<u>1419</u>	<u>1210</u>
	B6C3F1 mice	M/F	<u>1797</u>	<u>1521</u>
NCI 1976 ⁷³	B6C3F1 mice	M	417	119
		F	857	496
NTP 1990 ⁷⁴	B6C3F1 mice	M	107	74
		F	270	175
Geometric Mean: (inhalation only)			1258	926

^a Calculated using polynomial benchmark dose model

^b Estimated target tissue dose metric value associated with 10% extra risk (units for metrics are provided in Tables 6-8 for the corresponding tissue)

^c 95% lower confidence limit on estimated target tissue dose metric associated with 10% extra risk

^d Underlined entries indicate preferred (inhalation) estimates

A higher apparent potency for liver cancer from oil gavage was also observed in the case of vinyl chloride.³¹ In that case, risks of liver angiosarcoma estimated from the administration of vinyl chloride in the diet were in good agreement with those obtained from inhalation bioassays, showing good route-to-route correspondence of potency based on the pharmacokinetic dose metric. However, the estimates based on oral gavage of VC in vegetable oil were about 6-fold higher than those from either dietary or inhalation exposure. It has previously been noted in studies with chloroform that administration of the chemical in corn oil results in more marked hepatotoxic effects than observed when the same chemical is provided in an aqueous suspension.²⁷³ It has also

been demonstrated that administration of corn oil alone leads to an increase in peroxisomal oxidative enzyme activity in rats.¹⁹¹ The toxicity and oxidative environment created in the liver by continual dosing with large volumes of vegetable oil could serve to potentiate carcinogenic processes in the liver. In support of this suggestion,²⁷⁴ found that incorporation of corn oil into the diet increased the yield of aflatoxin B₁-induced tumors in rats. A similar phenomenon is likely to be responsible for the apparently higher potency of vinyl chloride and TCE when administered by oil gavage as compared to inhalation. Consistent with this rationale, the recent USEPA risk assessment for vinyl chloride³² based the inhalation potency on the inhalation bioassays and the oral potency on the dietary bioassay, and did not use the oil gavage bioassay in obtaining potency estimates. In the case of TCE there is no dietary bioassay, so the geometric mean of the LED₁₀s from the inhalation bioassays was used in this analysis as the point of departure for both the oral and inhalation liver cancer risk estimates. To obtain the human equivalent LED₁₀, the mouse LED₁₀ was divided by four, based on the observed ratio of free fraction TCA in the plasma,²⁴⁰ effectively adjusting for species differences in the relationship between the target tissue dose metric, AUC TCA in the liver, and the metric predicted by the model, AUC TCA in the blood.

In the case of the lung or kidney, based on an assumption of equivalent target tissue response across species, target tissue doses estimated from the animal bioassays are assumed to be appropriate for humans as well. All that remains is to use the appropriate human dose metrics from Tables 3-5 to convert the internal target tissue dose metrics to external human exposure concentrations. Using the geometric mean for the internal LED₁₀ for each target tissue and dose metric from Table 6, along with the human dose metrics from Tables 3-5, the external LED₁₀ was calculated for both inhalation and drinking water exposure. The results are shown in Table 7, where the units of the LED₁₀ are now $\mu\text{g}/\text{m}^3$ of TCE in air for continuous lifetime inhalation and $\mu\text{g}/\text{L}$ of TCE in drinking water for continuous lifetime exposure.

Using the linear option under the new USEPA guidelines, calculation of the exposure level associated with a risk of one in a million is simply a matter of dividing the LED₁₀ by 100,000. In the case of the nonlinear MOE option, the point of departure is simply divided by an environmental concentration or the RfD/RfC and the resulting MOE is evaluated for acceptability, using the considerations discussed above in the section on dose-response approaches.

Table 7: Comparison of Margin of Exposure (MOE) and Linear Estimates of Virtually Safe Concentrations for Continuous Lifetime Exposure to TCE*

Tissue	LED ₁₀ ^a ($\mu\text{g}/\text{m}^3$ or $\mu\text{g}/\text{L}$)	10 ⁻⁶ Risk Level ^b ($\mu\text{g}/\text{m}^3$ or $\mu\text{g}/\text{L}$)	MOE ^c	VSC ^d ($\mu\text{g}/\text{m}^3$ or $\mu\text{g}/\text{L}$)
Inhalation ($\mu\text{g}/\text{m}^3$)				
Lung	8.0x10 ⁶	80	10000	800^e
Kidney	2.4x10 ⁷	240	3000	8000
Liver	6.6x10 ⁴	0.66	1000	66
Drinking Water ($\mu\text{g}/\text{L}$)				
Lung	1.5x10 ⁸	1500	10000	15,000
Kidney	9.0x10 ⁷	900	3000	30,000
Liver	2.6x10 ⁵	2.65	1000	265

^a 95% lower bound on lifetime human exposure concentration corresponding to an extra risk of 10%

^b Lifetime human exposure concentration associated with an extra cancer risk of one in one-million based on linear extrapolation below the LED₁₀

^c Margin of exposure below LED₁₀ considered adequate (unitless)

^d Virtually Safe Concentration for lifetime human exposure based on adequate MOE below LED₁₀

^e Bolded results indicate preferred dose-response options

In the following sections, the mode-of-action information on each target tissue will be evaluated, and a recommendation will be made regarding the preferred dose-response option. In addition, in order to explore the issues that will be faced by the risk assessor and risk manager under the proposed guidelines, an attempt will be made to determine how much of an MOE would be necessary in each case to consider an exposure to be acceptable. For this purpose we will make use of the concept of a virtually safe concentration (VSC), which will be defined as (1) a concentration entailing a linear risk estimate of one in a million or less, or (2) a concentration that is below the LED₁₀ by an adequate MOE.

1. Liver

The most plausible mode of action for the carcinogenicity of TCE in the liver of the mouse appears to involve a selective mitogenic stimulus of enhanced cell proliferation in a subpopulation of hepatocytes by one or both of the metabolites, TCA and DCA. There is no evidence that mutagenicity from TCE or any of its metabolites plays a significant role in the production of these liver tumors. Just as there is an observable threshold for the induction of peroxisome proliferation by these chemicals, it is highly likely that there is a similar threshold for their mitogenic effects. Therefore, the nonlinear dose-response option would seem to be more appropriate. The MOE approach has been recommended for chemical

carcinogenesis associated with PPAR- α activation.²⁷⁵ Factors to be considered in determining an adequate MOE include 10 for human variability and 10 for the use of a severe endpoint (tumors). On the other hand, to the extent that TCA is responsible for the liver carcinogenicity of TCE there does not appear to be a need to increase the desired MOE by a factor representing uncertainty in the animal to human extrapolation of response. Given the markedly lower response of humans to the peroxisomal effects of chemicals, such as TCA, that interact with PPAR- α , it is likely that humans would be less, rather than more, sensitive to the mitogenic effects of this metabolite. In particular, human liver lysates have been found to contain more than 10-fold lower amounts of PPAR- α DNA binding activity than mouse liver lysates,²⁷⁶ suggesting at least a 10-fold reduction in the MOE would be justified. However, there is much more uncertainty regarding the mode of action, and therefore the relative human sensitivity, for DCA than for TCA, and it is not possible at this time to rule out a contribution of DCA to the liver tumorigenicity of TCE. Moreover, in the case of DCA there is unacceptable uncertainty regarding the estimation of the target tissue dose metrics in both mouse and human. Therefore, while an MOE of less than 100 might be considered adequate for a mode of action based solely on the effects of TCA, an MOE of 1000 seems preferable to consider the uncertainty regarding the potential role of DCA. The VSCs for liver tumors based on an MOE of 1000 are shown in Table 7.

2. Lung

The most plausible mode of action for the carcinogenicity of TCE in the lung of the mouse appears to involve sustained reparative hyperplasia secondary to repeated toxicity from CHL produced, but poorly cleared, from Clara cells. There is no evidence that mutagenicity from TCE or any of its metabolites plays a significant role in the production of these lung tumors, although mutagenicity from CHL could lead to a small but finite risk below the threshold for toxicity. Nevertheless, it seems unlikely that TCE would be a lung carcinogen in the mouse bioassays if it were not for the production of toxicity and resulting hyperplasia. Therefore, the nonlinear dose-response option would seem to be more appropriate. Factors to be included in determining an adequate MOE include 10 for human variability and 10 for the use of a severe endpoint (tumors). There does not appear to be a need to increase the desired MOE by a factor representing uncertainty in the animal to human extrapolation of response, since an average human is unlikely to be more sensitive than the mouse to the toxicity of CHL in the lung. On the other hand, there is multiple order of magnitude uncertainty regarding the estimation of the target tissue dose metrics in humans. Therefore, an MOE of 10000 might be desired in the case of a mode of action based on toxicity from CHL produced in the lung. As shown in Table 7, the VSCs for lung tumors based on this MOE are well above those for the liver.

3. Kidney

The most plausible mode of action for the carcinogenicity of TCE in the kidney of the rat appears to involve sustained reparative hyperplasia secondary

to repeated toxicity from a reactive thioketene produced from 1,2-DCVC by β -lyase in the kidney. There is also evidence that mutagenicity from 1,2-DCVC could play a significant role in the production of these kidney tumors. Nevertheless, it seems unlikely that TCE would be a kidney carcinogen in the rat bioassays if it were not for the production of toxicity and resulting hyperplasia. Therefore, the nonlinear dose-response option would seem to be more appropriate. Factors to be included in determining an adequate MOE include 10 for human variability and 10 for the use of a severe endpoint (tumors). There does not appear to be a need to increase the desired MOE by a factor representing uncertainty in the animal to human extrapolation of response, since humans are unlikely to be more sensitive than the rat to the toxicity of the thioketene in the kidney. However, there is significant uncertainty regarding the estimation of the target tissue dose metrics in both the rat and the human, and there is even some question whether the mode of action is well established. Therefore, an overall MOE of 3000 might be desired in the case of a mode of action based on toxicity from 1,2-DCVC in the kidney. The VSCs for kidney tumors based on this MOE, shown in Table 7, are well above those for the liver. However if the linear default is used, out of concern for the mutagenicity of 1,2-DCVC, the VSCs based on one in a million risk for the kidney would be similar to those derived using an MOE approach in the liver.

D. Risk Characterization

In the risk characterization, the information relevant to the carcinogenicity of the chemical must be summarized, with particular attention to the potential impact of uncertainty in both the qualitative and quantitative aspects of the risk assessment. The results of the quantitative risk assessment for TCE are shown in Table 7. Based on mode-of-action considerations, the nonlinear (MOE) approach is considered to be more appropriate for the liver and lung target tissues, while both the linear and nonlinear approach are considered appropriate for the kidney target tissue. The selection of these dose-response approaches for a TCE risk assessment is consistent with the recommendations of the expert panel convened by the USEPA to consider the application of the proposed cancer guidelines to TCE.²⁷⁷ Because of the range of MOEs considered to be adequate, VSCs for lifetime human exposure to TCE are 10- to 100-fold higher when the nonlinear approach is used instead of the linear approach.

Of the recommended dose-response options (nonlinear in liver and lung, linear and nonlinear in kidney) the lowest VSCs were obtained for the liver, assuming a mode of action involving the effects of TCA. The points of departure (lower bound estimates of the exposure associated with 10% tumor incidence) for lifetime human exposure to TCE, derived for mouse liver tumors and assuming a mode of action primarily involving the mitogenicity of TCA, are 66 milligrams TCE per cubic meter in air and 265 milligrams TCE per liter in drinking water. The associated linear unit risk estimates for mouse liver tumors are 1.5×10^{-6} for lifetime exposure to 1 microgram TCE per cubic meter in air and 0.4×10^{-6} for lifetime exposure to 1 microgram TCE per liter in drinking water. However, these risk estimates ignore the evidence that the human is likely to be

much less responsive to the carcinogenic effects of TCE than the mouse, and that the effects of TCE are unlikely to occur at low environmental exposures. Based on consideration of the most plausible carcinogenic modes of action of TCE, environmental exposures below 66 micrograms TCE per cubic meter in air and 265 micrograms TCE per liter in drinking water are unlikely to present a carcinogenic hazard to human health.

As shown in Table 8, these estimates of safe exposure levels, which use pharmacokinetic dose metrics and the nonlinear dose-response option, are roughly a factor of 100 higher than those previously estimated by the USEPA.^{22, 23} However, if the linear dose-response option had been selected, the estimates of safe exposure levels calculated in this study would have been essentially identical to those previously published by the USEPA. Thus the determination of an acceptable environmental exposure to TCE is highly dependent on the judgment regarding the nature of the dose-response for the carcinogenicity of TCE.

Table 8: Comparison of Estimated Virtually Safe Concentrations for Human TCE Exposures

<u>Basis</u>	<u>Inhalation</u> ($\mu\text{g}/\text{m}^3$)	<u>Drinking Water</u> ($\mu\text{g}/\text{L}$)
Linear: (10^{-6} risk)		
EPA 1985/1987 ^{22, 23} (Total metabolism scaled by body surface area)	0.6 – 0.8	3.1
This Study (AUC TCA in the liver)	0.66	2.65
Margin of Exposure: (1000 below LED₁₀)		
This Study (AUC TCA in the liver)	66	265

Uncertainty has been a major focus of the discussion already presented in this paper. One of the most frustrating aspects of evaluating uncertainty is that the areas with the greatest uncertainty are generally the most difficult to evaluate in terms of the potential quantitative impact of that uncertainty on the risk assessment. The most important quantitative uncertainties in the current risk assessment for TCE fall into two categories: pharmacokinetic and pharmacodynamic. The pharmacokinetic uncertainties result from the lack of data on the capacity and affinity of key enzymes associated with the dose metrics for the lung and liver target tissues. The pharmacodynamic uncertainties result from lack of data on the relative responsiveness of human liver cells to a mitogenic stimulus from DCA or TCA. Overlaid on these quantitative uncertainties are the substantial qualitative uncertainties that still remain regarding the mechanism of carcinogenicity of TCE in each of the primary target tissues (as well as in other potential target tissues suggested by human studies).

Figure 9 attempts to summarize the results of the present quantitative risk assessment 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 risk estimate, was suggested by Rodricks.²⁷⁸ 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 (MOE approach for the kidney).

Under the new USEPA guidelines, the principal product of the risk characterization is a short hazard narrative, which attempts to summarize the nature of the conditions under which the chemical might be carcinogenic in humans. In the case of TCE, the hazard narrative might consist of information similar to the following:

"Trichloroethylene (TCE) should be considered as if it were a likely human carcinogen, under some conditions of exposure, via both inhalation and oral routes. Evidence for the carcinogenicity of TCE comes primarily from animal studies: lung and liver tumors in mice, and kidney tumors in rats. Occupational exposures of workers to relatively high concentrations of TCE have sometimes been associated with increased incidences of liver/biliary tract tumors, non-Hodgkins lymphoma, or kidney tumors, although no single study has provided unequivocal evidence of carcinogenicity. None of the animal tumors have been reproduced in more than one species. The mouse tumors represent increased incidences of spontaneous lesions with a relatively high background incidence, while the rat kidney tumors represent a very low incidence of a relatively rare tumor type in a sensitive tissue.

In each of the animal target tissues for the carcinogenicity of TCE there is evidence for a mode of action involving enhanced cell proliferation due to the effects of one or more of the metabolites of TCE: mitogenicity from trichloroacetic acid and dichloroacetic acid in the liver, toxicity from chloral in the lung, and toxicity from a reactive thioketene in the kidney. Except in the kidney, where the mutagenicity of the reactive thioketene is of concern, there is no evidence that a genotoxic mode of action is responsible for the observed tumors. In general, the tumorigenicity of TCE does not appear to be relevant to low environmental exposures, and a nonlinear, margin of exposure approach is recommended for determining acceptable human exposures.

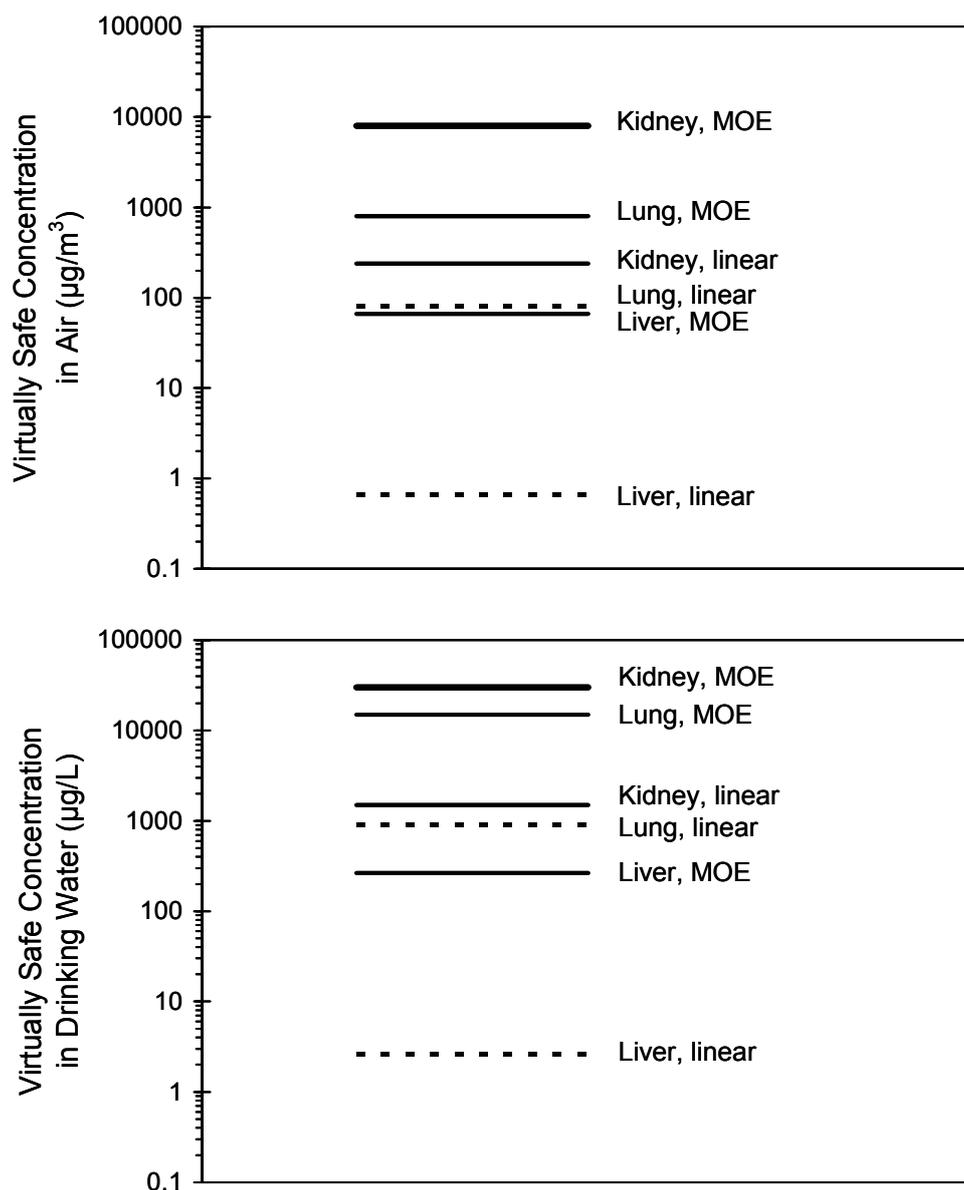


FIGURE 9. Comparison of virtually safe concentrations for trichloroethylene in air (top) and drinking water (bottom). Dashed lines indicate approaches that are not recommended. Broader solid line identifies the preferred approach based on biological plausibility.

The lowest points of departure (lower bound estimates of the exposure associated with 10% tumor incidence) for lifetime human exposure to TCE, derived for mouse liver tumors and assuming a mode of action primarily involving the mitogenicity of TCA, are 66 milligrams TCE per cubic meter in air and 265 milligrams TCE per liter in drinking water. The associated linear unit risk estimates for mouse liver tumors are 1.5×10^{-6} for lifetime exposure to 1 microgram TCE per cubic meter in air and 0.4×10^{-6} for lifetime exposure to 1 microgram TCE per liter in drinking water. However, these risk estimates ignore the evidence that the human is likely to be much less responsive to the carcinogenic effects of TCE than the mouse, and that the effects of TCE are

unlikely to occur at low environmental exposures. Based on consideration of the most plausible carcinogenic modes of action of TCE, environmental exposures below 66 micrograms TCE per cubic meter in air and 265 micrograms TCE per liter in drinking water are considered unlikely to present a carcinogenic hazard to human health. However, when present in the groundwater, TCE presents an additional concern due to the potential for bioconversion to vinyl chloride, a known human genotoxic carcinogen."

E. Identification of Data Gaps

Another feature of the new cancer guidelines is an emphasis on iterative risk assessment. Rather than delaying the risk assessment on a chemical indefinitely, waiting for additional information, an iterative process is described involving multiple risk assessments for a chemical over time, each based on the data available at the time and identifying the data that would improve the next risk estimate. In the case of TCE, a number of current data gaps have been described in this paper. Key pharmacokinetic data gaps that would significantly reduce uncertainty in the current risk assessment include:

(1) Data on the kinetics of DCA following administration of TCE in the mouse and human (and with lower priority, rats). Data on the kinetics of DCA following administration of TCA is also needed.

(2) Data on metabolism of TCE and its metabolites in the lungs of mice and humans (and with a lower priority, rats). Of highest priority is additional information on the production and clearance of chloral in the lung, involving the P450 enzymes, aldehyde dehydrogenase and glucuronyl transferase.

(3) Data on the glutathione-dependent metabolism of TCE in the liver and on the metabolism of products of this pathway in the kidney for rats and humans (and with lower priority, mice). Of highest priority is additional information on the production, intoxication, and clearance of 1,2-DCVC, involving liver glutathione transferase, and kidney β -lyase and N-acetyl-transferase.

VIII. DISCUSSION

The risk assessment for TCE has been presented in an attempt to illustrate the issues associated with implementing the new USEPA cancer guidelines. In the following discussion, some of these issues will be addressed in the more general context of risk assessments for other chemicals.

A. Target Tissue Correspondence

None of the tumors observed in TCE bioassays were reproduced in both mice and rats. This behavior can be contrasted with that of a "trans-species" carcinogen, such as vinyl chloride, which produces the same rare tumors (angiosarcoma) in the same target tissue (the liver) in all animal species tested, as well as in humans, with similar potency.²⁷⁹ The lack of site correspondence

across animal species for a carcinogen clearly has important implications for the expectation of site correspondence between animals and humans (which is implicit in the pharmacokinetic approach for risk assessment). In the case of such "opportunistic" carcinogens, which seem to exploit species-specific sensitivities, it should be considered possible that the target tissue in the human would also be a species-specific, sensitive tissue, which for the human might include tissues such as the hematopoietic and lymphatic systems, colon, lungs, mammary glands, and prostate.

B. Dose Metric Selection

Selection of the metric to be used to characterize target tissue dose is a crucial step in the quantitative risk assessment for a chemical. Whether intended or not, any dose metric will be appropriate for some modes of action and not for others. For example, the use of the AUC for the parent chemical is appropriate for a directly genotoxic chemical such as ethylene oxide, which is detoxified by metabolism; however, it would not be appropriate for a chemical like vinyl chloride, which requires metabolic activation to be genotoxic. In the latter case, increased metabolism would increase the exposure to the genotoxic species but would decrease a dose metric based on parent chemical AUC.

Dose metric selection has a particularly significant impact on the prediction of target tissue dose across species. The traditional USEPA dose metric, mg administered/kg/day scaled by body surface area, provides a reasonable estimate of the AUC for the parent chemical across species, and predicts that humans would receive a relatively greater target tissue dose for the same administered dose expressed in mg/kg. This dose metric is an appropriate default for chemicals that act through a mode of action involving the parent chemical itself (not requiring metabolic activation), such as ethylene oxide. Administered dose with body surface area scaling is also appropriate for a mode of action involving a stable metabolite, as in the case of TCE. In both cases, body surface area scaling of the administered dose provides a rough estimate of the AUC for the active form (parent chemical or stable metabolite). There is a wealth of literature on the cross-species scaling of toxicity from pharmaceuticals and other toxic chemicals that are detoxified by metabolism, showing that AUC is the appropriate cross-species measure of tissue exposure.²³⁹ Because metabolism falls off relative to body weight,²³⁹ the AUC in the human is relatively larger than in the animal for the same dose (i.e., the human receives the greater exposure).

However, the traditional USEPA dose metric provides incorrect predictions for chemicals like methylene chloride or vinyl chloride, which act through a mode of action involving a reactive metabolite. In this case, the same tendency of metabolism to fall off relative to body weight results in a lower rate of generation of the reactive metabolite in the human on a mg/kg/day basis. Thus for this mode of action the human receives a much lower exposure to the reactive metabolite than would be predicted by the traditional USEPA dose metric.

C. Cross-Species Scaling

In their application of a PBPK model in the cancer risk assessment for methylene chloride, USEPA chose to adjust the pharmacokinetic dose metrics by a body surface area scaling factor to consider potential species differences in pharmacodynamics.²⁴ More recently, however, the USEPA, in a joint effort with scientists from several other agencies, prepared a review paper on the issue of cross-species scaling of cancer,²³⁹ concluding that "...tissues experiencing equal average concentrations of the carcinogenic moiety over a full lifetime should be presumed to have equal lifetime cancer risk." Consistent with this conclusion, the recent USEPA risk assessment for vinyl chloride³² did not apply body surface area scaling to the pharmacokinetic dose metrics obtained from the PBPK model. The approach for cross-species scaling described in this paper similarly assumes that equal lifetime-average daily values of the pharmacokinetic dose metric in the target tissue of rodent and human entailed equal lifetime risk of cancer. This approach is scientifically reasonable and is consistent with empirical observations of cancer incidence across species.²³⁹

D. Use of Preneoplastic or Non-neoplastic Lesions

One of the more significant new options described in the guidelines is the possibility of using data on preneoplastic lesions or on a non-neoplastic process to inform the nature of the dose-response below the observable tumorigenic regime. However, it has yet to be demonstrated how this might be accomplished. In the case of data on preneoplastic lesions, the unknown quantity is the dose-dependent conversion rate of the preneoplastic lesions to neoplastic lesions. In the case of non-neoplastic data, such as generalized hyperplasia measured by a labeling index, the unknown quantity is the relationship between the rate of cell division and the production of tumors prior to normal death. There is no reason to suspect that either of these quantities can be represented by a linear function.

This is not to say, of course, that such data might not be useful for evaluating the general nature of the tumor dose response (linear or nonlinear), or to estimate an effective threshold for tumorigenicity based on the threshold for an obligatory precursor effect. What is not yet clear is how such data might be used in a quantitative fashion to extend the dose response below that of the tumor data. Ongoing studies with chloroform^{172, 180-183, 242} and formaldehyde^{243, 244} may produce the first opportunity to examine this question. As mentioned earlier, a similar problem exists with respect to the use of BBDR models in a cancer risk assessment. Although such models are given priority in the new USEPA guidelines, none currently exist. In fact, the development of a validated BBDR model capable of predicting tumor incidence in both rodents and humans for any chemical appears to be many years in the future.

E. Human Variability

The linear dose-response approach estimates the risk for an "average" individual, while the nonlinear approach applies an uncertainty factor of 10 to

account for human variability and the possibility of sensitive subpopulations. Clearly, human variability plays an important role in determining the actual risk to an individual as compared to the average risk to a population, even in the case of genotoxic carcinogens. By far the most important pharmacokinetic variability impacting target tissue dose is in metabolism. Studies of human populations have shown that the activity of the CYP enzymes can vary by more than a factor of ten between individuals,^{280, 281} and that there are genetic differences (polymorphisms) between individuals with high activity and low activity that are associated with a different susceptibility to cancer.²⁸² Genetic polymorphisms of the CYP enzymes across racial and ethnic groups have been observed,²⁸³ as have quantitative differences in metabolic capacity.²⁸⁴ Sex differences in the excretion of TCE metabolites have also been noted,²⁸⁵ with females excreting a larger proportion of TCA and a smaller proportion of TCOH than males.

F. Iterative Risk Assessment

The new USEPA guidelines describe an iterative risk assessment process, in which multiple risk assessments are performed on a chemical over time as significant new information becomes available. However, it is difficult to describe a general set of criteria to identify when it would be appropriate to re-evaluate a risk assessment on the basis of new biological or chemical-specific information. One criterion should certainly be evidence that the existing risk assessment is in some way inappropriate or inaccurate for the chemical of concern. For example, the knowledge that use of a low-dose linear approach is inappropriate for a nongenotoxic chemical whose carcinogenicity is mediated by cell proliferation provides a reason for attempting to identify an alternative approach. On the other hand, the inadequacy of the existing approach is not an acceptable justification for adopting a new approach that is also inadequate. For example, the linearized multistage model was developed in response to the observed instability of low dose extrapolation methods, in order to provide a stable statistical upper confidence limit on low dose risk.⁸⁶ The application of BBDR models for low dose extrapolation, without first addressing the instability issue, would clearly be premature. A second criterion for updating a risk assessment, then, is evidence that the proposed approach is scientifically sound and reasonably certain. This criterion, while easy to state, is hard to apply.

Even if there is not sufficient information on a chemical to support a full PBPK description including the metabolites, the use of a dose measure that is clearly not biologically correct is simply not supportable. If route-to-route extrapolation is required, such as in the calculation of drinking water risks from inhalation studies or vice versa, a parent chemical pharmacokinetic model may suffice to provide a meaningful measure of parent chemical dose. In other cases, simple semi-empirical models or classical compartmental descriptions may be adequate to provide an improved dose metric compared to administered dose or exposure concentration.

In summary, the incorporation of biological information in risk assessment requires a continuing interaction between scientific researchers and risk assessment scientists. Studies on chemicals with significant human risk implications should be designed while keeping the goal of impacting the quantitative risk assessment process in mind. In the words of one wit: "If you don't have a target, they're just arrows on the wall." Excellent examples of such risk assessment oriented studies are the ongoing investigations into the carcinogenicity of chloroform^{172, 180-182, 242} and formaldehyde^{243, 244} at the CIIT Centers for Health Research. Publication of preliminary suggestions for potential alternative risk assessment approaches based on biological information^{51, 243} is also helpful in moving the process forward, even when there is not yet sufficient data to perform an actual risk assessment with confidence. Such preliminary approaches promote consideration of the underlying assumptions and uncertainties, provide an opportunity for the exchange of ideas between the different disciplines, and suggest needed research.

In spite of the potential synergism associated with the interaction of scientific research and risk assessment, there is a key difference in the two processes with highly significant implications. The scientific method is an iterative process in which hypotheses are generated, tested, and revised in the light of contradictory data. This process necessarily entails a likelihood of false steps, and the history of science is replete with examples where the general consensus of scientists was wrong. Risk assessment, on the other hand, 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 is not the same as the level of certainty required to embrace a new risk estimate, particularly when the cost of being mistaken is at the expense of human health.

G. Recommendations

In general, both qualitative and quantitative studies provide important information for assessing the effects of chemicals on the body. 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. The next step in a biologically-based risk assessment procedure is to develop quantitative models of mode of action (pharmacodynamics) and tissue dosimetry (pharmacokinetics). These concepts are present in the proposed USEPA cancer risk assessment guidelines. The relationship between the qualitative concepts and quantitative tools are shown schematically in Figure 10. As defined in the guidelines, a BBDR model represents the ideal of having a fully developed and validated dose-response model combining relatively complete pharmacodynamic and pharmacokinetic descriptions. Although not clearly described in the guidelines, intermediate options between the full-information (BBDR) model and the no-information defaults can usually be described. Given the difficulty of developing a full BBDR model, these intermediate options are likely to provide

the main opportunity to use chemical-specific data to improve risk assessments in the foreseeable future.

Qualitative Information

Quantitative Tools

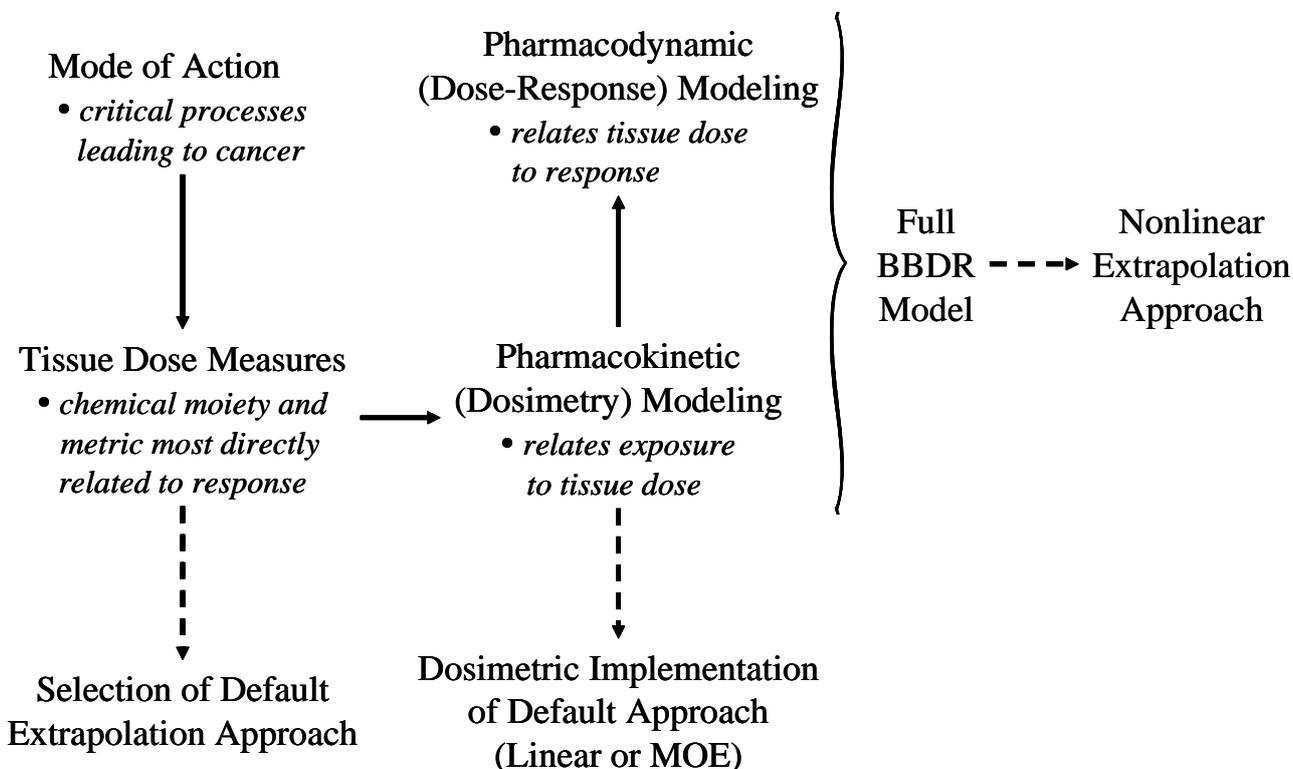


FIGURE 10. Incorporation of scientific information in cancer risk assessment under the proposed USEPA guidelines.

The new USEPA cancer guidelines describe the marshaling and interpretation of mode-of-action information to make qualitative decisions about low dose extrapolation strategies. As described in the guidelines, both modeling within the observable range and low dose extrapolation would preferably be based on a BBDR model, if available. 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 chemical exposure to risk of cancer. To do this successfully, the BBDR model must specify the pharmacokinetic relationship between applied and tissue dose as well as the pharmacodynamic relationship between tissue dose and response for different exposures and for different species.

In the absence of such a model a default approach is described in which tumor incidence data are analyzed to estimate a point of departure, adjustments are made to derive a human equivalent concentration, and then either linear extrapolation is used to estimate low-dose risks or MOE comparisons are made. This approach would be appropriate, for example, for a compound where mode-

of-action considerations were sufficiently well supported that a determination could be made regarding the method of low-dose extrapolation, but the nature of the toxic moieties associated with the effects remain unknown. This default approach, as described by the guidelines, employs dosimetry corrections that implicitly presume that the parent compound is the toxic species. The specific adjustments described in the guidelines are body weight to the three-quarters power scaling for oral administration and RfC-style human equivalent concentration calculations for inhaled compounds.

An intermediate approach, using a chemical-specific dosimetry model, is the option used in this analysis with TCE. In this case, the mode of action generally supported non-linear extrapolation, but the relationship of tumor incidence to measures of tissue dose was not known with certainty over a wide range of doses. In particular, no data were available to support quantitative cross-species extrapolation of this relationship with a BBDR model. Nevertheless, the mode-of-action analysis still played a crucial role in assessing the relevant measure of tissue dose. In the TCE example, the modes of action entailed altered proliferative responses due to mitogenic or cytotoxic interactions. Each target tissue was associated with a preferred internal dose measure (AUC TCA in liver, production of reactive thioketene in kidney, and AUC chloral in lung) associated with the cell proliferative responses. However, the exact relationship of the dose metric to tumor incidence was not known. In this case, the calculations from the pharmacokinetic model provided the tissue dose metrics associated with the responses in the animal bioassays. These tissue dose metrics were then used with an empirical dose-response model to estimate an LED_{10} in terms of tissue dose. For the linear option, the animal LED_{10} was used as the basis for linear extrapolation to derive the tissue dose associated with an acceptable risk. The human pharmacokinetic model was then used to estimate the exposure concentration/dose that would give rise to this value of the tissue dose metric.

In the analysis described in this paper, the non-linear option was performed differently than as described in the USEPA proposed guidelines. The MOE approach described in the guidelines is nothing more than a comparison of the LED_{10} with the RfD or RfC. Factors to be included in the determination of whether a particular MOE is adequate are discussed, but no formal determination of what would constitute an adequate MOE is performed. In the analysis performed here, the animal LED_{10} was adjusted by a set of factors to obtain a tissue dose providing an adequate MOE. As in the linear method, the human pharmacokinetic model was then used to estimate the exposure concentration/dose that would give rise to this value of the tissue dose metric. Our departure from the methodology in the proposed guidelines was motivated in part by our belief that the MOE should be based on target tissue dose rather than to the external exposure.

It should be noted that, in cases where either a full BBDR or just a pharmacokinetic (dosimetry) model are used, no additional calculation of a human equivalent concentration/dose, as described in the guidelines, is

necessary. Since in these cases the species differences in dosimetry are accounted for by the model, the application of default dosimetry adjustments, such as body-weight scaling of oral doses, would be redundant.

Trichloroethylene serves as an interesting and challenging case study in the application of mode-of-action information to shape a quantitative dose-response assessment strategy in the context of USEPA's proposed guidelines for cancer risk assessment. Issues that had to be faced included multiple tumors sites with divergent modes of action, complex metabolic pathways, and interactions of several active metabolites. In addition, as the implementation of the PBPK model for TCE was pursued, a number of quantitative dosimetry issues became more apparent. These issues reflect the difficult manner in which the qualitative information from the mode-of-action analysis is translated into a quantitative application of a pharmacokinetic model for interspecies extrapolation, and the intricacies of arriving at intermediate options between the two extremes of developing a full BBDR model and applying policy defaults. These difficulties in the application of the new USEPA guidelines are not the result of deficiencies in the guidelines, but simply exhibit the increased complexity of incorporating chemical-specific data in a risk assessment as compared to the use of generic default procedures. The reward of dealing with this additional complexity, of course, is a risk assessment that more accurately reflects the scientific information available on the carcinogenicity of the chemical and, hopefully, provides a more definitive estimate of human risk.

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Table 2: Summary of Bioassay Results for Trichloroethylene and Its Metabolites

Study	Strain/Species	Sex	Duration	Endpoint	Administered Dose ^a	Incidence
TRICHLOROETHYLENE - GAVAGE						
NCI 1976 ⁷³	B6C3F1 mice	M	5 d/w, 78 w	Hepatocellular carcinoma	0, 1169, 2339	1/20, 26/50*, 31/48*
	B6C3F1 mice	F	5 d/w, 78 w	Hepatocellular carcinoma	0, 869, 1739	0/20, 4/50, 11/47*
NTP 1990 ⁷⁴	B6C3F1 mice	M	5 d/w, 103 w	Hepatocellular carcinoma Hepatocellular adenoma and carcinoma	0, 1000	8/48, 31/50* 14/48, 39/50*
	B6C3F1 mice	F	5 d/w, 103 w	Hepatocellular carcinoma Hepatocellular adenoma and carcinoma	0, 1000	2/48, 13/49* 6/48, 22/49*
Van Duuren ²⁸⁶	Swiss mice	M/F	1 d/w, 622 d	c	0, 2.8	NS
Henschler ²⁸⁷	ICF Swiss mice	M	5 d/w, 18 m	c	0, 1900	NS
	ICF Swiss mice	F	5 d/w, 18 m	c	0, 1400	NS
NCI 1976 ⁷³	Osborne mendel rats	M/F	5 d/w, 78 w	c	0, 549, 1097	NS
NTP 1990 ⁷⁴	F344/N rats	M	5 d/w, 103 w	Renal tubular cell adenoma Adenoma and carcinoma	0, 500, 1000	0/48, 0/48, 3/49* ^b 0/48, 2/49, 3/49* ^b
NTP 1988 ²³⁵	ACI, August, Marshall, Osborne-Mendel rats	M/F	5 d/w, 103 w	c	c	Inadequate ^c

Maltoni ^{77, 78}	Sprague-Dawley rats	M/F	4-5 d/w, 52 w	c	0, 50, 250	NS
TRICHLOROETHYLENE - INHALATION						
Bell ⁷⁵	B6C3F1 mice	M	6 h/d, 5 d/w, 104 w	Hepatocellular carcinoma Adenoma and carcinoma	0, 100, 300, 600	18/99, 28/95*, 31/100*, 43/97*, 20/99, 35/95*, 38/100*, 53/93*
	B6C3F1 mice	F	6 h/d, 5 d/w, 104 w	Hepatocellular adenoma and carcinoma	0, 100, 300, 600	8/99, 9/100, 10/94, 17/99*
Henschler ²⁸⁷	HAN:NMRI mice	M	6 h/d, 5 d/w, 18 m	c	0, 100, 500	NS
	HAN:NMRI mice	F	6 h/d, 5 d/w, 18 m	Malignant lymphoma	0, 100, 500	9/29, 17/30*, 18/28*
Fukuda ⁷⁶	ICR mice	F	7 h/d, 5 d/w, 104 w	Lung adenocarcinoma Adenoma and adenocarcinoma	0, 50, 100, 450	1/49, 3/50, 8/50*, 6/46*, 6/49, 5/50, 13/50, 11/46 (NS)

Table 2 (continued)

Study	Strain/Species	Sex	Duration	Endpoint	Administered Dose ^a	Incidence
Maltoni ^{77, 78}	Swiss mice	M (BT 305)	7 h/d, 5 d/w, 78 w	Pulmonary tumors (mostly benign) Hepatomas	0, 100, 300, 600	10/90,11/90,23/90*, 27/90* 4/90,2/90,8/90,13/90*
	Swiss mice	F (BT 305)	7 h/d, 5 d/w, 78 w	c	0, 100, 300, 600	NS
Maltoni ^{77, 78}	B6C3F1 mice	F (BT 306 bis)	7 h/d, 5 d/w, 78 w	Pulmonary tumors (almost exclusively benign)	0, 100, 300, 600	4/90, 6/90, 7/90, 15/90*
	B6C3F1 mice	M (BT 306 bis)	7 h/d, 5 d/w, 78 w	c	0, 100, 300, 600	NS
Bell ⁷⁵	B6C3F1 mice	M/F	7 h/d, 5 d/w, 78 w	Hepatomas	0, 100, 300, 600	4/180, 5/180, 7/180, 15/180*
	Charles River rats	M/F	6 h/d, 5 d/w, 104 w	c	0, 100, 300, 600	NS
Henschler ²⁸⁷	HAN:WIST	M/F	6 h/d, 5 d/w, 18 m	c	0, 100, 500	NS
Fukuda ⁷⁶	Sprague- Dawley rats	F	7 h/d, 5 d/w, 18 m	c	0, 50, 150, 450	NS
	Sprague- Dawley rats	M (BT 304/304 bis)	7 h/d, 5 d/w, 104 w	Leydig cell tumors (benign) Renal tubular cell tumors	0, 100, 300, 600	6/135,16/130*,30/130*,31/129* 0/135,0/130,0/130,4/129
Maltoni ^{77, 78}	Sprague- Dawley rats	F (BT 304/304 bis)	7 h/d, 5 d/w, 104 w	c	0, 100, 300, 600	NS
TRICHLOROETHYLENE - DRINKING WATER						
Herren-Freund ⁸⁰	B6C3F1 mice	M	61 w	Hepatocellular carcinoma	0, 40 mg/L	0/22, 31/32
TRICHLOROACETIC ACID c DRINKING WATER						

Bull ³	B6C3F1 mice	M/F	52 w	C	0, 1, 2 g/L	NS
De Angelo 1992 ²²⁷	F344 rat	M	100-104 w	C	0, 3.6, 36, 378	NS
De Angelo 1997 ⁸⁴	F344 rat	M	104 w	C	0, 0.05, 0.5, 5.0 g/L	NS
Ferreira- Gonzalez ¹⁵⁷	B6C3F1 mice	M	104 w	Hepatocellular carcinoma	0, 4.5 g/L	19%, 73.3%
	B6C3F1 mice	F	360 d	Hepatocellular carcinoma	0, 2, 6.67, 20 mmol/L	0/40, 0/40, 0/19, 5/20*
Pereira ⁸³	B6C3F1 mice	F	576 d	Hepatocellular adenoma	0, 2, 6.67, 20 mmol/L	2/90, 4/53, 3/27, 7/18*
				Hepatocellular carcinoma		2/90, 0/53, 5/27*, 5/18*
Herren- Freund ⁸⁰	B6C3F1 mice	M	61 w	Hepatocellular carcinoma Hepatocellular adenoma	0, 5 g/L	0/22, 7/22* 2/22, 8/22*

Table 2 (continued)

Study	Strain/Species	Sex	Duration	Endpoint	Administered Dose ^a	Incidence
DICHLOROACETIC ACID - DRINKING WATER						
Herren-Freund ⁸⁰	B6C3F1 mice	M	61 w	Hepatocellular carcinoma Hepatocellular adenoma	0, 5 g/L	0/22, 21/26* 2/22, 25/26*
Daniel ⁸²	B6C3F1 mice	M	104 w	Hepatocellular carcinoma Hepatocellular adenoma and carcinoma	0, 93	2/20, 15/24* 3/20, 18/24*
Bull ³	B6C3F1 mice	M/F	52 w	Hepatocellular carcinoma	0, 1, 2 g/L	0/2, 0/1, 5/10*
	B6C3F1 mice	F	360 d	Hepatocellular adenoma	0, 2, 6.67 20 mmol/L	1/40, 0/40, 3/20, 7/20*
Pereira ⁸³	B6C3F1 mice	F	576 d	Hepatocellular adenoma Hepatocellular carcinoma	0, 2, 6.67 20 mmol/L	2/90, 3/50, 7/28*, 16/19* 2/90, 0/50, 1/28, 5/19*
	DeAngelo 1992 ²²⁷	B6C3F1 mice	M	104 w	Hepatocellular carcinoma Hepatocellular adenoma	0, 1, 3.5 g/L
DeAngelo 1999 ²³⁰	B6C3F1 mice	M	90-104 w	Hepatocellular carcinoma Hepatocellular adenoma and carcinoma	0, 0.5, 1, 2, 3.5 g/L	26%, 48%, 71%*, 95%*, 100%*
DeAngelo 1991 ⁸¹	B6C3F1 mice	M	60 or 75 w	Hepatocellular adenoma and carcinoma	0, 7.6, 77, 486	2/28, 7/29, 3/27, 25/28*
	B6C3F1 mice	F	60 or 75 w	Hepatocellular adenoma and carcinoma	0, 94, 437	8%, 20%, 100%*
DeAngelo 1996 ¹⁸⁵	F344 rat	M	100 w	c	0, 3.6, 40.2	NS
	F344 rat	M	103 w	Hepatocellular carcinoma	0, 139	1/33, 6/28*

DeAngelo 1992 ²²⁷	F344 rat	M	100-104 w	c	0, 4.3, 48, 295	NS
DeAngelo 1991 ⁸¹	F344 rat	M	60 or 75 w	Hepatocellular adenoma and carcinoma	0, 4.3, 40	6%, 0%, 28%*
	F344 rat	F	60 or 75 w	c	0, 295	NS
CHLORAL HYDRATE - DRINKING WATER						
Daniel ⁸²	B6C3F1 mice	M	104 w	Hepatocellular carcinoma Hepatocellular adenoma and Carcinoma	0, 166	2/20, 11/24* 3/20, 17/24*
George ²²⁸	B6C3F1 mice	M	104 w	Hepatocellular Adenoma Hepatocellular Carcinoma	0, 13.5, 65, 146.6	9/42. 20/46*, 20/39*, 16/32* 23/42. 25/46. 23/39, 27/32*

^a Reported in units of mg/kg/day for oral studies and ppm for inhalation studies unless otherwise indicated.

^b Only significant by Life Table and Incidental Tumor Tests.

^c NTP has considered this study to be an inadequate study of carcinogenic activity because of chemically-induced toxicity, reduced survival, and deficiencies in the conduct of the studies.

* Statistically significant (p #0.05).

NS Not statistically significant for any dose group.

Table A-1: Parameter Values Used in the PBPK Model for TCE ²⁵⁹

Parameter	Abbreviation	Units	Mouse	Rat	Human
Body weight	BW	kg	0.035* (0.02-0.035)	0.35* (0.19-0.35)	70.0
Alveolar ventilation	QPC	L/hr ^a	30.0	24.0	24.0* (18.0)
Cardiac output	QCC	L/hr ^a	18.0	15.0	16.5* (13)
Fractional Blood Flows to Tissues:					
All Rapidly Perfused	QRC	--	0.594	0.594	0.699
Gut	QGC	--	0.141	0.153	0.181
Liver	QLC	--	0.02	0.03	0.046
Tracheo-Bronchial	QTBC	--	0.005	0.021	0.025
All Slowly Perfused	QSC	--	0.406	0.406	0.301
Fat	QFC	--	0.07	0.07	0.052
Fractional Volumes of Tissues:					
All Rapidly Perfused	VRC	--	0.165	0.106	0.101
Gut	VGC	--	0.042	0.03	0.017
Kidney	VKC	--	0.017	0.007	0.004
Liver	VLC	--	0.057	0.034	0.026
Tracheo-Bronchial	VTBC	--	0.0007	0.0007	0.0007
All Slowly Perfused	VSC	--	0.638	0.718	0.651
Fat	VFC	--	0.072	0.124	0.214
Partition Coefficients:					
Blood/Air	PB	--	14.0	18.5	9.2
Fat/Blood	PF	--	36.0	27.5	73.0
Gut/Blood	PG	--	1.8	1.3	6.8
Liver/Blood	PL	--	1.8	1.3	6.8
Rich/Blood	PR	--	1.8	1.3	6.8
Slow/Blood	PS	--	0.75	0.5	2.3
TB/Blood	PTB	--	1.8	1.3	6.8

Table A-1 (continued)

Parameter	Abbreviation	Units	Mouse	Rat	Human
Oral Uptake of TCE:					
Stomach to liver	KAS	/hr	0.0	0.	0.0
Duodenum to liver	KAD	/hr	1.0* (0.27-1.1)	0.6* (0.2-0.6)	1.0
Stomach to duodenum	KTSD	/hr	10.0	10.0	10.0
Fecal excretion	KTD	/hr	0.0	0.0	0.0
TCE Metabolism:					
Capacity	VMC	mg/hr ^a	39.0* (39.0-60.0)	12.0* (12.0-20.0)	10.0* (6.0-10.0)
Affinity	KM	mg/L	0.25	0.25* (0.25-18.0)	1.5* (1.5-3.0)
Fraction TCA	PO	--	0.035* (0.035-0.1)	0.02* (0.02-0.06)	0.08
TCOH oxidation to TCA:					
Capacity	VMOC	mg/hr ^a	1.0* (0.5-1.5)	0.12* (0.08-0.25)	25.0 (15.0-25.0)
Affinity	KMO	mg/L	0.25	0.25	250.0
TCOH reduction to DCA:					
Capacity	VMRC	mg/hr ^a	1.0	0.1	0.1
Affinity	KMR	mg/L	10.0	10.0	10.0
TCOH glucuronidation:					
Capacity	VMGC	mg/hr ^a	100.0	100.0* (35.0-150.0)	5.0
Affinity	KMG	mg/L	25.0	25.0	25.0
Kinetics of glucuronide:					
Biliary excretion	KEHBC	/hr ^b	1.0	1.0	1.0
Reabsorption	KEHRC	/hr ^b	0.0	0.0* (0.0-0.3)	0.0
Urinary excretion	KUGC	/hr ^b	0.5	0.5	3.0
TCA reduction to DCA:					
Capacity	VMTC	mg/hr ^a	0.0* (0.0-0.1)	0.1* (0.0-0.1)	0.0
Affinity	KMT	mg/L	10.0	10.0	10.0
Urinary excretion	KUTC	/hr ^b	0.035* (0.035-0.1)	0.05	0.023

Table A-1 (continued)

Parameter	Abbreviation	Units	Mouse	Rat	Human
DCA reduction/elimination:					
Capacity	VMDC	mg/hr ^a	100.0	50.0	1730.0
Affinity	KMD	mg/L	1000.0	1000.0	1000.0
Urinary Excretion	KUDC	/hr ^b	0.035	0.05	0.023
DCVC Kinetics in Kidney:					
Production	KFC	/hr ^b	0.015	0.015	0.015
Activation	KBLC	/hr ^b	0.4	17.0	37.0
Clearance	KNATC	/hr ^b	0.5	1.1	19.0
Chloral Kinetics in Lung Clara Cells:					
Production capacity	VMTBC	mg/hr ^a	3.0	0.3	0.0045
Affinity	KMTB	mg/L	0.25	0.25	1.5
Clearance capacity	VMCTBC	mg/hr ^a	250.0	250.0	250.0
Affinity	KMCTB	mg/L	250.0	250.0	250.0
Volumes of distribution (fraction of body weight):					
TCA	VDTCAC	--	0.238	0.25	0.1
DCA	VDDCAC	--	0.2	0.2	0.1
TCOH	VDBWC	--	0.65	0.65	0.65
Fraction of lung containing Clara cells:					
	FCLARA	--	0.1	0.1	0.1

* Default value used for calculation of risk assessment dose metrics-- different values (shown in parentheses) were used for comparison with pharmacokinetic studies (see text)

^a Scaled by body weight to the 3/4 power

^b Scaled by body weight to the -1/4 power

Chapter 10

Evaluation of physiologically based pharmacokinetic models in risk assessment: an example with perchloroethylene

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ABSTRACT:

One of the more problematic aspects of the application of a physiologically based pharmacokinetic (PBPK) models in risk assessment is the question of whether the model has been adequately validated to provide confidence in the dose metrics calculated with it. A number of PBPK models have been developed for perchloroethylene (PCE), differing primarily in the parameters estimated for metabolism. All of the models provide reasonably accurate simulations of selected kinetic data for PCE in mice and humans, and could thus be considered to be "validated" to some extent. However, quantitative estimates of PCE cancer risk are critically dependent on the prediction of the rate of metabolism at low environmental exposures. Recent data on the urinary excretion of trichloroacetic acid (TCA), the major metabolite of PCE, for human subjects exposed to lower concentrations than those used in previous studies [1], make it possible to compare the high- to low-dose extrapolation capability of the various published human models. The model of Gearhart et al. [2], which is the only model to include a description of TCA kinetics, provided the closest predictions of the urinary excretion observed in these low-concentration exposures. Other models overestimated metabolite excretion in this study by 5- to 15-fold. A systematic discrepancy between model predictions and experimental data for the time-course of the urinary excretion of TCA suggested a contribution from TCA formed by metabolism of PCE in the kidney and excreted directly into the urine. A modification of the model of Gearhart et al. [2] to include metabolism of PCE to TCA in the kidney at 10% of the capacity of the liver, with direct excretion of the TCA formed in the kidney into the urine, markedly improved agreement with the experimental time-course data, without altering predictions of liver metabolism. This case study with PCE demonstrates the danger of relying on parent chemical kinetic data to validate a model that will be used for the prediction of metabolism.

I. INTRODUCTION

Pharmacokinetic and mechanistic information provide valuable insight into processes of primary interest in toxicology and risk assessment: receptor-specific target tissue doses and responses. Accordingly, the most recent guidelines, final or draft, for cancer and noncancer risk assessment [3, 4] have emphasized the importance of considering what is known about the pharmacokinetics and mode of action of a chemical to support quantitative dosimetry in the estimation of acceptable levels of chemical exposure. These changes in the guidelines facilitate the application of more sophisticated techniques for the incorporation of pharmacokinetic and mechanistic data into the dosimetric calculations performed in a risk assessment [5]. PBPK models are particularly attractive for this purpose because they can be used to quantitatively describe the metabolism and disposition of a chemical in both experimental animals and humans, resulting in more accurate dosimetry estimates [6, 7].

As the development of PBPK models has flourished, there have been increasing efforts to incorporate these models into the risk assessment process

[8]. The first use of a PBPK model in an agency risk assessment was in 1987, when the USEPA updated their inhalation risk assessment for methylene chloride using the PBPK model of Andersen et al. [9]. The model used in this case was relatively simple, with five tissue compartments and two metabolic pathways, but uncertainty regarding model structure and parameterization was nevertheless a major source of controversy [10]. Since this first consideration of a PBPK model in risk assessment, more complex models have been proposed for use, driven by the need to quantitatively describe relevant physiological processes [8]. For example, upper respiratory tract dosimetry models have now been developed [11, 12] that divide the nasal cavity into multiple compartments to describe the exposure of the tissue areas most vulnerable to chemical insult. In general, model structure should be as complex or as simple as necessary to describe the pertinent *in vivo* processes; however, it is critical that these model structures rely on sufficient data in their development to validate their use in the context of the risk assessment [13]. Evaluation of the suitability of a particular PBPK model for use in a risk assessment can be a difficult undertaking, particularly when the model is being used to predict a dose metric that is not directly measurable, such as the average tissue concentration of a reactive intermediate produced during metabolism. In such cases, where it is necessary to validate the model based on predictions of quantities other than the preferred dose metric, additional care is needed to assure that the validation data are adequately informative regarding the performance of the model for the intended use [14]. Sensitivity and uncertainty analysis are useful techniques for performing this evaluation [15, 16].

Often when a risk assessment is to be conducted, more than one PBPK model is available for the chemical of interest. This was the case for the methylene chloride risk assessment [10], and it is currently an issue for several chemicals for which regulatory toxicity criteria are in the process of being developed, including trichloroethylene (TCE) [17-20], 2,3,7,8-tetrachlorodibenzo-p-dioxin (Leung et al. 1990; Kohn et al. 1993; Andersen et al. 1997; Wang et al. 2000; Kim et al. 2002; Maruyama et al. 2002), and perchloroethylene (PCE) [2, 21-30]. The alternative models will typically differ in the data used for their development and validation, and may have structural differences reflecting both the desired level of detail and the nature of the intended application. In addition, the alternative models may address different routes of exposure or simulate kinetics in different species or strains. Whenever multiple models are available, questions will naturally arise as to which model provides the most accurate estimates of the dose metrics to be used in the risk assessment. Differences in model structure and parameterization can have a significant impact on the estimation of dose metrics, and hence on the estimation of acceptable levels of chemical exposure [31, 32]. In these cases, it is critical that all of the available quantitative data on the pharmacokinetics of a given chemical, and not just the data used for the development of a specific model, be considered during comparative model evaluation.

PCE provides a particularly suitable example of the issues associated with comparative model evaluation. A number of applications of PBPK modeling in a

risk assessment for PCE have been described [2, 21, 24, 33, 34]. Several analyses have also been performed to evaluate the uncertainty and/or variability in PBPK models of PCE and the implications of this uncertainty/variability for a PCE risk assessment [23, 24, 29, 31, 32, 35]. This issue has been a major concern in ongoing risk assessment efforts for PCE both at the U.S. Environmental Protection Agency (EPA) [36] and the California EPA [34]. In particular, variability in the prediction of human dosimetry was a critical issue in the recent derivation of a Public Health Goal (PHG) for PCE [34] by the California EPA's Office of Environmental Health Hazard Assessment (OEHHA). PHGs are intended to be based on risk assessments conducted using the most current principles, practices, and methods [37]. In the case of PCE, OEHHA determined that the level of variability associated with predictions of the desired human dose metric (fraction metabolized) justified the use of upper-bound estimates from a published population variability analysis [23].

The purpose of this review is to conduct a comparison of the various published PBPK models for PCE, and to identify critical issues to be considered when applying these models in a risk assessment.. After a brief summary of the most pertinent information regarding the carcinogenicity and pharmacokinetics of PCE, the available PBPK models will be described. The importance of the data with which the models are validated will be demonstrated by comparing the predictions of the various models with recently published data on human exposures at relatively low concentrations. This evaluation of the PBPK models for PCE provides an example of how it is possible to deal with the problem of PBPK model structure and parameter selection without resorting to upper-bound approaches, such as those employed in the derivation of the PHG for PCE.

A. Carcinogenicity of PCE

Epidemiological studies provide conflicting evidence regarding the carcinogenic potential of PCE in humans following inhalation or oral exposure [38-47]. In general, these studies either did not indicate statistically significant increases in cancer incidence or were largely confounded by co-exposure to other chemicals [48-51]. A recent comprehensive review concluded that "the current epidemiological evidence does not support a conclusion that occupational exposure to PCE is a risk factor for cancer of any specific site" [51]. Because the results of epidemiological studies are inconclusive, estimates of the human carcinogenic potential of PCE have relied mainly upon chronic studies conducted in rodents [33, 34, 52].

Only two chronic bioassays addressing the potential carcinogenicity of PCE have been conducted that are usable for risk assessment purposes: an oral gavage study [53] and an inhalation study [54]. The published USEPA [33, 52] risk assessments for PCE, as well as the PHG for PCE [34], are based on the tumorigenicity observed in these studies (Table 1).

Table 1: Tumor Incidence in Chronic Bioassays with Perchloroethylene

Study	Sex/Species	Doses and Incidence			
NCI, 1977 – oral [53]	Male Mouse	Hepatocellular Carcinoma			
		Dose mg/kg/day	Fatal Tumors	Incidental Tumors	# of Animals At Risk
		0	0	2	20
		536	19	13	50
	1072	17	10	50	
	Female Mouse	Hepatocellular Carcinoma			
		Dose mg/kg/day	Fatal Tumors	Incidental Tumors	# of Animals At Risk
		0	0	0	20
386		8	11	50	
772	13	6	50		
NTP, 1986 – inhalation [54]	Male Mouse	Hepatocellular Adenoma or Carcinoma			
		Concentration (ppm)	Fatal Tumors	Incidental Tumors	# of Animals At Risk
		0	1	16	49
		100	17	14	49
	200	14	27	50	
	Female Mouse	Hepatocellular Adenoma or Carcinoma			
		Concentration (ppm)	Fatal Tumors	Incidental Tumors	# of Animals At Risk
		0	2	2	48
		100	5	12	50
	200	24	14	50	
	Male Rat	Mononuclear Cell Leukemia			
		Concentration (ppm)	Fatal Tumors	Incidental Tumors	# of Animals At Risk
		0	19	9	50
		200	26	11	50
	400	28	9	50	
	Female Rat	Mononuclear Cell Leukemia			
Concentration (ppm)		Fatal Tumors	Incidental Tumors	# of Animals At Risk	
0		8	9	50	
200		20	10	50	
400	19	10	50		

In the chronic oral study [53], groups of male and female Osborne-Mendel rats or B6C3F₁ mice were administered two dose levels of PCE in corn oil by gavage five days per week for 78 weeks, followed by 32 (rats) or 12 (mice) weeks of observation. Accounting for several dose adjustments made during the study, time-weighted average doses were 471 and 941 mg/kg/day in male rats, 474 and 949 mg/kg/day in female rats, 536 and 1072 mg/kg/day in male mice, and 386 and 772 mg/kg/day in female mice. Statistically significant increases in the incidence of hepatocellular carcinomas were observed in treated mice of both sexes, compared to control incidences (Table 1). Survival was significantly lower in treated mice than in controls, suggesting that the optimum dose may have been exceeded. Significant early mortality was also observed in rats, mainly due to PCE-related toxic nephropathy. Because of the reduced survival, the rat study was not considered adequate for evaluation of the carcinogenicity of PCE [53]. This study has also been questioned because the PCE administered was stabilized with epichlorohydrin, a strong alkylating agent that is itself an animal carcinogen.

In the inhalation study [54], both B6C3F₁ mice and Fischer-344 rats were exposed to PCE six hours/day, five days/week for 103 weeks. Mice were exposed to concentration of 0, 100, or 200 ppm PCE, while rats were exposed to concentrations of 0, 200, or 400 ppm. In mice of both sexes, there were statistically significantly increased incidences of hepatocellular adenomas and carcinomas (combined) in treated animals compared to corresponding controls (Table 1). In rats, the only endpoint that was statistically significantly increased compared to concurrent controls was mononuclear cell leukemia in both males and females. NTP [54] considered these tumors as contributing to the evidence of carcinogenicity of PCE in rats; however, the U.S.EPA Science Advisory Board concluded that these results did not provide a scientific basis to associate mononuclear cell leukemia in rats with inhalation exposure to PCE [55]. This type of leukemia, which occurs spontaneously in Fischer-344 rats, does not occur in humans; therefore, its relevance to human health is uncertain [56]. A non-significant increase in renal tubular cell adenomas and carcinomas observed in male rats only was considered to be treatment-related [54]. In contrast, no tumors were observed in groups of 96 male and 96 female Sprague-Dawley rats exposed to PCE concentrations of 300 or 600 ppm, 6 hours per day, 5 days per week for 52 weeks and observed for the rest of their lives [57]. The reason for this apparent discrepancy is unknown, but may be related to differences in rat strain and/or exposure duration.

In 1985, the U.S.EPA classified PCE in Weight-of-Evidence Group "C – Possible Human Carcinogen", and calculated risk estimates based on the incidence of hepatocellular carcinoma in mice in the oral gavage study [53], using the then-current default cross-species scaling based on body surface area [52]. An inhalation unit risk of $4.8 \times 10^{-7} (\mu\text{g}/\text{m}^3)^{-1}$ was obtained using estimates of metabolized dose (based on total urinary excretion of metabolites) from pharmacokinetic studies in mice [58] and humans [59]. A drinking water unit risk of $1.5 \times 10^{-6} (\mu\text{g}/\text{L})^{-1}$ was also derived assuming 100% metabolism of ingested PCE. In 1986, the Agency revised their PCE inhalation risk estimate [33] based on the results of the inhalation bioassay [54]. The risk estimates were

again calculated on the basis of metabolized dose using the same approach as the previous assessment. The resulting inhalation unit risk, based on the incidence of liver adenoma/carcinoma, was $5.8 \times 10^{-7} (\mu\text{g}/\text{m}^3)^{-1}$, almost identical to the value derived from the oral study. Similar unit risks were also obtained for the other endpoint evaluated: leukemia in male and female rats.

B. Metabolism and Mode of Action of PCE

PCE is a volatile lipophilic compound that is readily absorbed following inhalation, oral or dermal exposure in both animals and humans, and is distributed primarily to the fat [49]. Based on urinary excretion of metabolites, the metabolism of PCE is expected to be limited. For example, after an oral dose of 800 mg/kg in rats, excretion of metabolites in the urine accounted for only 2% of the dose [60]. Metabolism of PCE in humans is also very limited, based on metabolites measured in the urine [61, 62]. The metabolic pathway of PCE appears to be qualitatively similar in mice, rats, and humans (Figure 1).

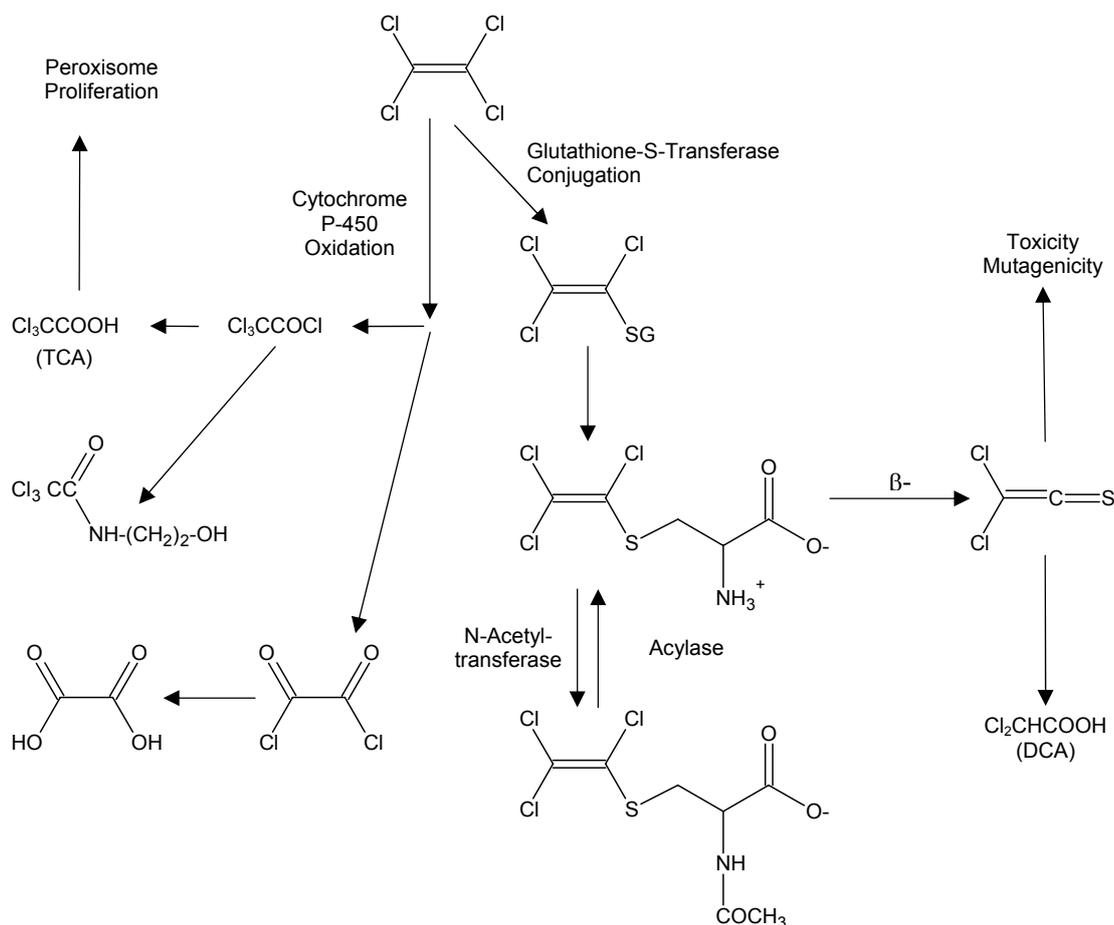


Figure 1: Metabolism of PCE (adapted from Vamvakas et al. 1993 [60]).

However, the significant quantitative differences in the rate and extent of metabolism among the species are thought to be responsible for corresponding species differences in target organ doses, and hence responses [1, 49, 63-68]. Formation of TCA via cytochrome P450 (CYP) oxidation is the principal route of metabolism in all three species, representing approximately 60% of total metabolism in both rats and mice [69], and essentially all of the urinary excretion observed after PCE exposure [60, 70]. Excretion of TCA in urine therefore provides a useful measure of PCE metabolism.

Two alternative (but not mutually exclusive) modes of action have been frequently discussed to explain the liver carcinogenicity of PCE in mice: (1) cytotoxicity associated with reactive intermediates produced during the oxidative metabolism of PCE [33, 52], and (2) metabolism to TCA, a peroxisome proliferator [64]. CYP oxidation of PCE results in formation of PCE oxide and trichloroacetyl chloride (Figure 1); both can react with macromolecules [67, 71, 72]. Oxidative metabolism of PCE is quantitatively greater in mouse than rat hepatocytes [64], and greater in rat than in human liver [1, 32]. Trichloroacetylated protein residues have been detected in the livers of mice [73] and rats [67, 71, 72], indicating reaction of some trichloroacetyl chloride with proteins. Adduct formation in human liver has not been directly examined. Since trichloroacetylated proteins were much lower in blood from human volunteers exposed to 10 and 40 ppm PCE than from similarly exposed rats, humans should also have a correspondingly lower level of hepatic binding [67]. While the toxicological significance of these protein adducts is unclear, oxidative damage to lipids and DNA provides a more direct indication of potential for cellular toxicity. PCE-induced oxidative stress has not been examined in mice; however, vitamin E partially protected mice receiving very high doses of PCE (3,000 mg PCE/kg orally for 15 days) from liver toxicity [74, 75]. Rats administered single doses of PCE (100, 500, or 1,000 mg/kg) showed no evidence of either lipid peroxidation or oxidative DNA damage [76].

Of some interest, a recent study comparing indices of oxidative stress in female drycleaners exposed to time-weighted average concentrations below 5 ppm vs. laundry workers matched by age, race, and smoking status reported significantly decreased levels of leukocyte 8-hydroxydeoxyguanosine (an extensively used biomarker of exposure to oxygen-based radicals) in the former group [77]. Several experimental observations offer potential mechanistic explanations for reduced levels of the marker in these workers. First, pretreatment of rats with clofibrate (a peroxisome proliferating hypolipidemic drug) or TCA markedly mitigated the lipoperoxidative response following acute challenge with TCA [78]. Since this effect was linked to both markers of peroxisome proliferation and increased expression and activity of CYP4A, the observation indicates that it is mediated by peroxisome proliferator-activated receptor α (PPAR α). The authors suggested that the observed reduction of the peroxidative response in pretreated animals was due to the shift in the expression of CYP isoforms from those that participate in lipid peroxidation to those that do not [78]. Human hepatocytes contain lower levels of PPAR α than those of rodents and do not undergo peroxisome proliferation. However, the

increases in CYP4A in response to peroxisome proliferators, including TCA, have been reported in cultured human cells [79, 80]. TCA produced by PCE metabolism in humans might exert a similar PPAR α -mediated protective effect. PPAR α ligands inhibit inflammatory processes in many tissues, acting via a variety of molecular mechanisms [81-83]. Although no studies have demonstrated any anti-inflammatory efficacy of TCA, it may share this property with other peroxisome proliferators.

Although some of the kidney toxicity of high doses of PCE observed in male rats is attributable to α 2 μ -globulin accumulation [84], kidney tumorigenicity has been proposed to be associated with formation of the glutathione conjugate in the liver followed by conversion to the cysteine conjugate in the kidney, where it is activated by β -lyase to form a highly reactive metabolite, dichlorothioketene [67, 69, 84-86]. This pathway appears to be significantly less active in humans than in rats, suggesting that humans may be less susceptible to PCE-induced nephrotoxicity [1, 66, 68].

C. PBPK Modeling of PCE

With few exceptions, the PBPK models for PCE share the simple four-compartment structure (liver, fat, rapidly perfused tissues, and slowly perfused tissues) and steady-state description of lung equilibration developed by Ramsey and Andersen [87] for styrene. Only one of the published models [2] provides a description of the kinetics of TCA, the major metabolite of PCE. None of the models provide a description of the glutathione conjugation metabolic pathway that has been implicated in the kidney lesions produced by PCE. Therefore it is not possible to estimate dose metrics for kidney toxicity as has been done in the case of TCE [88]. For the most part, the differences between the models reflect the different data used by the authors in their development. To simplify comparison, animal and human modeling studies will be described separately, followed by a discussion of studies focusing on the uncertainty and variability in the models.

1. Animal Modeling Studies

Several pharmacokinetic modeling studies have been performed to characterize the kinetics and metabolism of PCE in the mouse [2, 21, 22, 29] and, in some cases, the rat [21, 22, 29]. Two of these models have been applied in the estimation of internal dose metrics for use in risk assessment [2, 21]. Development of PBPK models of PCE has also been conducted in the rat and dog by Dallas et al. [25-28]. These rat and dog models, which were developed to describe blood and exhaled air concentrations of PCE, would likely be useful to provide parent chemical dosimetry estimates for toxicity studies conducted in these species. However, since the cancer risk assessment for PCE is based on tumors in the mouse, these models of PCE kinetics in the rat and dog have not been considered in the present analysis.

Chen and Blancato [21] developed a PBPK description of the pharmacokinetics of PCE as part of a USEPA [33] risk assessment. Their model structure was based on the styrene model of Ramsey and Andersen [87]. Physiological parameters were taken from a compilation of values used in previously published PBPK models [89], and partition coefficients were obtained from the Air Force toxicology laboratory at Wright-Patterson Air Force Base [90]. Metabolism parameter values in rat and mouse were estimated by fitting the model to published data on total metabolism following inhalation and oral exposures to radiolabeled PCE [70, 91], assuming a single oxidative CYP pathway that followed Michaelis-Menten kinetics. No attempt was made to compare predictions of the model with other kinetic data. Using this model, Chen and Blancato [21] estimated daily production of total metabolites in male and female mice following exposure to PCE either by gavage or inhalation under the bioassay exposure conditions [53, 54].

Ward et al. [22] developed a similar PBPK model for PCE in the mouse and rat. Metabolism parameter values were estimated by fitting the model to experimental data sets on total metabolism and total metabolite excretion. In addition to the disposition data of Pegg et al. [91] and Schumann et al. [70] relied on by Chen and Blancato [21], data on total urinary excretion of metabolites (Buben and O'Flaherty 1985) were used, assuming that urinary excretion accounted for 65% of metabolism. Again, no attempt was made to compare the model with other kinetic data. In contrast to the model of Chen and Blancato [21], which included only a saturable Michaelis-Menten description of the metabolism of PCE, the Ward et al. [22] model included both a saturable and a linear component in the equation for metabolism. They assumed that the saturable pathway represented oxidative CYP metabolism and that the linear pathway represented conjugation with glutathione; however, no experimental data were presented to support this assumption.

Gearhart et al. [2] conducted targeted experimental studies to support the development of a more comprehensive mouse PBPK model for PCE. Unfortunately, the publication does not provide a complete documentation of the model structure and parameters; however, it was possible to obtain a copy of the actual model code from the principal developer (J. M. Gearhart, personal communication). As a check on the model code, it was possible to reproduce each of the figures in Gearhart et al. [2]. A diagram of the Gearhart et al. [2] model is shown in Figure 2, and the parameters are provided in Table 2. The Gearhart et al. [2] model differed from the earlier models in that it included two fat compartments with different perfusion ratios. The value of including multiple fat compartments has since been demonstrated for a related compound, TCE [92]. Another refinement was the use of a two-compartment description of oral uptake (Figure 2); a similar description has also been used successfully for TCE [18].

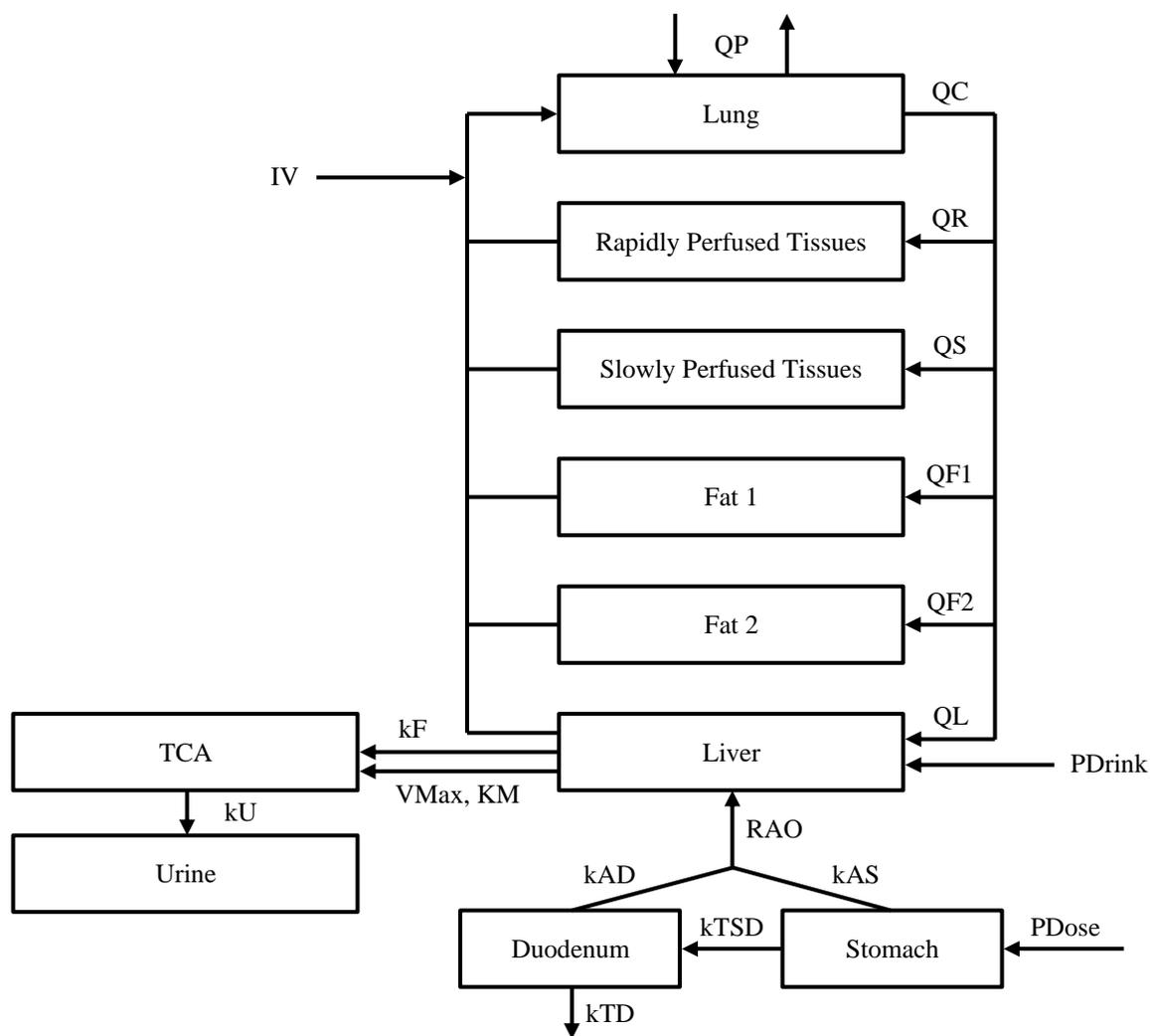


Figure 2: Structure of PCE PBPK model of Gearhart et al. (1993) [2] A description of the parameters is provided in Table 2. For some parameters, a "C" has been added to the abbreviation to indicate a "scaled" value..

Table 2: Parameters for Perchloroethylene PBPK Model of Gearhart et al. (1993) [2].

Name	Definition	Mouse	Human
BW	Body weight (kg)	0.035	70.0
QCC	Cardiac output (L/hr/kg ^{0.75})	18.0	13.0 ^a
QPC	Alveolar ventilation rate (L/hr/kg ^{0.75})	30.0	18.0 ^a
Blood Flows (fraction of cardiac output)			
QFC1	Fat tissue 1	0.04	0.03
QFC2	Fat tissue 2	0.03	0.02
QLC	Liver	0.16	0.23
QRC	Rapidly perfused tissues	0.59	0.70
QSC	Slowly perfused tissues	0.41	0.30
Tissue Volumes (fraction of body weight)			
VDC	TCA volume of distribution	0.24	0.1
VFC1	Fat tissue 1	0.05	0.16
VFC2	Fat tissue 2	0.02	0.05
VLC	Liver	0.057	0.026
VRC	Rapidly perfused tissues	0.21	0.11
VSC	Slowly perfused tissues	0.64	0.65
Partition Coefficients			
PB	Blood:air	20.0	11.58
PF	Fat:blood	75.0	125.2
PL	Liver:blood	2.4	5.28
PR	Rapidly perfused tissue:blood	2.2	5.06
PS	Slowly perfused tissue:blood	3.3	6.11
Metabolism Parameters			
VMaxC	Maximum rate of metabolism (mg/hr/kg ^{0.75})	0.2	0.28
KM	Michaelis-Menten affinity constant (mg/L)	2.0	7.7
kFC	Pseudo-first order metabolism (kg ^{0.25} /hr)	2.0	0.0
FTCA	Fraction of PCE metabolized to TCA in liver	0.6	0.6
Oral Absorption Parameters (/hr)			
kAS	Absorption from stomach	0.0	-- ^b
kAD	Absorption from duodenum	0.3	--
KTSD	Transfer from stomach to duodenum	2.0	--
kTD	Excretion from duodenum	0.0	--
Urinary Clearance Parameter (kg ^{0.25} /hr)			
kUC	TCA urinary clearance	0.035	0.12 ^c

^a Values used for simulation of experimental subjects. Values used for human dose metric calculations were 16.5 for QCC and 24.0 for QPC.

^b Human oral exposure from drinking water described by continuous zero-order intake

^c Original value used in Gearhart *et al.* (1993) [2] model. Revised human model and dose metric calculations used a value of 0.023, based on value used for TCA in a PBPK model of trichloroethylene (Clewell *et al.* 2000) [18]. Revised model also assumed metabolism of PCE in the kidney with a VMaxC of 0.028.

More significantly, the Gearhart *et al.* [2] model also described the kinetics of the principal metabolite, TCA, using a single-compartment model with first-order urinary excretion, and assuming that the amount of TCA produced represents 60% of the total amount of PCE metabolized [69]. The kinetic parameter values for TCA were taken from a description of the same metabolite in a model of TCE [17]. To determine the metabolic parameters for the mouse model, Gearhart *et al.* [2] conducted closed-chamber PCE clearance studies at concentrations of 200, 1000 and 3500 ppm PCE. In addition, concentrations of PCE and TCA were measured in the blood of mice following gavage administration of doses comparable to those used in the NCI [53] study. These oral data were used to validate the kinetic parameters for PCE and TCA. The description of metabolism in the model was similar to that used by Ward *et al.* [22], including both saturable and linear metabolism components. However, both components were assumed to represent oxidative CYP metabolism, producing TCA. This assumption was necessary to provide a consistent description of both the closed-chamber PCE clearance data and the data on TCA concentrations following oral gavage with PCE. The parameter values for this model are shown in Table 2.

Reitz *et al.* [29] conducted six-hour inhalation studies with PCE in the mouse and rat. Metabolism parameter values were then estimated by fitting a PBPK model to the post-exposure time-course for the exhalation of PCE, assuming Michaelis-Menten kinetics. The resulting estimates for both the capacity (V_{max} , mg/hr) and affinity (K_m , mg/L) of PCE metabolism were roughly a factor of two higher than the values estimated by Chen and Blancato [21] from disposition data, but the estimated clearance (V_{max}/K_m) was similar. The model was then used with the estimated parameter values to predict closed-chamber gas uptake studies. The metabolism parameter values estimated for the rat provided model predictions that were in good agreement with the closed-chamber data. However, in order to obtain agreement with the closed-chamber data in the mouse it was necessary to double the K_m estimated from the post-exposure exhalation data.

2. Human Models

A number of studies have been performed to develop PBPK models of PCE in the human [2, 21, 22, 29, 30], and in two cases [2, 21] the resulting models have been applied in the estimation of internal dose metrics for use in risk assessment.

Chen and Blancato [21] developed a PBPK description of the pharmacokinetics of PCE in the human as part of a USEPA [33] risk assessment. This model used the same structure as their rodent model. Physiological parameter values were taken from a compilation of values used in previously published PBPK models [33], and partition coefficients were obtained from the Air Force toxicology laboratory at Wright-Patterson Air Force Base [90]. Metabolism parameter values in the human were estimated from fitting of published data on total urinary excretion of the metabolite TCA for 72 hours

following inhalation exposures to PCE [93, 94], assuming that the excretion of TCA over the 72 hours post-exposure represented 30% of total metabolism. This estimate, which is lower than would be expected from disposition data in animals, was based on the fit of the model to data on exhaled air concentrations of PCE post-exposure [93]. No attempt was made to compare predictions of the model with other published kinetic data.

Ward et al. [22] also developed a model for PCE in the human. In contrast to their description of the rat and mouse, which included both saturable and linear metabolism, the human model only included a saturable oxidative pathway. Metabolism parameter values were estimated by fitting the model to data on the urinary excretion of TCA from workers exposed to PCE in the course of their work [95]. The predictions of the model were then compared with published data on exhaled air concentrations for controlled inhalation exposures ranging from 72 to 198 ppm [93, 94, 96]. The authors concluded that the human metabolic parameters could be predicted by assuming equal K_m across species and scaling the rat V_{max} by body weight raised to the three-quarters power, and that similar scaling of V_{max} from the mouse overestimated human metabolism. However, the human metabolic parameter values estimated in this study must be viewed with caution because they were estimated from data for individuals exposed under uncontrolled conditions in the workplace, with exposure concentration estimates based on the average of measurements taken at various sites in different workshops.

Gearhart et al. [2] developed a human model of PCE that, like the mouse model on which it was based, includes two fat compartments in the parent chemical description, and also describes the production, distribution, and excretion of the principal metabolite, TCA. The parameters for the metabolism of PCE in the human were estimated by fitting the model to data on the time-course of urinary excretion of TCA following inhalation exposure to PCE [93], assuming that TCA represents 60% of the total metabolism of PCE in the human, as it does in the mouse and rat [69]. As in the case of the Ward et al. [22] model, only a single saturable pathway was described in the human. The predictions of the model were then compared with published data on exhaled air concentrations for controlled inhalation exposures [93, 96].

Reitz et al. [29] developed a human version of their PBPK model for PCE by estimating *in vivo* metabolic parameter values in humans based on a parallelogram approach using *in vivo* data in mice and rats plus *in vitro* data in mice, rats, and humans. A sensitivity analysis of the PBPK model revealed that the most significant uncertainties were in the techniques used to estimate rates of PCE metabolism in humans.

Loizou [30] developed a PBPK model for PCE in the human to investigate workplace exposures. Using the PCE metabolism and partitioning parameters from Gearhart et al. [2], he was able to successfully simulate experimental data on exhaled PCE concentrations following both inhalation [93] and dermal [97] exposures, as well as data on the time-course of blood concentrations and

urinary excretion of TCA following inhalation of PCE [1, 94]. The model was then used to analyze occupational exposure data from dry-cleaning operations.

3. Uncertainty Analyses

Farrar et al. [35] re-parameterized the USEPA [33] model using distributions of parameters rather than single values, and conducted a Monte Carlo analysis to evaluate the uncertainty in risk estimates resulting from the uncertainty in the parameters. They found that an upper-bound estimate (the 97.5th percentile) of the human dose metric for amount of PCE metabolized per volume of liver was approximately four-fold higher than the median estimate.

Bois et al. [24] conducted a similar analysis using the same model structure evaluated by Farrar et al. [35]. As in Farrar et al. [35], instead of estimating single values for each parameter in the model, probability distributions were specified. Estimates of metabolic parameter values in the rodent were obtained by fitting multiple disposition studies [58, 69, 70, 98], assuming saturable Michaelis-Menten metabolism. Estimates of metabolized doses in the mouse and rat were similar to those estimated by Chen and Blancato [21]. In the case of the human, Bois et al. [24] relied on urinary excretion data from occupational studies of Japanese workers [61, 95] to estimate maximum likelihood estimates of the metabolism parameter values. The fraction of total metabolite excreted in the urine was assumed to be 65%, based on data from animals [69, 91, 98, 99]. Bois et al. [24] then used the model to estimate metabolized dose of PCE in rats and mice, following inhalation exposure in the NTP [54] study, and in humans exposed to 1 ng/L in air. As in Farrar et al. [35], parameter values were described by probability distributions rather than fixed values, with a Monte Carlo approach used to generate a distribution of estimates. The model was coupled with a multistage model to evaluate the precision of the resulting risk assessment. In looking at the variability in the model parameters, they assessed risks that ranged from 0.04 per million (5th percentile) to 6.8 per million (95th percentile), with a median risk estimate of 1.6 per million, very similar to the results of Farrar et al. [35].

Hattis and co-workers [31, 32] compared parameters and results from a number of published and unpublished PBPK models for PCE in the human, including the models of Chen and Blancato [21], Ward et al. [22], and Farrar et al. [35]. All of the models compared were variations on same basic framework described by Chen and Blancato [21]; that is, they all represented models of parent chemical absorption, distribution, and clearance, with no description of metabolite kinetics. The model of Gearhart et al. [2], which includes a description of the kinetics of the principal metabolite, TCA, was not available at the time this comparison was conducted. Some models did, however, include a non-specific compartment for total un-excreted metabolites, to facilitate modeling of data on total urinary metabolite excretion. The most important differences noted between the various models were in their descriptions of the metabolism of PCE, particularly in the use of linear or saturable metabolism, or both. In the first study, Hattis et al. [32] evaluated the variability in predictions

of total metabolism in animals exposed at the lowest concentrations used in the NTP bioassays and in humans exposed at 1 ppm. While predictions of the various models were in relatively good agreement in the mouse (within a factor of three), the authors found substantial (60-fold) differences in model predictions of metabolism in the human, which they concluded were primarily related to the choice of data used for estimating the metabolic parameter values. In the second study [31], predictions of the various human models were compared with data on fractional absorption of inhaled PCE, as well as measured concentrations of PCE in alveolar air and venous blood. Overall, the model predictions showed a systematic departure from the observed alveolar air and blood levels that the authors suggested might be resolved with more sophisticated description of the fat compartment.

Gearhart et al. [2] performed a Monte Carlo analysis with their mouse and human models, following the approach of Farrar et al. [35], and incorporating estimates of the uncertainty in the parameters from statistical analyses of repeated measurements conducted as part of the experimental effort. They found that, in general, the upper- and lower-bound estimates for model-predicted dose metrics were within a factor of 2 of the median estimates. These results are consistent with the conclusions of Farrar et al. [35], who found a somewhat greater variability for risk estimates, because the observed variation in the risk estimates reflects the variation in both the animal and human dose metrics.

Bois et al. [23] applied the Markov chain Monte Carlo (MCMC) technique to evaluate the population variability of the metabolism of PCE in the human, using the same model structure as in their earlier study [24]. In this hierarchical Bayesian approach, prior estimates of the distributions for each of the parameters, as well as for the uncertainty in the distributional parameters (mean and variance) themselves, are used as input to the MCMC algorithm along with experimental data sets that are considered informative regarding the population distribution of the parameters. The algorithm then combines the information from the priors and the data in a Bayesian framework to obtain posterior estimates of the population distributions of the parameters. In the case of the analysis conducted by Bois et al. [23], only one experimental data set was used in the MCMC: measured concentrations of PCE in blood and exhaled air for one week following exposures to 72 or 144 ppm PCE in an inhalation chamber for 4 hours [94].

The resulting estimates of fractional metabolism obtained by Bois et al. [23] were strongly dependent on exposure concentration. For exposure at 50 ppm, the predicted 95% confidence interval for fractional metabolism ranged from 0.52 to 4.1%, while for exposure at 1 ppb the 95% confidence interval ranged from 15 to 58%. The authors concluded that the model predictions for fractional metabolism at the higher concentration were consistent with the data in Monster et al. [94]. Indeed, the average excretion of TCA in these studies was approximately 6 and 11 mg at 72 and 144 ppm, respectively, while the corresponding average net uptake of PCE reported by Monster et al. (1979) was

455 and 945 mg; therefore, excretion of TCA accounted for around 1% of the net uptake of PCE at both exposure levels. Bois et al. [23] attributed the difference in fractional metabolism between high and low exposure concentrations to saturation occurring between 1 and 10 ppm.

II. EVALUATION OF ALTERNATIVE PBPK MODELS FOR USE IN A PCE RISK ASSESSMENT

The appropriate evaluation of a PBPK model is strongly dependent on its intended application. The criteria for a model intended to codify quantitative hypotheses and support experimental design and inference in conjunction with mechanistic studies are likely to be quite different from those for a model intended to perform the dosimetric extrapolations needed in a risk assessment. In this review, the evaluation of the various PBPK models is conducted from the viewpoint of their potential use in a cancer risk assessment for PCE, and does not consider their value as a tool for conducting research on the metabolism and mechanism of toxicity of PCE.

A. Results of Parameter Sensitivity Analyses

Given the relatively large number of parameters in a PBPK model, even the simple models that have been developed for PCE, a parameter-by-parameter comparison would be impractical. Fortunately, only a few of the parameters typically have a significant impact on the dose metric predictions of the models [15]. Based on sensitivity analyses conducted with several of the PBPK models for PCE [2, 24, 29], it is possible to restrict consideration to the key parameters for the prediction of metabolized dose in the animal bioassays and in human steady-state exposure conditions.

Based on the observed correlation between the model parameters and the predicted rate of metabolism during continuous human exposures, Bois et al. [24] found that the most important parameters were those for metabolism, partitioning, and, to a lesser extent, the ventilation rate, while those for tissue volumes and blood flows were relatively unimportant. Using the same correlational approach for estimating sensitivity, Gearhart et al. [2] obtained similar results for both continuous human ingestion and mouse gavage. Reitz et al. [29] determined the analytical sensitivity of predictions of metabolism to each of the model input parameters for inhalation exposure of mice and humans. Their results were consistent with the correlation analyses, in that the most critical parameters for the prediction of amount metabolized were found to be the capacity and affinity of metabolism, the blood:air partition coefficient, and, at low concentrations in the human, the ventilation rate. Of these critical parameters, those for metabolism are clearly the most uncertain. The blood:air partition coefficients for PCE are well-characterized in both mice and humans [2], and ventilation rates, while varying across individuals and workloads, are also reasonably well characterized [100, 101]. Therefore, it is concluded that evaluation of the alternative models should focus on their metabolism parameters.

B. Comparison of Mouse Models

Based on the evaluation performed by Hattis et al. [31, 32], it is clear that the greatest differences in the predictions of the PBPK models for PCE are in the case of the human rather than the mouse. Hattis et al. [32] found only a three-fold variation in the model predictions for metabolism in the mouse, while similar predictions in the human varied by 60-fold. Nevertheless, there are a few points worth considering with regard to the modeling of metabolism in the mouse. In particular, any comparison of the metabolism predictions of the PBPK models for mice is complicated by the fact that some of the models use a single saturable term, while others use both saturable and linear terms. Moreover, the assignments of the pathways in the two-pathway models differ. Ward et al. [22] assumed that the saturable pathway represented oxidative CYP metabolism while the linear pathway represented conjugation with glutathione, whereas Gearhart et al. [2] assumed that both pathways represented oxidative CYP metabolism. In both cases only a single saturable pathway was used for the human. A similar use of multiple components in the description of CYP oxidation in the mouse and rat, but not the human, was also required in the PBPK model for another haloethene, vinyl chloride [14]. In that case, two saturable components were included, one representing high-affinity, low-capacity metabolism by CYP 2E1, and the other representing lower affinity, higher capacity metabolism, presumed to be due to other isozymes of the CYP family. The observation that multiple CYP isozymes contribute to the metabolism of PCE in the mouse and rat [102, 103] provides support for this description. Therefore, it is likely that the combination of saturable and linear components in the model of Gearhart et al. [2] represents an empirical description of PCE metabolism by multiple CYP isozymes with widely different affinities and capacities. This interpretation may also apply to the metabolism description in the model of Ward et al. [22].

From the viewpoint of using the models in a risk assessment, the main interest is the ability of the model to estimate amount metabolized in the PCE bioassays. Therefore, an approach similar to that used by Hattis et al [32] for comparing the various models would seem to be the most reasonable. That is, the predictions of the models for the dose metric of interest for the risk assessment, the amount metabolized in this case, can be compared for an exposure similar to those used in the bioassays. The exposure chosen for the present analysis is shown in Figure 3, which compares the measured blood concentrations of TCA produced by a 6 hour inhalation exposure of mice to PCE at 400 ppm [64] with the predicted TCA blood concentrations obtained using the PBPK model of Gearhart et al. [2]. This data set, which reproduces the highest exposure in the inhalation bioassay [54], was not used in the development of any of the models being compared. The estimates of total metabolism obtained with several different models (Table 3) are within a factor of two, consistent with the results obtained by Hattis et al. [32]. This agreement is not surprising since most of the mouse models have been calibrated against data on metabolite formation and/or excretion that are highly dependent on the extent of metabolism.

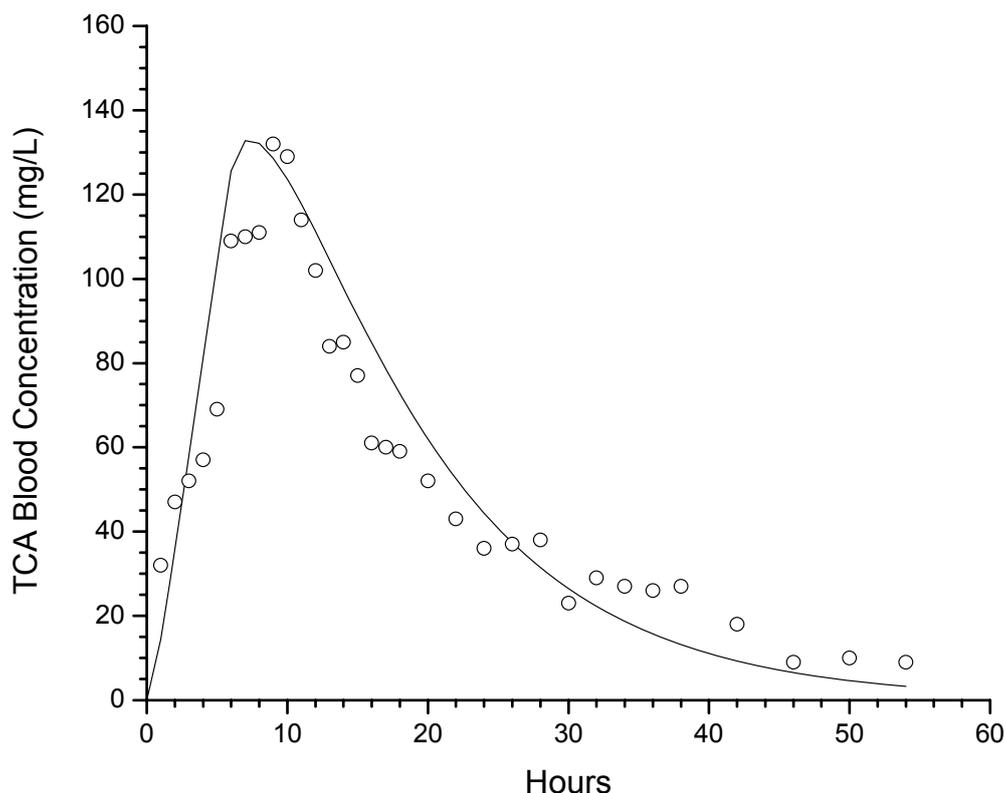


Figure 3: Predicted (curve) and experimental (symbols) blood concentration of TCA for a 6 hour inhalation exposure of mice to perchloroethylene at 400 ppm (Odum et al., 1988) [64], using the PBPK model of Gearhart et al. (1993) [2].

Table 3: Total Metabolism Following 6-hour Inhalation Exposure of Mice to Perchloroethylene at 400 ppm, Predicted with Different PBPK Models

<u>Model</u>	<u>Total Metabolism</u>
Chen and Blancato, 1987 [21]	2.09
Ward <i>et al.</i> , 1988 [22]	2.43
Ward <i>et al.</i> , 1988 ^a [22]	1.66
Bois <i>et al.</i> , 1990 [24]	2.06
Gearhart <i>et al.</i> , 1993 [2]	2.28
Reitz <i>et al.</i> , 1996 [29]	3.09

^a Predicted metabolism by saturable pathway only

C. Comparison of Human Models

The much greater variability in metabolism predictions observed by Hattis et al. [32] in the human reflects the greater variety of data used to calibrate the human models. Also, few human data are available that provide information concerning the rate of metabolism. There are no studies of radiolabel disposition in the human, and the interpretation of urinary excretion data is complicated by the longer half-life of metabolites in humans vs. rodents [1, 104]. Therefore, most of the human models have relied to some extent on data for the *in vitro* or *in vivo* kinetics of the parent chemical to infer the rate of metabolism. For example, in the human model of Bois et al. [23], the metabolic parameters are based on blood and exhaled air concentrations of PCE after the end of an inhalation exposure [94].

The use of post-exposure parent chemical concentration data for metabolism parameter estimation is problematic because the predictions of PBPK models for volatile chemicals like PCE during the post-exposure period in human inhalation studies provide only an indirect indication of the amount metabolized, particularly for compounds with relatively low blood:air partition coefficients [14]. Moreover, the human post-exposure kinetics of volatiles is typically dependent on a number of uncertain parameters apart from those for metabolism. For example, in the case of methylene chloride inhalation in the human [15] some of the largest sensitivities for predictions of blood concentrations during the post-exposure period are to the parameters for the volume, blood-flow, and partitioning of the fat, and for the blood-flow to the liver. In the case of vinyl chloride [14], varying the metabolic clearance 10-fold had little impact on the fit of the PBPK model to data on parent chemical concentrations during and after a constant concentration inhalation exposure to 2.5 ppm vinyl chloride for 30 minutes. However, data from inhalation exposures to similar concentrations of the same compound using a closed re-breathing chamber provided a much better estimate of metabolic clearance. These studies demonstrate that highly uncertain parameters are likely to have more influence than the metabolic parameter values on the post-exposure PCE concentrations predicted by the model for human inhalation exposures.

Another important determinant of post-exposure parent chemical kinetics for volatiles such as PCE is the subject's activity level, due to its effects on both ventilation rate and the associated redistribution of blood-flows [105]. Therefore, even less confidence can be placed in metabolic parameter values estimated from studies conducted in the workplace, such as those used to parameterize the human models of Ward et al. [22] and Bois et al. [24].

Subsequent to the publication of the human PBPK models for PCE described above, a new experimental study was reported in which human subjects were exposed to lower concentrations of PCE than in previous studies (10, 20, or 40 ppm for 6 hours), and the urinary excretion of TCA was measured for 72 hours post-exposure [1]. Blood concentrations of TCA were also reported at two time-points following the 10- and 40-ppm exposures. Since this study

was not used in the development of any of the published models, and since it was conducted at lower concentrations than previous studies, it provides an opportunity to validate the metabolism predictions of the various models. In order to make use of this time-dependent metabolite formation and excretion data to infer total metabolism, the PBPK model of Gearhart et al. [2], which includes a description of TCA formation and excretion, was exercised for the conditions of the exposure. The predictions of the Gearhart et al. [2] model, using the original parameter values (Table 2), are compared with the experimental data in Figure 4. With no adjustment of the parameter values, the predicted blood concentrations of TCA are in good agreement with the experimental data, while the model over-predicts the rate of excretion by roughly a factor of two.

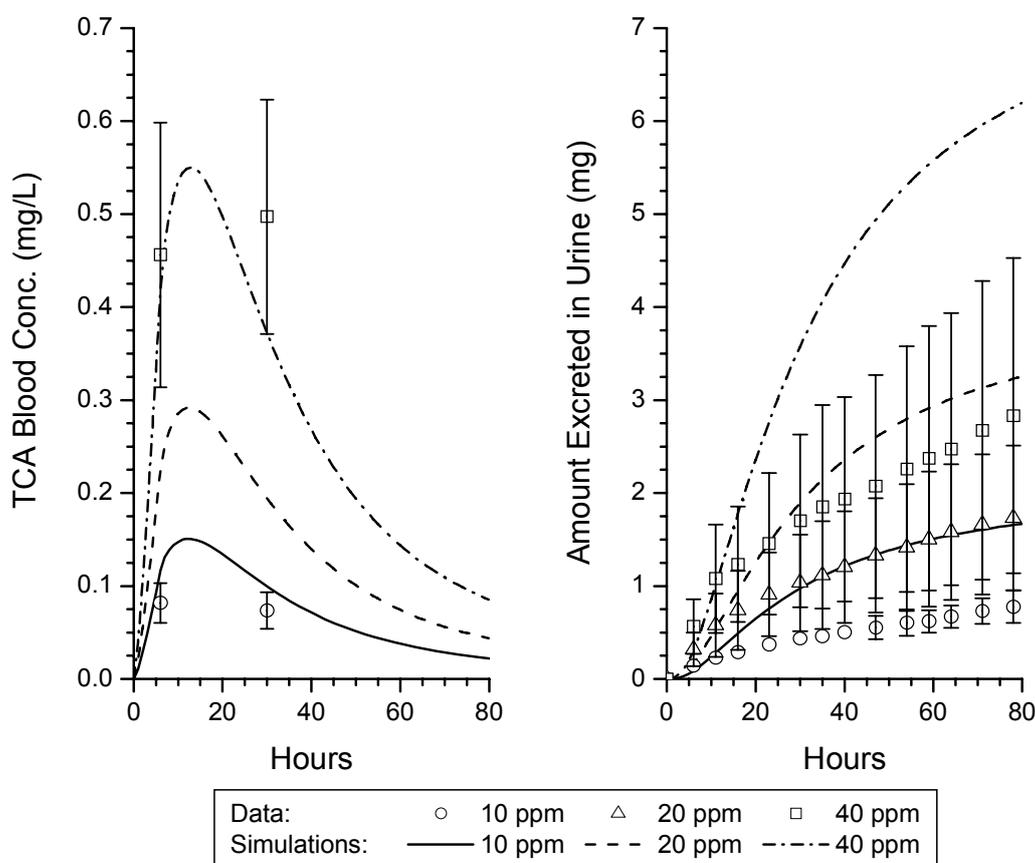


Figure 4: Predicted (curves) and experimental (symbols) blood concentrations (left) and urinary excretion (right) of TCA for 6 hour inhalation exposures of human subjects to PCE at 10, 20, and 40 ppm (Volkel et al. 1998) [1], using the original PBPK model of Gearhart et al. (1993) [2]. Data are plotted as means \pm one standard deviation. Error bars do not appear where the standard deviation was smaller than the symbol used to plot the mean.

Although the purpose of this review was not to develop yet another PBPK model for PCE, there appeared to be a systematic discrepancy between the predictions of the Gearhart et al. [2] model and the experimental data for the time-course of urinary excretion of TCA [1]. Specifically, the model was only

able to simulate the rapid early excretion of TCA at the expense of overestimating excretion at longer times (Figure 4). Reducing the urinary excretion rate for TCA from the original value of 0.12 to the value of 0.023 used in a PBPK model for trichloroethylene [18], resulted in better agreement with the urinary excretion of TCA at the end of the measurement period, but underestimated the initial rate of excretion (not shown). The nature of these discrepancies suggested the need to include metabolism of PCE to TCA in the kidney, with direct excretion of the TCA formed in the kidney into the urine (rather than into the systemic volume of distribution). Accordingly, the model of Gearhart *et al.* [2] was modified to incorporate these elements, and the relative capacity of PCE metabolism in the kidney was estimated by fitting the data of Volkel *et al.* [1]. Better agreement with the experimental time-course for TCA excretion was obtained when the metabolic capacity of the kidney was assumed to be 10% of the capacity of the liver, and the value of V_{maxc} in the liver was reduced from 0.28 to 0.24 (Figure 5).

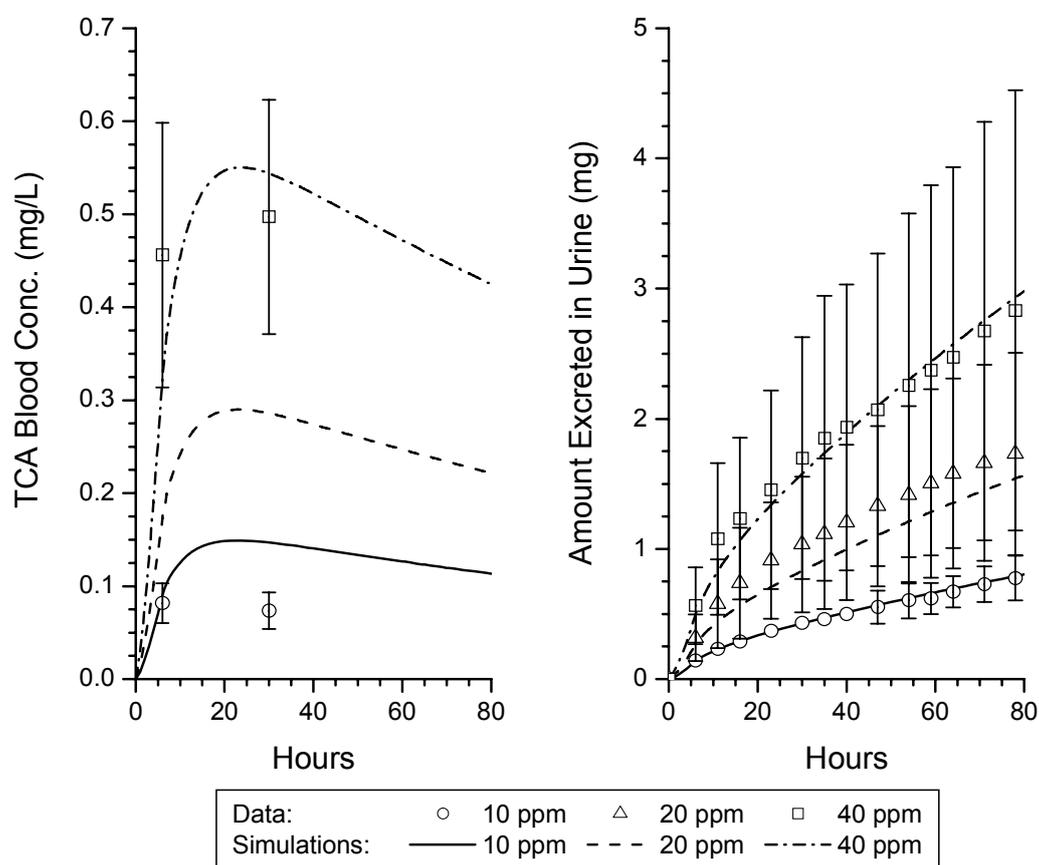


Figure 5: Predicted (curves) and experimental (symbols) blood concentrations (left) and urinary excretion (right) of TCA for 6 hour inhalation exposures of human subjects to perchloroethylene at 10, 20, and 40 ppm (Volkel *et al.* 1998) [1], using a modification of the PBPK model of Gearhart *et al.* (1993) that included metabolism of PCE to TCA in the kidney, with direct excretion of the TCA formed in the kidney into the urine. Published pharmacokinetic parameters for TCA (Clewell *et al.* 2000) [18] were used. To obtain the simulations shown in

this figure, values of 0.24 and 0.024 were used for V_{\max} in the liver and kidney, respectively. Data are plotted as means \pm one standard deviation. Error bars do not appear where the standard deviation was smaller than the symbol used to plot the mean.

The pharmacokinetic parameters for TCA in the modified model were taken from a published PBPK model for trichloroethylene [18]. While the agreement of the modified model with the data on TCA excretion does not necessarily imply that the kidney contributes to the metabolic clearance of PCE, such a possibility is supported by data indicating that several CYP isoforms, including 2E1, 2A6, and 3A4, contribute to the metabolism of anesthetics in human kidney [106].

In order to evaluate the predictions of the other human PBPK models, the selected PCE model was coupled to a sub-model of TCA pharmacokinetics from a PBPK model for trichloroethylene [18]. The results of this approach are illustrated in Figure 6, where the PCE parent chemical model is from Ward et al. [22].

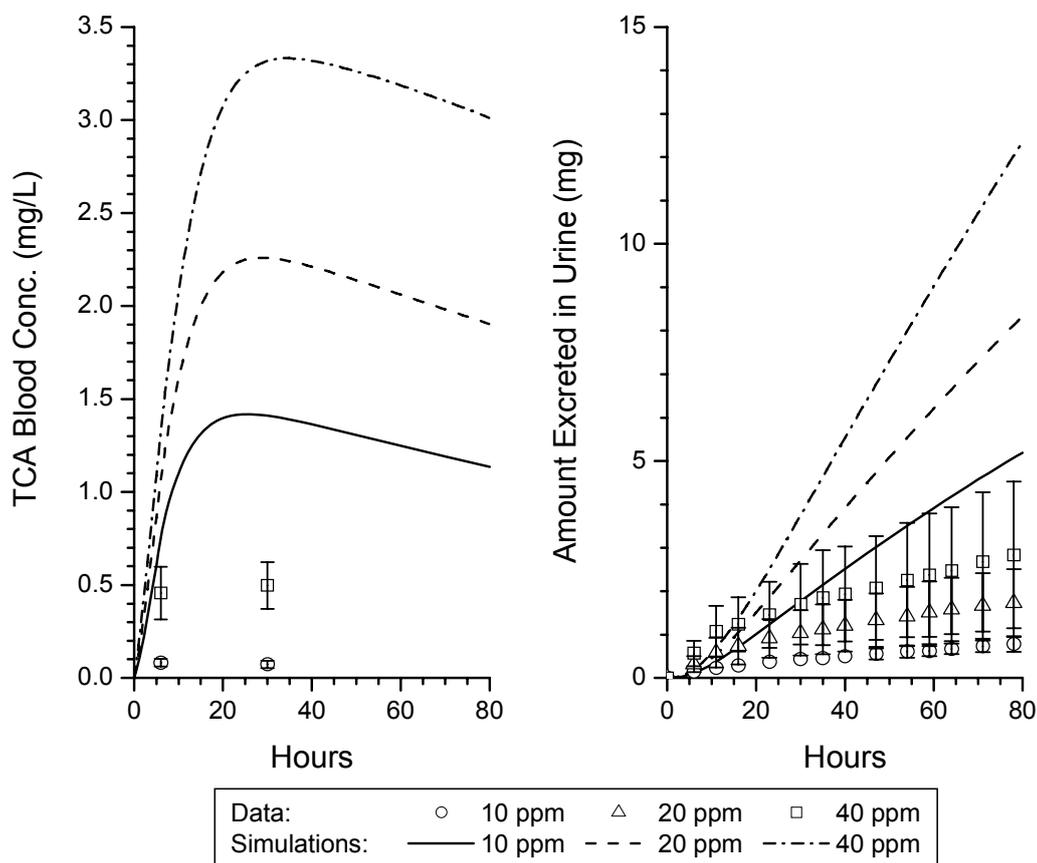


Figure 6: Predicted (curves) and experimental (symbols) blood concentrations (left) and urinary excretion (right) of TCA for 6 hour inhalation exposures of human subjects to perchloroethylene at 10, 20, and 40 ppm (Volkel et al. 1998) [1], using the PCE parameter values from the PBPK model of Ward et al. (1988) [22], together with a published pharmacokinetic submodel for TCA (Clewell *et al.* 2000) [18]. Data are plotted as means \pm one standard deviation. Error bars do

not appear where the standard deviation was smaller than the symbol used to plot the mean.

A comparison of the predictions across the human models is presented in Figure 7. All of the human models over-predict the urinary excretion of TCA in the Volkel *et al.* [1] study, ranging from a factor of two for the model of Gearhart *et al.* to a factor of more than ten for the model of Reitz *et al.* [29]. More importantly, two of the models [22, 23] demonstrate a concentration dependence (reflected in the slope of the lines connecting the predictions) that differs significantly from that of the experimental observations. These two models have in common the use of lower values for the metabolism affinity parameter (K_m 's less than 1 mg/L) as compared to the models that more closely predict the observed concentration dependence (K_m 's greater than 1 mg/L).

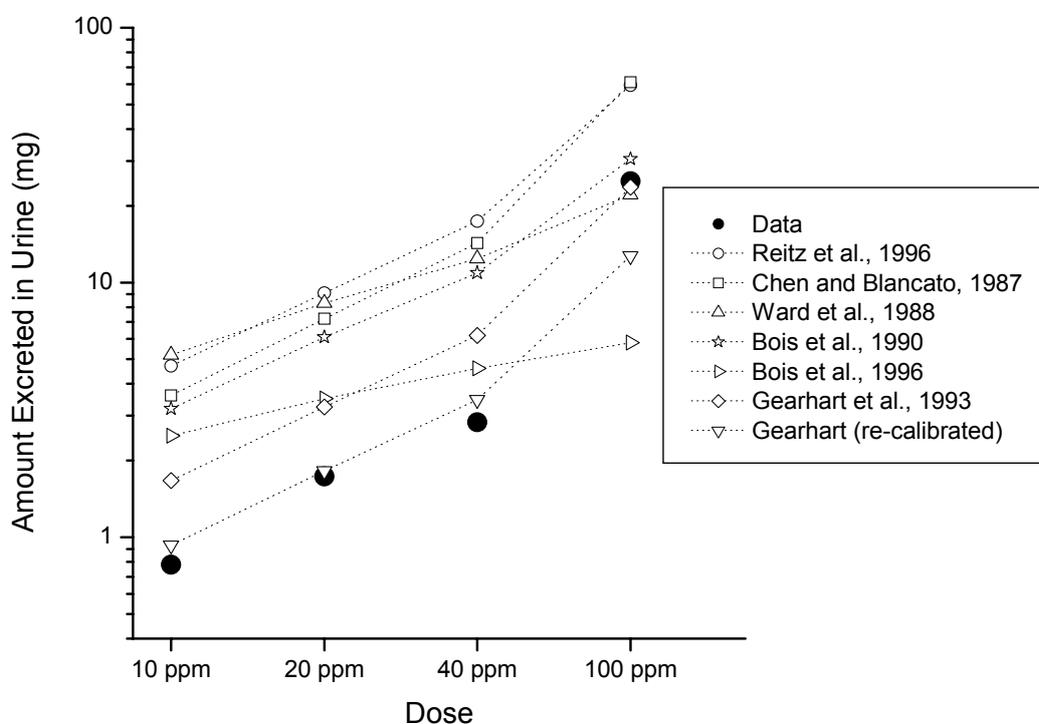


Figure 7: Predicted (dotted lines and open symbols) and experimental (solid symbols) total urinary excretion of TCA at 72 hours post-exposure, for inhalation exposures of human subjects to PCE at 10, 20, and 40 ppm for 6 hours (Volkel *et al.* 1998) [1] and at 100 ppm for 8 hours (Fernandez *et al.* 1976) [93]. Predictions were obtained using the PCE parameter values from the indicated PBPK model, together with a published pharmacokinetic submodel for TCA (Clewell *et al.* 2000) [18]. The re-calibrated Gearhart *et al.* (1993) model includes metabolism in the kidney, as described in the text.

The predictions of the human models for the fraction of PCE metabolized for continuous inhalation at 1 ppb or steady-state ingestion of 2L/day of drinking water at 1 $\mu\text{g/L}$ are presented in Table 4. The relationships of the model predictions, which span more than an order of magnitude, are similar to those in

Figure 7, except for the two models with the shallower concentration dependence of metabolism [22, 23], which predict relatively higher fractional metabolism at these very low exposures. Based on the performance of the various models in predicting the data of Volkel *et al.* [1], it can be concluded that metabolism estimates obtained with the model of Gearhart *et al.* [2] would provide the most reliable dose metrics for a PCE risk assessment.

Table 4: Fraction Metabolized During Continuous Human Exposure to Perchloroethylene at 1 ppb in Air or 1 µg/L in Water, Predicted with Different PBPK Models

<u>Model</u>	<u>Fraction Metabolized</u>	
	<u>Inhalation</u>	<u>Oral</u>
Bois <i>et al.</i> , 1996 [23]	0.36	0.54
Ward <i>et al.</i> , 1988 [22]	0.114	0.265
Reitz <i>et al.</i> , 1996 [29]	0.077	0.179
Bois <i>et al.</i> , 1990 [24]	0.069	0.142
Chen and Blancato, 1987 [21]	0.053	0.123
Gearhart <i>et al.</i> , 1993 [2]	0.011	0.026

III. EVALUATION OF RISK ASSESSMENT IMPLICATIONS

As discussed earlier, cancer risk assessments for PCE have generally been based on the liver tumors observed in the mouse bioassays [53, 54], using metabolized dose as the metric. The rationale for the use of metabolized dose as the measure of internal exposure is that the liver carcinogenicity of PCE is associated with the production of reactive moieties during the oxidative metabolism of PCE [33, 52]. In order to compare metabolized doses across species it is necessary to define the basis for equivalence. In the published USEPA [33, 52] risk assessments, the basis of equivalence for lifetime cancer risk was assumed to be the lifetime average daily amount of PCE metabolized per unit body surface area (actually calculated as body weight raised to the 2/3 power). That is, the amount metabolized in the human assumed to be equivalent to an amount metabolized in the mouse was obtained by multiplying the mouse value (in mg) by the ratio of the human and mouse body weights (in kg) raised to the 2/3 power. This scaling approach is referred to as body surface area scaling, and for many years it was the default method of cross-species scaling for cancer risk assessment used by the USEPA. The justification for its use was uncertainty regarding potential differences in sensitivity between rodents and humans [107]. In the OEHHA [34] PHG derivation, body surface area scaling (to the $\frac{3}{4}$ power) is also applied to the amount metabolized per unit body weight estimated by PBPK modeling. However, the recent USEPA [108] cancer guidelines indicate that when pharmacokinetic tissue dosimetry is used in

a risk assessment, no body surface area scaling should be performed. This change in policy reflects the conclusions of an interagency working group analysis of cross-species scaling [109]. The appropriate pharmacokinetic dose metric for a lesion produced by a short-lived reactive product of metabolism is the daily amount metabolized divided by the volume of the tissue in which it is produced [9], in this case the liver.

Another proposed mode of action for the liver carcinogenicity of PCE is the production of the stable metabolite, TCA [64]. In fact, TCA, a peroxisome proliferating compound that has been shown to be hepatocarcinogenic in mice, is considered a leading candidate as the basis for the mouse liver carcinogenicity of TCE [110]. An appropriate dose metric for liver carcinogenicity under this mode of action would be the integral over time of the concentration of TCA (and/or DCA) in the liver, or as a surrogate, in the blood [110]. This integral is referred to as the area under the curve (AUC), and if divided by the time period over which it is calculated yields the average concentration over that period.

Having defined the alternative dose metrics, it is necessary to select the PBPK model that should be used to estimate them. The present evaluation of the published PBPK models for PCE is consistent with the results of earlier comparisons that the predictions of metabolism in the mouse obtained with different models are in reasonable agreement, but the predictions of metabolism in the human vary substantially. Therefore, it is important that the estimates of metabolized dose used in a risk assessment for PCE be obtained with a model that has been adequately validated for that purpose. Based on the comparative evaluation of the predictive capability of the human models for the data of Volkel et al. [1], the model of Gearhart et al. [2] appears to provide the most reliable dosimetry in the human. In addition, the predictions of the Gearhart et al. [2] model for the kinetics of TCA have been validated in both the mouse and the human (Figures 3 through 5). The revision of the Gearhart et al. [2] model to include metabolism in the kidney, described above, did not significantly alter predictions of liver metabolism, but did affect predictions of AUC for TCA. In the case of TCA AUC, the use of the revised model produced a higher (more conservative) result. Therefore, the revised model was used for the risk assessment calculations performed in this study.

The dose metrics calculated with the model of Gearhart et al. [2], as revised to include kidney metabolism, are provided in Table 5. The parameter values used for these calculations are listed in Table 2. In order to reflect liver exposure to TCA, the model-predicted human dose metrics for AUC TCA in the blood would need to be adjusted for the differences in plasma binding between mice and humans. Data on the binding of TCA in the plasma of rats and humans, [111] suggest that TCA in plasma is bound to a much greater extent in the human (~80%) than in the rat (~50%). More recent data [104] confirm these results and show that the fraction bound in the mouse is even lower (~20%). Assuming binding of TCA in liver cells is similar across species, it can be estimated that the liver to plasma TCA concentration ratio in the human would be about 25% of the ratio in the mouse. Moreover, the observed dose-response

for the binding is such that at lower human exposures the binding would be greater than 80%, while at higher mouse exposures the binding would be lower than 20%. Thus using the average daily AUC of TCA in the plasma as the dose metric would overestimate the liver exposure to TCA in the human by at least 4-fold, and possibly more. A pragmatic resolution of this problem is to simply adjust the model-predicted human dose metrics for AUC TCA by the ratio of the mouse and human free TCA fractions in plasma; that is, the human dose metrics should be divided by a factor of four [110].

Table 5: Perchloroethylene Dose Metrics (Lifetime Average Daily Dose)^a

<u>Study</u>	<u>Species</u>	<u>Concentration or Dose</u>	<u>BW</u>	<u>Mg metabolized per kg liver</u>	<u>Mg metabolized per kg body weight</u>	<u>TCA AUC (mg-hr/L)</u>
NTP [54]	Male Mouse	100 ppm	0.037	286	16.3	502
		200 ppm		536	30.6	944
NTP [54]	Female Mouse	100 ppm	0.032	296	16.9	501
		200 ppm		555	31.6	941
	Human	1 ppm	70	0.88	0.0228	4.18 ^c
		1 µg/m ³		1.30e-4	3.36e-6	6.15e-4 ^c
		1 mg/kg/day		1.00	0.026	4.80 ^c
		1 mg/L ^b		0.029	0.00075	0.138 ^c

^a Dose metrics estimated using Gearhart et al. (1993) [2] model, as revised to include kidney metabolism. Model parameter values are listed in Table 2.

^b Assuming 70 kg individual consuming 2 L/day drinking water

^c Human dose metric for TCA in blood obtained from model has been divided by 4 to adjust for differences in mouse and human plasma binding of TCA (Clewell and Andersen 2004) [110].

Lifetime risk estimates obtained with the three different dose metrics are presented in Table 6. These estimates were based on the incidence of hepatocellular adenoma/carcinoma in the mouse in the PCE inhalation bioassay [54] using a time-to-tumor model to consider animal survival (TOX_RISK, V3.5, ICF Consulting, Inc., Fairfax, VA). The inhalation bioassay results were used for both inhalation and oral risk calculations to avoid the potential confounding effects of the corn oil vehicle on risk estimates derived from oil gavage studies [14]. This approach has also been recommended for TCE [110]. In the absence of definitive information on the mode of action, the conservative default approach in the new USEPA [108] cancer guidelines, linear low-dose extrapolation, was used. In this approach, a 95% lower bound on the dose associated with a 10% increase in tumor incidence is calculated, and the risk R at dose D is estimated from the LED₁₀ by the formula $R = 0.1 * D / LED_{10}$. In this case, both the LED₁₀ and D represent metabolized doses, so the PBPK model must be used to calculate the human metabolized dose, D, associated with a given environmental exposure.

Table 6: Lifetime Risk Estimates for Continuous Exposure to Perchloroethylene

Dose Metric	Mg metabolized per kg liver			Mg metabolized per kg body weight			TCA AUC		
	Male	Female	Geo-metric Mean ^a	Male	Female	Geo-metric Mean	Male	Female	Geo-metric Mean
LED ₁₀	25.8	45.2	34.1	1.5	2.6	2.0	45.4	76.3	58.9
Unit Risks:									
per ppm	3.4e-3	2.0e-3	2.6e-3	1.6e-3	8.8e-4	1.2e-3	9.2e-3	5.5e-3	7.1e-3
per µg/m ³	5.0e-7	2.9e-7	3.8e-7	2.3e-7	1.3e-7	1.7e-7	1.4e-6	8.1e-7	1.0e-6
per mg/kg-day	3.9e-3	2.2e-3	2.9e-3	1.8e-3	1.0e-3	1.3e-3	1.1e-2	6.3e-3	8.1e-3
per µg/l ^b	1.1e-7	6.4e-8	8.5e-8	5.1e-8	2.9e-8	3.8e-8	3.0e-7	1.8e-7	2.3e-7

^a Bolded values indicate preferred dose metric (mg metabolized in liver per kg liver)

^b Assuming 70 kg individual consuming 2 L/day drinking water

Risk estimates are provided in Table 6 using all three dose metrics; however, the risk estimates based on the different metrics should not be considered equally likely. There is not actually any biological justification for the use of amount metabolized per unit body weight as a dose metric. As discussed by Andersen et al. [9], the use of amount metabolized in the liver per unit liver weight provides a reasonable surrogate for the steady-state concentration of a reactive intermediate produced during metabolism, assuming constant stoichiometry and sufficiently high reactivity to rule out transport away from the target tissue before reaction. No similar rationale can be made for dividing the amount metabolized in a particular tissue (e.g., the liver) by the body weight. On the other hand, although the AUC for TCA has been suggested as a dose metric for the liver carcinogenicity of TCE [110], there does not appear to be equal justification in the case of PCE. The liver toxicity of PCE is greater than that of TCE for the same level of metabolite generation [58]. If the liver carcinogenicity of both PCE and TCE were due to the effects of TCA, one would expect similar potency estimates (LED₁₀s) for these two chemicals using the AUC TCA dose metric. In fact, for TCE-induced mouse liver tumors in the inhalation bioassays, the mean of the LED₁₀s for AUC TCA is 926 mg-hrs/L [110], as opposed to the LED₁₀ values of 45.4 and 76.3 mg-hrs/L obtained for male and female mice, respectively, for the PCE inhalation bioassay (Table 6). Similarly, the average daily AUC for TCA at its lowest carcinogenic dose in the mouse (1 g/L TCA in drinking water) is on the order of 1,600 mg-hrs/L [110]. Thus, the production of TCA in the PCE bioassays does not appear to be adequate, in itself, to account for the observed tumorigenicity, leaving the generation of reactive metabolites, or a combination of processes that includes the generation of reactive metabolites, as the most likely mode of action.

Using the preferred dose metric, amount metabolized in the liver per unit liver weight, the mean inhalation unit risk predicted using the PBPK model is $3.8 \times 10^{-7} (\mu\text{g}/\text{m}^3)^{-1}$, which is very similar to the inhalation unit risk of $5.8 \times 10^{-7} (\mu\text{g}/\text{m}^3)^{-1}$ estimated by the USEPA [33] in their last published risk assessment for PCE. The USEPA [33] inhalation unit risk was also based on the mouse liver tumors in the NTP [54] bioassay, but was obtained using estimates of metabolized dose (based on total urinary excretion of metabolites) from pharmacokinetic studies in mice [58] and humans [59], and assuming equivalence across species on a mg metabolized per body surface area per day basis. The mean drinking water unit risk predicted using the Gearhart *et al.* [2] PBPK model is $8.5 \times 10^{-8} (\mu\text{g}/\text{L})^{-1}$, which is roughly an order of magnitude lower than the unit risk of $1.5 \times 10^{-6} (\mu\text{g}/\text{L})^{-1}$ estimated by the USEPA [52] in their last published oral risk assessment for PCE. This USEPA [52] unit risk was based on the mouse liver tumors in the NCI [53] bioassay, with equivalence across species defined on a mg ingested per body surface area per day basis, assuming 100% metabolism of ingested dose. Given the similar predictions of the alternative PBPK models in the mouse, coupled with the tendency of several of the human models to overestimate metabolism for low human exposures by roughly 5- to 15-fold (Figure 7), risk estimates obtained with models other than that of Gearhart *et al.* [2] could be expected to produce risk estimates as much as an order of magnitude higher than those in Table 6.

It is also of interest to evaluate the impact of variation in the PBPK model estimates of fractional metabolism in the human on the PHG for PCE derived by OEHHA [34]. In their risk assessment, OEHHA [34] converted estimates of PCE intake via inhalation, oral, and dermal exposure routes, expressed as drinking water volume equivalents, to metabolized doses using upper-bound estimates (95% upper confidence limits) of the fraction of PCE metabolized from the PBPK uncertainty analysis of Bois *et al.* [23]. These upper-bound estimates were 58% and 79%, for the inhalation and oral routes, respectively [34]. However, as discussed earlier, it appears that the model of Bois *et al.* [23] greatly overestimates fractional metabolism in humans at the low exposures of interest for risk assessment (Figure 7, Table 4). Therefore the upper-bound estimates of fractional metabolism obtained with this model must be considered highly suspect.

Using the Gearhart *et al.* [2] model, the estimates of the fraction of PCE metabolized in the liver following inhalation and oral exposure are 1.1%, and 2.6%, respectively (Table 4), about 50- and 30-fold lower than the upper-bound estimates used by OEHHA (2001). Using intakes derived using these estimates of fractional metabolism (Table 7) with the mean of the male and female potency estimates from Table 6 for the metric selected by OEHHA, mg metabolized per kg body weight ($0.1/\text{LED}_{10} = 0.049$), the PHG estimated with the Gearhart *et al.* (1993) model is 14 $\mu\text{g}/\text{L}$:

$$C = \frac{1 \times 10^{-6} \times 70\text{kg}}{(0.049 \times 0.054\text{L}/\text{d}) + (0.049 \times 0.0467\text{Leq}/\text{d})} = 0.014\text{mg}/\text{L} = 14\text{ppb}$$

This PHG estimate is about 240-fold higher than the OEHHA [34] proposal of 0.056 µg/L. Of this difference, about a factor of 7 is due to the inappropriate use of body surface area scaling in the derivation of potency estimates by OEHHA [34], and the rest is primarily due to the different estimates of fractional metabolism in the human.

Table 7: Estimated Exposure to Perchloroethylene in Drinking Water for an Average California Resident

	Avg. Dose (mg/kg/day)	Exposure : Vol. Equiv. (L/day)	Fraction Metabolize d (UCL)	Metabolize d Vol. Equiv. (L/day)	% of Total Dose
Inhalation	1.27×10^{-6}	3.54	0.011	0.039	38.7 %
Ingestion: Water	7.16×10^{-7}	2.00	0.026	0.052	53.6 %
Produce, meat, etc	2.48×10^{-8}	0.07	0.026	0.002	
Total Ingestion	7.41×10^{-7}	2.07	0.026	0.054	
Dermal Uptake	2.51×10^{-7}	0.70	0.011	0.0077	7.7%
Dose Sum	2.26×10^{-6}	6.31		0.1007	100%

IV. CONCLUSIONS

PCE provides a useful case study to highlight some of the issues associated with the comparative evaluations of PBPK models for use in a risk assessment. A number of structurally similar PBPK models have been developed for PCE, differing primarily in the parameter values estimated for metabolism, although one of the models [2] also includes a description of the kinetics of the principal metabolite, TCA. All of the models provide reasonably accurate simulations of some of the pharmacokinetic data available for PCE in mice or humans, and could therefore be considered, to some extent, to be validated. However, while similar predictions of metabolism are obtained with the alternative models in the mouse, predictions of metabolism in the human with different models vary considerably. This species difference in the variation of the PBPK model estimates of metabolism seems to stem from the different kinds of data used to identify the metabolism parameter values in mice and humans. All of the mouse models made use of data that were highly informative regarding metabolism, including radiolabel disposition, metabolite excretion, or closed chamber clearance studies. Many of the human models, on the other hand, relied on parent chemical kinetic data that do not directly reflect metabolism.

The use of parent chemical kinetic data to infer metabolic parameter values or validate model estimates of metabolism in the human can be highly

misleading, because it is often the case that other uncertain parameters can strongly influence model predictions of parent chemical kinetic behavior. Therefore, demonstrating the agreement of a human model with data on the kinetics of the parent chemical may give a false sense of validation if the use of the model in the risk assessment is to predict the rate of metabolism. To avoid this problem it is crucial that time-dependent sensitivity analysis be conducted with the model under the conditions of the exposure to assure that the metabolic parameter values are identifiable from the available data [15].

The use of *in vitro* studies to identify human metabolic parameter values is limited by the difficulty of obtaining reliable estimates of the *in vivo* affinity, making it necessary to adjust the *in vitro* affinities measured in human tissues on the basis of the relationship between *in vitro* and *in vivo* estimates in the rodent [29]. A more direct approach would be to use estimates of the maximum velocity of metabolism from *in vitro* studies together with estimates of intrinsic clearance (V_{max}/K_m) from *in vivo* re-breathing studies.

In the case of the evaluation of the PCE human models, recent data [1] on the urinary excretion of TCA, the major metabolite of PCE, at lower exposure concentrations (10 to 40 ppm) than had been reported previously, made it possible to compare the high- to low-dose extrapolation capability of the various published models. We found that the model of Gearhart et al. [2], which was the only model to include a description of TCA kinetics, gave the closest predictions of the urinary excretion observed in the low-concentration exposures (within a factor of two). Other models over-estimated metabolite excretion in this study by 5- to 15-fold. Clearly, there are several advantages associated with the addition of a description of metabolite kinetics to a parent chemical model, even when the risk assessment is based on the rate of parent chemical metabolism rather than tissue exposure to the metabolite. Including a description of a major metabolite allows data on the time-course of metabolite kinetics and excretion to be more readily used for metabolism parameter estimation or validation, and reduces the uncertainty associated with the tendency in human studies to collect urine for too short a time to ensure that all of the metabolite has been excreted.

Based on the systematic nature of the discrepancy between the predictions of the model of Gearhart et al. [2] and the data on the time-course of the urinary excretion of TCA in the Volkel et al. [1] study, we suspected that TCA generated from the metabolism of PCE in the kidney was being excreted directly into the urine. Adding a quantitative description of this hypothesis to the model greatly improved the ability of the model to simulate the data. Although the success of the description does not in itself demonstrate the validity of the hypothesis, several CYP isoforms, including 2E1, 2A6, and 3A4, have been shown to contribute to the metabolism of compounds similar to PCE in human kidney [106].

The wide discrepancies between the predictions of the human PCE models resulted in large part from the fact that the metabolic parameter values in each model were estimated on the basis of only a small subset of the available kinetic

data; in some cases only a single study. Undoubtedly, if all of the data used in the development of previously published models were included in the development of subsequent models, the predictions of the later models would inevitably come closer together. Ideally, a PBPK model should be compared with all relevant data regarding the parameters to which the dose metric predictions are sensitive, rather than focusing only on one or two studies. This pre-supposes the use of sensitivity and uncertainty analysis to identify the parameters of concern (those that have the most influence on the dose metric estimate and are the least certain), as well as of time-dependent sensitivity analysis [15] to select the experimental data that are most informative for those parameters.

Cancer risk estimates for PCE based on liver tumors in mice in the NTP study were estimated with the Gearhart et al. [2] model, using lifetime average daily amount metabolized per volume liver as the dose metric. The resulting inhalation risk estimate for lifetime exposure to PCE at one microgram per cubic meter was 0.4 per million. The corresponding risk for oral exposure at one milligram per kilogram per day was 3 per thousand. Risk estimates using other published PCE PBPK models would be higher by as much as an order of magnitude, due to the tendency of the models to over-estimate human metabolism.

Finally, it is important to note that PBPK analyses alone provide limited insight into species differences in another response element critical for human health risk assessment: pharmacodynamics. In seeming contrast with the results of high-dose rodent studies, available epidemiological studies do not support a conclusion that PCE is carcinogenic in humans. Unfortunately, neither the mouse nor the rat appears to be an appropriate animal model for prediction of potential human carcinogenic responses to PCE, due both to differences in metabolic capabilities and to less well understood differences in cellular responses [49]. Thus, further studies to elucidate the mechanism(s) of action of PCE in different species will be of great value in improving the quality of human health risk assessments for this important compound.

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Chapter 11

On the Incorporation of Biokinetic and Mechanistic Data in Modeling for Risk Assessment: Discussion

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As stated in the introduction, the goal of the research described in this thesis was to develop approaches for fostering the increased use of emerging scientific information and innovative methods in chemical risk assessments, in order to assure the protection of public health while limiting the economic and social consequences of over-regulation. The following section provides a brief summary of the more important results of this research. This will be followed by a more extended discussion of three related topics:

- Lessons learned regarding the issues faced by regulators with regard to the use of chemical-specific data and modeling in risk assessment
- Directions for future research to foster the more rapid incorporation of new chemical-specific data and models in risk assessment
- Directions for future research to advance the state of the art for the application of mathematical modeling in risk assessment

Synopsis of the Research Presented in this Thesis

The research described in this thesis was comprised of a number of individually published studies that were conducted with the common goal of advancing the state of the art for the incorporation of biokinetic and mechanistic data in modeling for risk assessment. These studies were divided into three sections that reflect the different nature of the research described.

The first section included three studies that were performed to foster the broader use of biokinetic and mechanistic data and mathematic modeling approaches in risk assessment.

In the study presented in *Chapter 2*, a critical review was conducted of the use of mode of action in risk assessment, and examples from the literature of the application of mode-of-action evaluation were examined to assess the potential value of this concept. The principal conclusion of the review was that the mode of action concept represents an important advance toward increasing the biological basis of chemical risk assessments for both cancer and noncancer endpoints. Detailed analyses of two case studies, on risk assessments for trichloroethylene and inorganic arsenic, identified a number of key issues that need to be addressed in order to assure optimal use of mode of action evaluation in future risk assessments. Among these were the need for regulators to make regular use of outside expertise to assure the quality of their risk assessments in the face of increasing technical complexity, and the need for research on quantitative, biologically-based dose-response approaches that can provide an alternative to current default methods.

In the U.S., quantitative dose-response analysis has been used for many years to estimate cancer risks; however, this approach has not been widely applied to estimates risks for noncancer effects. In the study described in *Chapter 3*, quantitative dose-response analyses were conducted for the

noncancer effects of two chemicals: inorganic arsenic and methylmercury. These analyses demonstrate that empirical dose-response analysis can be of value for putting noncancer risks in perspective and to support cost-benefit analysis. It is important to note, however, that while it is argued that quantitative risk estimates obtained with empirical dose-response models can be of value in risk assessments for noncancer as well as cancer endpoints, it is not recommended that empirical dose-response modeling should be used to support extrapolation of risk estimates below the range of experimental observation for either cancer or noncancer endpoints. For both cancer and noncancer effects, the shape of the dose-response at very low doses remains unknown and, perhaps, unknowable (Crump et al., 1997).

The study documented in *Chapter 4* developed an approach for the incorporation of biokinetic and metabolic data in risk assessment using PBBK modeling. The proposed approach makes use of mode of action analysis and can be consistently applied across both cancer and noncancer endpoints. Using this consistent approach, PBBK modeling can be a highly effective tool for incorporating biokinetic and mechanistic data in both cancer and noncancer risk assessments.

The second section included three studies that were performed to examine the extent to which chemical-specific properties and human variability interact to produce different risks across individuals in a population.

Chapter 5 described a study that combined Monte Carlo uncertainty analysis with PBBK modeling to estimate the population distribution of safe doses for the effects of methylmercury. The toxicity of concern was the potential for neurodevelopmental effects from fetal exposure to methylmercury resulting from maternal ingestion of contaminated fish. To address this concern, a PBBK model was developed that included a description of the time-dependent changes in mother and fetus during the period of gestation, the critical window of susceptibility for the developmental effects of this chemical. The combination of Monte Carlo analysis and PBBK modeling made it possible to characterize the impact of human interindividual variability on the distribution of risks across the general population and to identify the key factors that contribute to increased risk for an individual. In the case of methylmercury, the principal clearance process was identified as incorporation and elimination in hair. The ratio of risks for average and sensitive individuals (in terms of biokinetics) was roughly a factor of two, somewhat less than the usual default factor of three.

The study documented in *Chapter 6* consisted of a critical review of the available data on the age- and gender-dependence of human physiology, metabolism, and clearance, coupled with an evaluation of the potential impact of these differences on tissue dosimetry, and hence risk. The study concluded that age- and gender-specific differences in physiology, metabolism, and clearance can lead to significant variations in the internal dose, and hence risk, resulting from the same exposure to a chemical. However, the direction and extent of the

differences depend on the properties and mode of action of the chemical, and are difficult to determine confidently without the use of quantitative PBBK modeling.

Chapter 7 described an effort that made use of the data and analyses from Chapter 6 to develop a PBBK model that could be used to examine the extent to which chemical-specific properties, physiology, and age-dependent biochemical processes interact to produce different risks across individuals in a population at different ages, from birth to 70 years of age. The study demonstrated that PBBK models that include age- and gender-dependent parameters can be used to predict the relative risk of individuals at different lifestages. The PBBK model developed for this study includes fully age-dependent descriptions of all of the physiological and biochemical parameters, and can be used to estimate the relative sensitivity of children or the elderly using chemical-specific data on partitioning and metabolism. For the chemicals examined in this study, which were selected to represent a variety of properties and modes of action, the standard default uncertainty factor of 3 for human variability in biokinetics appeared to be adequate.

The final section included three studies that attempted to make optimal use of the available biokinetic and mechanistic data in cancer risk assessments for three chloroethylenes using the methodologies emphasized in this research: mode of action evaluation, PBBK modeling, and dose-response modeling.

Chapter 8 described a cancer risk assessment performed for vinyl chloride, a chemical with a well-documented, genotoxic mode of action. The analysis provided a comparison of predicted human risks using data from different species and different routes of exposure. PBBK modeling of the liver carcinogenicity of vinyl chloride resulted in similar estimates of human risk on the basis of studies from different species (mouse, rat, and human) and routes of exposure (inhalation and dietary), demonstrating the value of risk assessment approaches based on target tissue dose.

Chapter 9 presented a cancer risk assessment conducted for trichloroethylene, a chemical with poorly-understood, non-genotoxic modes of action for several different cancer endpoints. This analysis required an extensive mode-of-action evaluation and the development of a fairly complex PBBK model. This study concluded that PBBK modeling, in itself, cannot provide meaningful risk estimates unless the risk assessment approach is consistent with the mode of action for the chemical and endpoint of concern. In the case of trichloroethylene, the modes of action in each of the cancer target tissues (lung, liver, and kidney) appear to be driven primarily by non-genotoxic effects (cytotoxicity in the lung and kidney, receptor activation in the liver), and are more consistent with a threshold risk assessment approach. Failure to incorporate this mode-of-action information can result in risk estimates that are seriously misleading regarding the relative hazards from trichloroethylene as compared to other compounds, such as vinyl chloride, where there is clear evidence of a genotoxic mode of action.

Chapter 10 demonstrated an approach for conducting a critical evaluation of alternative PBBK models, using perchloroethylene as a case study. The evaluation demonstrated that the proper evaluation of PBBK models for potential application in risk assessment is a difficult and complex process that not only requires evaluation of the model itself, but also of the mode(s) of action for the effect(s) of the chemical and the adequacy of the data available for model parameterization and validation. A modification of the most successful model, which provides improved fits to the available data, was then used to conduct a cancer risk assessment for perchloroethylene. In the case of perchloroethylene, the mode of action appears to involve the production of a reactive metabolite. The crucial data was from low-concentration exposures of human subjects to perchloroethylene in which the production of the principal metabolite, trichloroacetic acid, was measured. Models with parameters identified solely from data for the parent chemical at higher concentrations over-predicted the metabolite data at the lower concentrations, and would therefore be expected to seriously overestimate risks at even lower environmental exposures.

Overall, the research described in this thesis demonstrates that the use of mode-of-action evaluation, PBBK modeling, and quantitative dose-response modeling can greatly increase 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.

Lessons Learned for Researchers and Regulators

Looking back over the work described in this thesis, the process of incorporating chemical-specific data in risk assessment has been a contentious but productive 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.

It is also clear, however, that 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; the history of science is replete with examples where the general consensus of scientists was wrong (phlogiston and spontaneous generation are famous examples).

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 at the expense 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 alternative possibilities. 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.

Another lesson learned over the seven years in which this research was conducted is that the state of the art for chemical risk assessment is advancing rapidly. For example, a recent study by the Health Council of the Netherlands recommends 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.

Table 1

Some of the Scientific Skills and Experience Potentially Required
for a Major Risk Assessment

- Mode of action evaluation
 - 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
- Dose-response modeling
 - Quantitative epidemiological dose-response analysis vs. meta-analysis of multiple studies
 - Benchmark modeling of nested quantal developmental data vs. continuous data with multiple covariates
 - Low-dose extrapolation of cancer bioassay data with the Linearized Multi-Stage model vs. the Armitage-Doll model (for time-to-tumor data)
 - Categorical regression of dose-response-severity for acute toxicity vs. concentration-time extrapolation
 - Biologically based dose-response modeling
- Evaluation of a biokinetic model
 - 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

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 modeling. To assure that these powerful techniques fulfill their intended purpose (i.e., to increase the accuracy of the risk assessment), it is obvious that 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. It is imperative that regulatory agencies adhere 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 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, it will become increasingly necessary for regulatory agencies 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 modeling 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. It is unreasonable to expect regulators 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. As the well-known toxicologist and pharmacokineticist, Melvin Andersen, has said: "If you don't have a target, they're just arrows on the wall."

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 are concerns about perceived uncertainties associated with their use. To some extent these 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 a tool for quantitatively characterizing the range of plausible risk estimates and objectively identifying a preferred estimate.

As an example, Figure 1 (taken from Chapter 9) 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 (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 (MOE approach for the kidney).

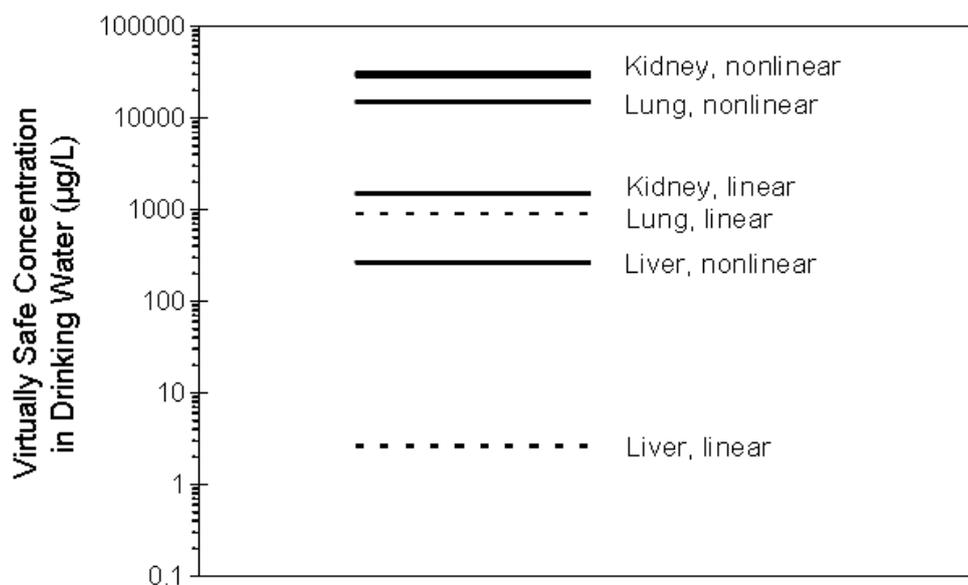


Figure 1. Comparison of virtually safe concentrations for trichloroethylene in drinking water. Dashed lines indicate approaches that are not recommended. Broader solid line identifies the preferred approach based on biological plausibility. (Adapted from Clewell and Andersen, 2004)

Of course, the presentation of alternative risks in figure 1 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 (Figure 2).

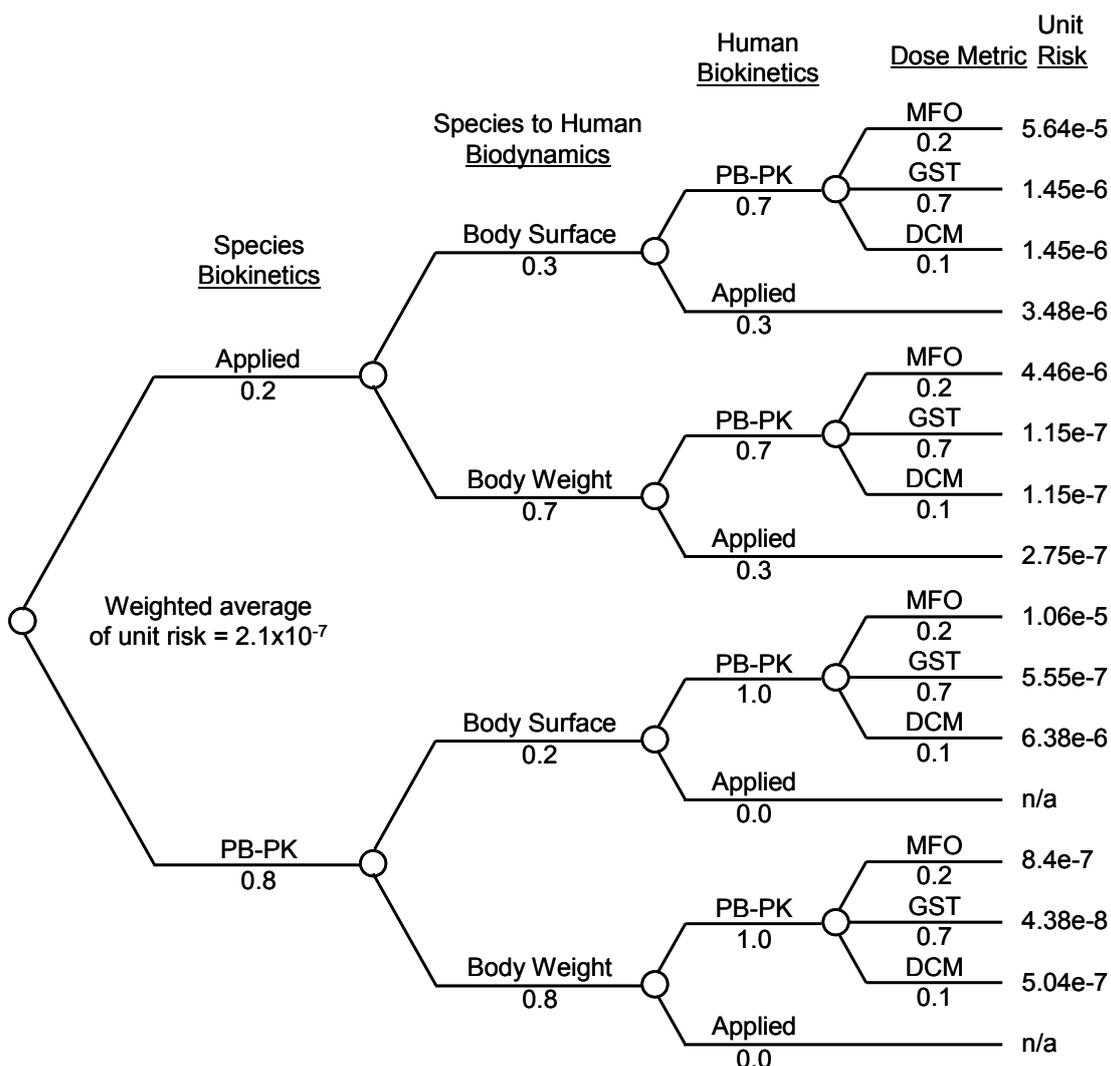


Figure 2: Decision tree diagram for the cancer risk assessment for methylene chloride. Each branch of the tree is annotated with the probability assigned to that alternative (i.e., the level of belief that it is the correct alternative). See text for explanation of terms and abbreviations. (Adapted from Clement and Tatman, 1990)

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) the PBBK model for that chemical (Andersen et al., 1987) in the risk assessment. These decisions, as illustrated in the tree diagram in Figure 2, included whether to use the PBBK (PB-PK in Figure 2) model or applied dose in the animal, whether to scale from animal 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).

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 wider group of individuals.

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 $1 \mu\text{g}/\text{m}^3$) associated with the selected weighting for the alternative approaches (Figure 3).

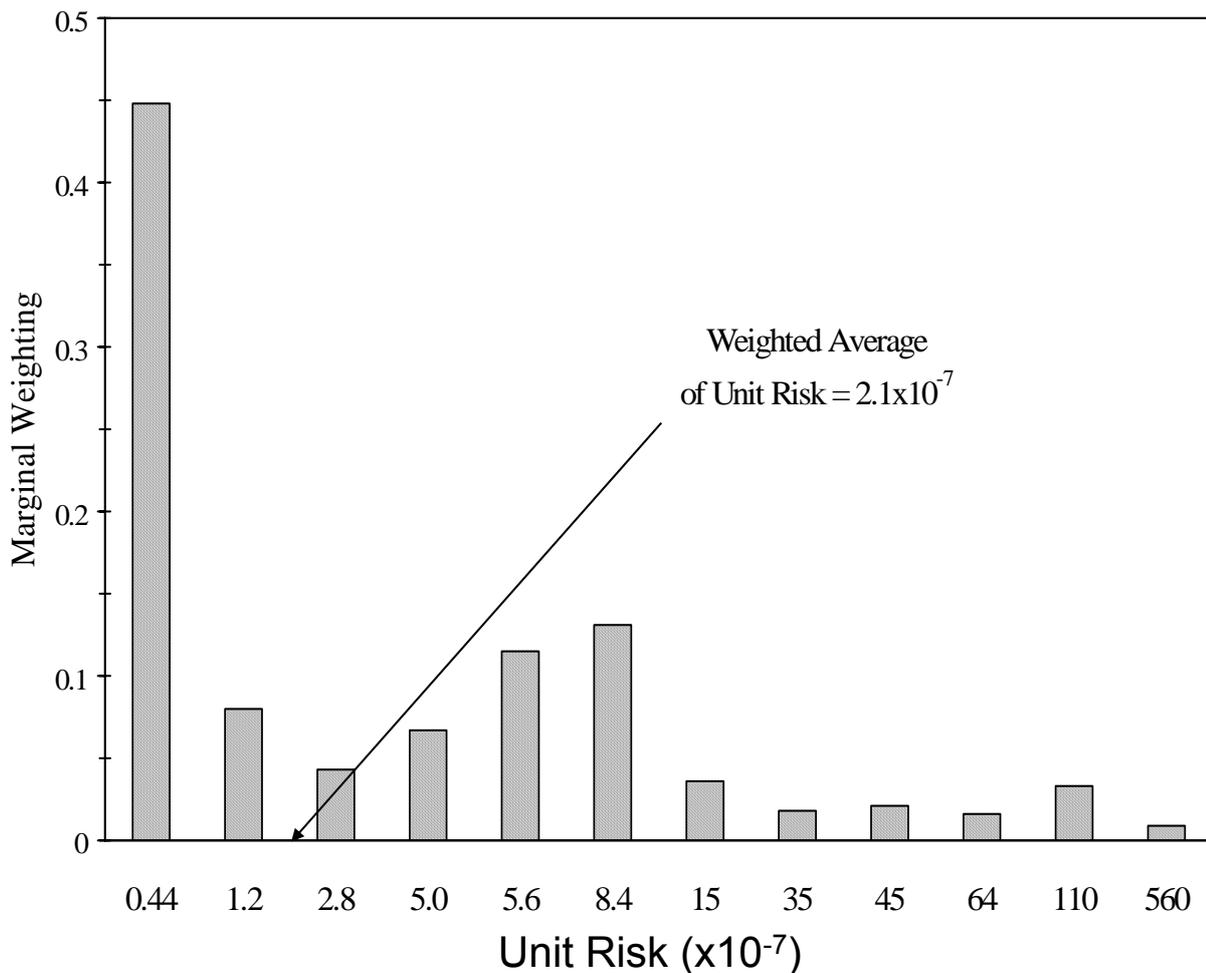


Figure 3: Distribution of unit risk estimates associated with decision tree diagram for the for the cancer risk assessment for methylene chloride. Unit risk in this case is defined as the increased lifetime risk associated with continuous exposure at $1 \mu\text{g}/\text{m}^3$ (Adapted from Clement and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

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 modeling 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.

Research to Expand the Application of Mathematical Modeling

The risk assessment oriented research described in this thesis focused primarily on the application of two mathematical modeling approaches: empirical dose-response modeling and biologically based modeling. There are, of course, a number of other mathematical and statistical modeling 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 these two methodologies. A few of the potentially fruitful areas of investigation are listed in Table 2.

Table 2

Research Directions for Expanding the Application of Dose-Response and Biologically Based Modeling

- Dose-response modeling (Zeiss et al.2002):
 - Bayesian Model Averaging (Bailer et al., 2005)
 - Dose-response analysis of genotoxicity endpoints (Shipp et al., 2006)
 - Dose-response analysis of genomic data (Clewell and Thomas, 2006)
- Biologically based modeling
 - 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 modeling of essential or endogenous toxicants (Andersen et al., 1999)
 - Simulation of cellular dosimetry and transport (Hack et al., 2007)
 - Extension of biokinetic modeling to biodynamics (Tan et al., 2003)
 - Biologically based dose-response modeling (DeWoskin et al., 2001)

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 modeling 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 biodynamic processes has the potential to revolutionize risk assessment to an even greater extent than the progress that has resulted from the modeling of biokinetics.

Final Thoughts

The desire to increase the biological basis of chemical risk assessments has driven the development of new methodologies, such as PBBK modeling (Reddy et al., 2005). The development and application of PBBK models in turn demands well-formulated statements about the chemical mode of action. It is this requirement for an explicit, mechanistic hypothesis that 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 led 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 and methods for their quantitative evaluation continues as the emphasis inexorably shifts from modeling of biokinetics to biodynamics

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Major Conclusions

- The use of mode-of-action evaluation to inform and direct the risk assessment process represents a major advance in increasing the biological basis of chemical risk assessments (*Chapter 2*).
- Quantitative risk estimates obtained with empirical dose-response models can be of value in risk assessments for both cancer and noncancer endpoints; however, empirical dose-response modeling should not be used to support extrapolation below the range of experimental observation for either cancer or noncancer endpoints (*Chapter 3*).
- Physiologically based biokinetic (PBBK) modeling is a highly effective tool for incorporating biokinetic and mechanistic data in risk assessments for both cancer and noncancer endpoints (*Chapter 4*).
- PBBK modeling can provide important insights into the effect of age- and gender-specific differences in physiology, metabolism, and clearance on the variation in internal dose, and hence risk, from an exposure (*Chapters 5-7*).
- PBBK modeling of the liver carcinogenicity of vinyl chloride resulted in similar estimates of human risk on the basis of studies from different species (mouse, rat, and human) and routes of exposure (inhalation and dietary), demonstrating the value of risk assessment approaches based on target tissue dose (*Chapter 8*).
- PBBK modeling, in itself, cannot provide meaningful risk estimates unless the risk assessment approach is consistent with the mode of action for the chemical and endpoint of concern (*Chapter 9*).
- In order for the mode-of-action-directed risk assessment approach to be implemented broadly, research is required on objective approaches for dealing with, and quantitatively characterizing, the propagation of uncertainty in the risk estimate (*Chapter 10*).

Samenvatting

Het doel van het onderzoek zoals beschreven in dit proefschrift was het ontwikkelen van innovatieve methoden in de risicobeoordeling van chemicaliën. Deze methoden zijn binnen bereik gekomen door de aanzienlijke uitbreiding van onze kennis op een aantal terreinen. Deze nieuwe methoden dienen enerzijds bij te dragen aan de bescherming van de volksgezondheid en anderzijds te voorkómen dat overregulering leidt tot onaanvaardbare economische en sociale gevolgen.

Het proefschrift bestaat uit een aantal onafhankelijk gepubliceerde onderzoeken, die gezamenlijk een beeld geven van deze toename in de kennis van zowel de biokinetische processen in organismen als gegevens over de mechanismen van toxiciteit van stoffen, zoals gebruikt in de biokinetische en toxicodynamische modellering. De algemene introductie geeft een beschrijving van de verschillende methoden voor de toepassing van stof-specifieke informatie in de risicoschatting en –beoordeling: het werkingsmechanisme, de zgn. *benchmark dose modeling*, biokinetische modellering en stof-specifieke onzekerheidsfactoren.

Overzicht van het proefschrift

Het eerste deel bestaat uit drie studies die werden uitgevoerd om de acceptatie van het toepassen van biokinetische en toxicodynamische data en rekenkundige modelmatige benaderingen in de risicobeoordeling te vergroten.

Hoofdstuk 2 geeft een kritisch overzicht van het gebruik van gegevens over werkingsmechanismen in de risicoschatting en –beoordeling. De voornaamste conclusie is dat het gebruik van werkingsmechanismen een belangrijke stap voorwaarts is in de risicobeoordeling van stoffen, zowel voor kanker-gerelateerde als voor andere toxische effecten. De gedetailleerde analyse van de risicobeoordeling van trichloorethyleen en anorganisch arseen, bracht een aantal punten aan het licht die nader onderzocht moeten worden voor een optimaal gebruik van werkingsmechanismen in de toekomstige risicobeoordeling. Een van deze kritische punten was de constatering dat autoriteiten, belast met de toelating van stoffen, continu gebruik zouden moeten maken van externe expertise om de kwaliteit van uitgevoerde risicobeoordelingen te waarborgen. Dit in het licht van de toegenomen technische complexiteit en de behoefte aan onderzoek op het gebied van kwantitatieve dosis-respons benaderingen op basis van biologische gegevens. Een dergelijke aanpak kan dan een alternatief zijn voor de momenteel in gebruik zijnde methoden, die veelal gebruik maken van standaard onzekerheidsfactoren.

In de Verenigde Staten van Amerika wordt al geruime tijd gebruik gemaakt van kwantitatieve dosis-respons analyses om het risico op kanker in te schatten. Deze methode is echter niet uitgebreid toegepast voor de risicoschatting van niet-kanker gerelateerde toxiciteit. In het onderzoek, beschreven in hoofdstuk 3, worden kwantitatieve dosis-respons analyses uitgevoerd voor de niet-kanker effecten van twee chemicaliën: anorganisch arseen en methylnitrosamine. Deze analyses tonen aan dat empirische dosis-respons analyse waardevol kan zijn om de niet-kanker risico's in perspectief te plaatsen. Dit biedt tevens de mogelijkheid om een betere afweging te maken van de kosten en de opbrengsten van eventueel te nemen risicoverminderende maatregelen. Het is hierbij van belang aan te merken dat, hoewel kwantitatieve risicoschattingen op basis van empirische dosis-respons modellen waardevol kunnen zijn voor de risicobepaling voor zowel kanker als niet-kanker eindpunten, het niet aan te bevelen is om deze modellen te gebruiken voor de extrapolatie van risicoschattingen tot ver onder de grens van waarnemingen in experimenten. Voor zowel kanker als niet-kanker eindpunten blijft de vorm van dosis-respons curve bij erg lage doses onbekend en wellicht zelfs onbepaalbaar.

De studie, zoals beschreven in hoofdstuk 4, ontwikkelde een benadering voor de toevoeging van biokinetic en metabolistische data in de risicobeoordeling op basis van de PBBK-modellering. De voorgestelde benadering maakt gebruik van de werkingsmechanismenanalyse en kan consequent worden toegepast bij zowel kanker als niet-kanker eindpunten. Door het consequente gebruik van deze benadering, kan PBBK-modellering een zeer effectieve werkwijze leveren voor de toevoeging van biokinetic en toxicodynamische data in kanker en niet-kanker risicoschattingen.

Het tweede deel van dit proefschrift omvat drie studies die werden uitgevoerd om te bepalen in hoeverre stof-specifieke eigenschappen en menselijke variabiliteit op elkaar inwerken bij de bepaling van de verschillende risico's voor individuen in een populatie. Hoofdstuk 5 beschrijft een onderzoek waarin de zgn. Monte Carlo onzekerheidsanalyse wordt gecombineerd met PBBK-modellering om de populatiespreiding van veilige doses te schatten voor methylnitrosamine. Het toxische effect waar het om ging, was de mogelijke kans op neurologische effecten door blootstelling van het embryo aan methylnitrosamine bij consumptie van gecontamineerde vis door de moeder tijdens de zwangerschap. Hiertoe is een PBBK-model ontwikkeld waarin een beschrijving wordt gegeven van de veranderingen in de moeder en de foetus tijdens de zwangerschapsperiode en van het kritische venster voor de gevoeligheid voor de ontwikkelingseffecten van deze stof. De combinatie van de Monte Carlo berekening met de PBBK-modellering maakte het mogelijk om de invloed van inter-individuele variatie voor de mens in de spreiding van de risico's voor de algehele populatie te bepalen en om de sleutelfactoren die bijdragen aan de risicotename voor een individu te identificeren. In het geval van methylnitrosamine werd als belangrijkste klaringsroute de opname en uitscheiding in haar gevonden. De verhouding tussen het risico in gemiddelde en gevoelige individuen (in biokinetic termen)

was ongeveer een factor twee, iets minder dan de gebruikelijke standaard factor van drie.

De studie beschreven in hoofdstuk 6 bestond uit een kritische evaluatie van de beschikbare gegevens ten aanzien van leeftijds- en geslachtsafhankelijke humane fysiologie, metabolisme en klaring, gekoppeld aan een evaluatie van de mogelijke impact van deze verschillen op huiddosimetrie en risico. De conclusie was dat leeftijds- en geslachtsspecifieke verschillen in fysiologie, metabolisme en klaring kunnen leiden tot significante verschillen in de interne dosis, en dus het risico, veroorzaakt door een gelijke blootstelling aan een stof. De richting en omvang van de verschillen hangen echter af van de eigenschappen en het werkingsmechanisme van de stof, en ze zijn moeilijk te betrouwbaar te bepalen zonder het gebruik van kwantitatieve PBBK-modellering.

Het werk beschreven in hoofdstuk 7 maakte gebruik van de gegevens en de analyses uit hoofdstuk 6 voor het ontwikkelen van een PBBK-model dat gebruikt kon worden om te onderzoeken in welke mate de stof-specifieke eigenschappen, fysiologie en leeftijdsafhankelijke biochemische processen samenhangen voor de verschillende risico's tussen individuen in een populatie op verschillende leeftijden, van geboorte tot zeventigjarige leeftijd. De studie toonde aan dat PBBK-modellen, die leeftijd- en geslachtsafhankelijke parameters bevatten, gebruikt kunnen worden om de relatieve risico's te voorspellen voor individuen in de verschillende leeftijdsgroepen. Het PBBK-model, dat ontwikkeld werd voor deze studie bevat volledig leeftijdsafhankelijke beschrijvingen van alle fysiologische en biochemische parameters. Het model kan gebruikt worden voor de schatting de relatieve gevoeligheid van kinderen of ouderen als gebruik wordt gemaakt van de stof-specifieke gegevens voor verdeling en metabolisme. Voor de stoffen onderzocht in deze studie, die werden geselecteerd zodat ze tezamen een verscheidenheid aan eigenschappen en werkingsmechanismen representeren, bleek de standaard gebruikte onzekerheidsfactor van 3 voor humane variabiliteit in biokinetiek geschikt te zijn.

Het laatste deel van dit proefschrift omvat drie studies die werden uitgevoerd met het optimale gebruik van biokinetische en toxicodynamische data in kankerrisicobeoordelingen. Dit werd gedaan voor drie verschillende chloorethylenen, waarbij de volgende methoden werden: evaluatie van werkingsmechanismen, PBBK-modellering, en dosis-respons modellering. Hoofdstuk 8 beschrijft een kankerrisicobeoordeling uitgevoerd voor vinylchloride, een stof waarvan het genotoxische werkingsmechanisme uitgebreid beschreven is in de literatuur. De analyse vergelijkt de voorspelde humane risico's op basis van data van gegevens verkregen met verschillende diersoorten en verschillende blootstellingroutes. PBBK-modellering van de levercarcinogeniteit van deze stof resulteerde in vergelijkbare schattingen van het humane risico, waarbij men gebruik kan maken van gegevens uit onderzoek met verschillende species (muis, rat en mens), en de blootstellingroutes (via inademing of via voedsel) Dit geeft het belang aan van risicobenaderingen op basis van doelorgandosis.

In hoofdstuk 9 wordt een schatting van het kankerrisico gepresenteerd uitgevoerd voor trichloorethyleen. Trichloorethyleen is een stof met niet goed begrepen, niet-genotoxische werkingsmechanismen voor verschillende kanker-eindpunten. In deze analyse was het daarom noodzakelijk om een uitgebreide evaluatie van werkingsmechanismen en om een nogal complex PBBK-model te ontwikkelen. De conclusie is dat PBBK-modellering op zich geen waardevolle risicoschattingen kan opleveren tenzij de risicobenadering samenhangt met het werkingsmechanisme voor de stof en de bestudeerde eindpunten. In het geval van trichloorethyleen blijken de werkingsmechanismen in alle kankerdoelorganen (long, lever en nier) hoofdzakelijk te worden aangestuurd door niet genotoxische effecten (cytotoxiciteit in de long en nier, en receptor-activatie in de lever). Deze zijn in overeenstemming met de drempelwaarde risicobenadering. Het weglaten van de informatie over het werkingsmechanisme kan leiden tot een risicoschatting die ernstig misleidend is ten aanzien van de relatieve risico's van trichloorethyleen, dit in tegenstelling tot de evaluaties met andere stoffen zoals vinylchloride, waarvoor een duidelijk genotoxisch werkingsmechanisme bekend is.

Hoofdstuk 10 omschrijft een kritische evaluatie van alternatieve PBBK-modellen, met perchloorethyleen als voorbeeld. De evaluatie van deze stof demonstreerde dat de goede evaluatie van PBBK-modellen voor de mogelijke toepassing in risicoschatting een moeilijk en complex proces is dat niet alleen vraagt om een evaluatie van het model zelf. Tevens moet gekeken worden naar de werkingsmechanismen voor de effecten van de chemicaliën en de beschikbaarheid van adequate gegevens voor de parametrisering en validatie van het model.

Aanpassing van het meest succesvolle model, dat een verbetering van de beschrijving van de beschikbare experimentele gegevens geeft, is vervolgens gebruikt voor de risicoschatting van perchloorethyleen. In het geval van perchloorethyleen blijkt het werkingsmechanisme de vorming van een reactieve metabool te behelzen. De cruciale gegevens kwamen uit studies van humane blootstelling aan lage concentraties van perchloorethyleen waarbij de vorming van een belangrijke metabool (trichloorethyleen) werd gemeten. Modellen die alleen parameters gebruiken op basis van de stof zelf, bij hogere concentraties geven een overschatting van de metaboolvorming bij lagere concentraties. Daarom kan verwacht worden dat die modellen een grove overschatting van de risico's bij lagere milieublootstellingen geven.

Conclusies

Het onderzoek, zoals beschreven in dit proefschrift, geeft aan dat het gebruik van gegevens over de werkingsmechanismen, van PBBK-modellering en van kwantitatieve dosis-respons modellering veel kan bijdragen aan het verbeteren van de risicobeoordeling van stoffen. Dit zal resulteren in een verbetering van de risicoschatting voor de te beoordelen stoffen en daardoor een meer accurate benadering van de mogelijke risico's verbonden aan de humane blootstelling. In het optimale geval zorgt toegenomen wetenschappelijke kennis voor verbeteringen in de risicobeoordelingen en zullen omgekeerd ook de

inzichten verkregen in het risicobeoordelingsproces kunnen leiden tot een meer gerichte aanpak van onderzoek naar de meest belangrijke onderzoeksgebieden bij het bestuderen van werkingsmechanismen.

Er moet echter erkend worden dat er een fundamenteel verschil is tussen enerzijds wetenschappelijk onderzoek en anderzijds het proces van risicobeoordelingen en dit heeft aanzienlijke en vergaande consequenties. De wetenschappelijke methode is een opzettelijk herhalend proces waarin hypothese gegenereerd en getest worden, welke herhaaldelijk worden herzien in het licht van nieuwe tegenstrijdige data. Daar staat tegenover het proces van risicobeoordeling: dit is een proces waarin onjuiste conclusies mogelijkerwijze kunnen leiden tot aanzienlijke kosten. Daarom is het niveau van de veiligheid ingebouwd in een wetenschappelijke hypothese niet vergelijkbaar met het noodzakelijke zekerheidsniveau waarmee men beslist om een nieuwe risicoschatting te aanvaarden. Dit geldt in het bijzonder als de kosten van foute beslissingen kunnen resulteren in aantasting van de humane gezondheid. Dit verschil leidt regelmatig tot verschillen van mening tussen wetenschappers en toelatingsautoriteiten.

Als gevolg van de snelle ontwikkeling van de moderne wetenschap en technologie kunnen complexe en *state of the art* risicoschattingen niet meer worden uitgevoerd door enkele personen, deze vragen om teams van wetenschappers met verschillende achtergronden en expertise vanuit meerdere disciplines, uiteenlopend van biologie en scheikunde tot wiskunde en statistiek. In het licht hiervan kunnen twee suggesties gedaan worden. Ten eerste is het noodzakelijk dat er een nauwgezette kwaliteitsbewaking is van alle aspecten van een complexe humane gezondheidsrisicobenadering. Het vereist dat wet- en regelgevende instanties blijven hameren op formele kwaliteitsborgingprocedures voor humane gezondheidsrisicobenadering, inclusief onafhankelijke controle en verificatie van alle belangrijke kwantitatieve analyses. Ten tweede is de borging van de kwaliteit en nauwkeurigheid van de voornaamste humane gezondheidsrisicobenadering te belangrijk om dit alleen over te laten aan de gebruikelijke interne controlesystemen binnen beoordelingsinstanties, met een kort proces van externe beoordelingen en de mogelijkheid tot het geven van commentaar door belanghebbenden. Het succesvol volbrengen van een ingewikkelde humane gezondheidsrisicobenadering hangt volledig af van de continuïteit van het kwaliteitsborgingproces gedurende het gehele proces. In aansluiting hierop zal het steeds belangrijker worden voor beoordelende instanties om permanent te zoeken naar medewerking en controle door externe experts voor alle relevante methoden.

Aanbevelingen voor toekomstig onderzoek

De belangrijkste belemmering voor de acceptatie door regelgevende instanties en voor de toepassing van nieuwe gegevens en biokinetische modellen voor de risicobenaderingen is de zorg rondom de mogelijke onzekerheden die gepaard kunnen gaan met het gebruik van deze modellen. Tot op zekere hoogte kan deze zorg 'behandeld' worden door het ontwikkelen van geaccepteerde

methoden voor het opstellen van modellen. Een ander belangrijk antwoord op deze bedenkingen is de verdere ontwikkeling van de methoden voor kwantitatieve onzekerheidsanalyses en het aantonen dat zo ook werkelijk werken. Hiertoe zijn eerder onder andere parameter gevoeligheidsanalyses en de Monte Carlo onzekerheidsanalyses gebruikt. Recent is een zgn. "hiërarchische Bayesiaanse analytische methode", bekend als Markov-chain Monte Carlo analyse, gebruikt voor de combinatie van parameterschattingen met onzekerheidsanalyses.

Deze methoden verschaffen echter alleen informatie over kwantitatieve onzekerheden die verbonden zijn met een enkele modelstructuur en een enkele risicobenadering. Ze helpen niet om de kwantitatieve implicaties van onzekerheden ten aanzien van de goede modelstructuur objectief te bepalen en ook zijn ook niet de meest geschikte toepassing van het model om alternatieve hypothesen voor werkingsmechanismen te ondersteunen. Onderzoek naar deze bredere vraagstelling is dringend nodig om toelatingsinstanties te voorzien van handvaten voor de kwantitatieve karakterisering van de mogelijke pausibele risicoschattingen en het objectief bepalen van de beste parameterschattingen. De methode van formele beslisboomanalyse lijkt een zeer hoopvolle mogelijkheid om aan de vraag van deze instanties tegemoet te komen om een kwantitatieve karakterisering van de onzekerheid bij de toepassing van nieuwe data en modellen in de risicoschatting. Verdere ontwikkeling en toepassing van deze methoden en andere technieken afkomstig van beslissingsanalyse is een belangrijk onderzoeksgebied.

Het onderzoek naar risicobeoordeling, zoals beschreven in dit proefschrift, richt zich hoofdzakelijk op de toepassing van twee mathematische modelbenaderingen: empirische dosis-respons modellering en biologische modellering. Uiteraard zijn er nog andere mathematische en statistische modelbenaderingen die gebruikt kunnen worden in de risicoschatting, maar een goed bruikbaar onderzoek kan alleen gedaan worden op basis van de uitbreiding van de toepassing van de twee eerder genoemde methoden.

Ten aanzien van de toepassing en het gebruik van stof-specifieke eigenschappen in de risicobenadering, is mogelijk het belangrijkste onderzoeksgebied de verdere ontwikkeling van biologische modellen voor de primaire aangrijpingspunten van stoffen in weefsel en de reacties op celniveau die daaruit voortvloeien. Het relatief nieuwe veld van systeem-biologie heeft een raamwerk opgeleverd voor een beter begrip en de beschrijving van de reacties in cellen en dit heeft een fundamentele wijziging teweeg gebracht in de toxicologische kennis. De toxische effecten van een stof worden nu gezien als een verstoring van de normale celfunctie, die leidt tot voorspelde veranderingen in de celregulering en -signaaloverdracht. De kwantitatieve beschrijving van deze biodynamische processen heeft de potentie om de risicobeoordeling revolutionair te veranderen, zelfs in een grotere mate dan de vooruitgang op het gebied van biokineticische modellering heeft veroorzaakt.

De wens om te komen tot een betere biologische basis van de chemische risicobeoordeling heeft geleid tot de ontwikkeling van nieuwe methoden, zoals PBBK-modellering. De ontwikkeling en toepassing van PBBK-modellen vragen op hun beurt om nauwkeurig beschreven uitspraken over de chemische werkingsmechanismen. Het is deze vraag naar een expliciete hypothese voor het toxische werkingsmechanisme die biologische modellen hun statistische kracht geven, en aan de andere kant de grootste belemmering zijn voor acceptatie door wet- en regelgevers.

Modellen op biologische basis maken ook andere onzekerheden in de risicoschatting meer zichtbaar, zoals de inter-individuele variatie. In sommige gevallen heeft deze zichtbaarheid geleid tot verbetering van de standaard risicobeoordeling. Door slecht gedefinieerde onzekerheden, in de standaard benadering, te vervangen door gedefinieerde modelonzekerheden hebben biologische modellen de ontwikkeling en toepassing van meer gecompliceerde technieken voor onzekerheidsanalyse bevorderd. Men kan hierbij denken aan de Monte Carlo analyse en de hiërarchische Bayesiaanse analyse, die thans worden gebruikt voor een beter begrip van de reikwijdte van risicoschattingen gebaseerd op de beschikbare informatie van een bepaalde stof. Het is van uiterst belang dat deze gelijktijdige ontwikkeling van biologisch onderbouwde beschrijvingen en methoden voor de kwantitatieve evaluatie doorgaat terwijl de nadruk onverbiddelijk verschuift van biokinetische modellering naar biodynamische modellering.

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Curriculum vitae

Harvey Clewell was born on 30 June 1947, in Berwyn, Illinois, USA. He studied chemistry at Bradley University, Peoria, Illinois, graduating *cum laude* in May 1969. In 1971 he received a Master of Arts in Chemistry from Washington University, St. Louis, Missouri, before entering the U.S. Air Force as an officer in the Biomedical Sciences Corps.

During the course of his Air Force career he conducted research in the areas of environmental modeling, toxicology, and risk assessment. In his later years he served as the Deputy Director of the Air Force Toxic Hazards Division, Director of Hazardous Materials Safety for the Air Force Aeronautical Systems Center, and Consultant to the Air Force Surgeon General on Chemical Risk Assessment. He retired as a Lt. Colonel in 1991.

After a brief period working as a contractor in the Air Force Toxic Hazards Division, he joined the K.S. Crump Group in Ruston, Louisiana, from 1992 to 2005.¹³ The research described in this thesis was performed during this period.

In 2005 he joined the CIIT Centers for Health Research, now known as the Hamner Institutes for Health Sciences, as the Director of the Center for Human Health Assessment.

He was certified as a Diplomate of the American Board of Toxicology in 2001 and 2005. In 2007 he received the Society of Toxicology's Arnold J. Lehman Award for his contributions to risk assessment and the regulation of chemical agents, including pharmaceuticals.

A major focus of his research over the last 25 years has been in the application of physiologically based pharmacokinetic (PBPK) modeling in risk assessment. To foster greater use and acceptance of this technique, he has developed and conducted numerous courses on PBPK modeling and its applications, from 1986 to the present.

His current research interests include the application of biokinetic modeling together with *in vitro* data as an alternative to live animal testing, the incorporation of genomic dose-response information in quantitative risk assessment, and the use of hierarchical Bayesian analysis in the development and evaluation of biokinetic and biodynamic models.

¹³ During this time, the K.S. Crump Group changed its affiliation from ICF Consulting, Inc., to ENVIRON International Corp.

