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This paper recapitulates the means by which a physiologically based risk assessment for dichloromethane (DCM, methylene chloride) evolved. Two somewhat different physiologically based pharmacokinetic (PBPK) models had been published by Angelo et al. in 1984 and Andersen et al. in 1987. The paper discusses the rationale for the selection and implementation of a PBPK model for purposes of estimating the delivered or effective dose. Also discussed is the rationale used for selecting the scaling method for estimating the equivalency of delivered dose between test species and humans.

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The Impact of Pharmacokinetics on the Risk Assessment of Dichloromethane

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Although not EPA's first use of pharmacokinetic data and physiologically-based pharmacokinetic models (PBPK) for risk assessment purposes, dichloromethane is certainly the most well publicized and closely watched. Using administered dose as a basis for risk extrapolation is not the method of choice when other means and data are available. For example, administered dose ignores obvious pharmacokinetic differences between doses and species, and in fact, may not always result in the most conservative assessment, as is commonly thought.

The risk assessment process involves several extrapolation procedures, including going from relatively high concentrations in the mouse bioassays to lower concentration for the expected potential human exposure. In performing such extrapolations many assumptions are made and questions of uncertainty arise. The understanding of pharmacokinetic differences between species and between doses eliminates only some of these uncertainties.

We did not and do not hesitate to use PBPK models in the risk assessment process but rather ask questions regarding which model or models best represent the true process in the body, how such models are validated, and how are the model's respective parameters most accurately determined. Even after adequately addressing these pharmacokinetic issues one is left with deciding how to apply the results of such PBPK models to the risk assessment process.

In formulating a PBPK model several things must be considered: First, what are the available data? Are they adequate to support formulation and testing of a rational model which is congruent with known physiology and anatomy? What is known about the mechanism of action? What delivered dose does the risk assessor need to know? Is there evidence that there are significant non-linearities between administered dose and delivered dose. If not, then administered dose may serve as a very adequate surrogate for the effective dose at the molecular level. Experience has shown, however, that most internal biological processes are not linear with administered doses and thus, some accounting for or quantifying of the non-linearities is crucial for proper assessment.

Two physiologically-based pharmacokinetic models have been formulated for dichloromethane, each with its own peculiarities and each revealing interesting aspects of this compound's pharmacokinetic behavior. One, the Reitz-Andersen model (Andersen et al., 1987), describes flow-limited conditions in several of the body's organs. The other, developed by Angelo et al. (1984), is a hybrid model; for certain exposure conditions it described some of the organs as having lipid containing regions which would cause dichloromethane to sequester for relatively long times.

In analyzing, validating, and applying pharmacokinetic models for the dichloromethane risk assessment several important issues arise regarding model formulation and validation. In such physiologically-based pharmacokinetic models there are many crucial thermodynamic, biochemical, and physiological parameters. The range of values for some, such as blood flows and organ sizes, are fairly well documented in the literature. Others, such as partitioning ratios and

metabolic rate constants, need to be determined for each chemical of interest. Alternative methods for determining many of these may exist. For dichloromethane some question arose regarding the reliability of in-vitro determination of both partition coefficients and metabolic rate constants. Before spending an inordinate effort to fine-tune the exact values, sensitivity analyses may show the relative importance of particular parameters. Those to which the model is most sensitive should be most carefully determined in the laboratory.

Particularly problematic are those parameters which must be extrapolated from in-vitro to in-vivo conditions and those which must be extrapolated from one species to another. Generally, the literature has shown that total body clearance scales along a body weight to the 0.7 to 0.75 power. It is not so evident however, how rate constants for individual metabolic pathways scale. The models for compounds which are biotransformed will be most sensitive to such rate constants. A challenge exists then, to develop guidance and methodologies to best determine these metabolic rate constants. It is probable that species-to-species extrapolation will only be accurate with accompanying in-vitro studies. It is not clear, however, how to decide which in-vitro experiment will provide the most suitable information in a particular case and how to best utilize information gained from such studies in in-vivo pharmacokinetic models.

Model validation has only been lightly considered. One can easily adjust a number of parameters to fit a particular endpoint, thus yielding a model which can be called, under specific circumstances, validated. However, the choice of endpoint used for validation can have dramatic impact on the criteria of validation. For example, as learned from this case, blood levels of dichloromethane do not accurately reflect its tissue levels. Thus, if the tissue level of dichloromethane is the desired delivered dose, validation of the model against blood levels might be considered to have limited usefulness. In this particular case the endpoint of concern was the amount of toxic metabolite produced. The difficulty here is that this endpoint could not easily be measured in the laboratory, thus little or no data were available for direct validation of the endpoint of concern. Because the metabolite was rapidly formed in the liver and lung it might be concluded that its formation is closely related to concentration of the substrate, in this case parent dichloromethane, in the blood. Under such conditions one could use blood concentration of dichloromethane as the product for validation. Since the original model was published, new data have become available which may allow accurate estimation of metabolite formed by each pathway. If these data are deemed reliable, they, rather than the blood level of dichloromethane, should be used as the measure for evaluating the accuracy of the model.

Another area of potentially fruitful research is in regard to model simplification. Often in the process of model formulation there is a tendency to formulate high resolution models, i.e., ones that include a multitude of tissues and organs. In practical situations however, sufficient data to support the estimation of the necessary parameters is often lacking. This leads to either expensive and time consuming laboratory experiments or to models which are over-parameterized and thus no longer truly physiologic. Guidance is needed that will counsel modelers on the best way to simplify models in a systematic way that is consistent with sound mathematical principles and is congruent with the known physiology of the system.

The first step in the process of applying pharmacokinetic models to risk assessment is to decide what should be used as the delivered dose. The appropriate definition of delivered dose may depend upon the specific case. Delivered dose may simply be the administered dose corrected for less than 100% absorption efficiency. It may be a measure of the amount of metabolites resulting from biotransformation of the compound entering the body. In fact, it may be a concentration of a chemical or one or all of its metabolites in a particular organ, tissue, or cell type. PBPK models can be formulated to describe and predict a variety of these delivered dose measures. The actual delivered dose selected as the basis on which to conduct the risk assessment depends upon the understanding of the mechanisms of toxicity, the test species, the

exposure conditions, and the levels of data that are available. For purposes of this discussion, effective dose will be defined as the dose causing the particular mechanism at the molecular site of action. This effective dose should ideally be the desired end point of any exposure assessment, but in reality is almost never realized.

In the case of dichloromethane the evidence indicates that, while the exact mechanism is unknown, the glutathione mediated biotransforming pathway leads to reactive metabolites which are carcinogenic. Thus, for this particular case, the delivered dose used for risk assessment purposes was the total metabolite produced as a result of this biotransforming pathway. This pathway, although exhibiting linear kinetics at the relevant exposure conditions, competes with another pathway, mediated by the P-450 system, which exhibits saturation kinetics. The interaction between the two pathways, the difference in predominance of the pathways at different doses and in different species affected the risk assessment for this compound. A risk assessment based on administered dose, in contrast, would imply that the kinetics (not the dose-response curve) were linear at all exposure conditions.

Once a satisfactory definition of delivered dose has been formulated, and once the ability to estimate this dose using pharmacokinetic modelling is judged sufficient, one is faced with the task of using this information in revising the extrapolation of risk from bioassay mice to humans at low doses. How to use pharmacokinetic information in risk extrapolation is not self evident. The experience with dichloromethane has revealed several points of view and has helped to define and sharpen the issues. The balance of the present paper outlines the steps used in EPA's revised cancer risk assessment for methylene chloride. The rationale for the method is presented along with a discussion of its implications and assumptions.

In EPA's pharmacokinetically-based revision of the dichloromethane unit cancer risk (USEPA, 1987) the following steps were taken: First, delivered dose was defined as the daily amount of reactive intermediate formed during the course of biotransformation by the GST-mediated pathway per liter of tissue in organs at risk in the NTP bioassay, namely liver and lung. The chemical instability of this intermediate molecular species (which is presumably responsible for its toxicity) indicates that it quickly and spontaneously reacts without leaving the site of formation. Thus, the total amount formed per liter over the course of an exposure (a "virtual concentration" in Reitz's terminology) is an index of target tissue exposure. (If the proximate toxin were a more stable metabolite, its residence time in the tissue would also be at issue, and species differences in its clearance should enter the definition of delivered dose.)

The second step was to use the Reitz-Andersen pharmacokinetic model to calculate delivered dose estimates for liver and lung in mice exposed to dichloromethane vapor according to the protocol of the NTP inhalation bioassay. Then, the multistage model procedure was used to define a low-dose upper bound on the dose-response curve of tumor incidence as a function of this delivered dose. Separate analyses were necessary for the two organs at risk since they receive different delivered doses for a given inhalation exposure. It should be noted that the need for low-dose extrapolation is not removed by using delivered doses and that non-linearities in the dose-response relationship do not arise solely from pharmacokinetic non-linearities.

Finally, the human version of the pharmacokinetic model was used to determine the delivered doses resulting from continuous exposure to 1 ppm of dichloromethane vapor. The risk engendered by such human delivered doses (combined for both liver and lung) constitutes a unit risk per ppm for dichloromethane. At such low exposures, delivered dose is linearly related to air concentration, and risk is (by the low-dose extrapolation assumption) linearly related to delivered dose. The problematic and controversial question is: What is the appropriate estimate of human risk for a given daily tissue exposure vis-à-vis mice with the same delivered dose?

It is important to appreciate that pharmacokinetics does not obviate the question of scaling doses across species--it addresses the relative delivered doses in mouse and man that result from a given exposure to dichloromethane vapor, but it does not address the relative risks arising from those tissue-level exposures. It is not at all clear that equal "virtual concentrations" experienced for a lifetime (in units of mg-eq metabolized/L of tissue/day) should engender equal risk in mouse and man; human organs have about 2000 times the volume, and presumably 2000 times the number of cells at risk, only one of which need be transformed to initiate a tumor. Moreover, the daily tissue exposure continues over a lifetime that is 35 times longer in a human than in a rodent. These factors alone suggest that humans should be extraordinarily sensitive to carcinogens--an assertion that is not supported by epidemiological findings. Clearly, other factors are at work as well. Thus, fully assessing the impact of pharmacokinetics on relative risk across species depends crucially on a remaining question, that of the comparative pharmacodynamics of carcinogenic response across species. This constitutes a major research need in the field of risk assessment.

Faced with this problem, our approach has been to examine the contribution of the pharmacokinetic component alone. This entails defining a "usual" or "default" expectation about the contribution of pharmacokinetic differences across species to the relative carcinogenic potencies of chemicals. Actual pharmacokinetic results, then, should change our potency calculations to the degree that they show these prior presumptions to be incorrect. We choose an assumption about the pharmacodynamic component that will leave our old risk estimates (calculated on the basis of administered dose) unchanged in cases where the pharmacokinetics turns out to be in line with the prior expectations based on general principles.

First consider external or administered dose. This constitutes the amount of compound breathed in (albeit not necessarily absorbed) during the course of exposure, and forms the traditional basis of dose calculation against which the pharmacokinetic approach is being compared. Administered dose is calculated by multiplying the breathing rate (in L of air/day) times the vapor concentration (in mg/L), and dividing by body weight to give a dose in mg/kg/day. Since breathing rate varies in approximate proportion to the surface area/volume ratio of the body, mice experience about a 13-fold higher administered dose than humans for a given episode of breathing air contaminated with a given concentration of vapor.

Starting from widely accepted general principles, one can show that the fraction of this administered dose that is metabolized should be about equal across species, despite the allometric scaling of metabolic rates. Briefly, during an inhalation exposure to a compound (such as dichloromethane) that is readily absorbed and readily expired, the blood and tissue concentrations rise until they reach a steady state, at which new net absorption is limited to the replacement of material lost to metabolism and non-pulmonary excretion. In essence, steady state represents the equilibration of the amount of compound dissolved in the external air and in the tissues. The concentration in the tissues is determined by the relative solubilities of the compound in tissue, blood, and air. These solubilities are physicochemical properties of the compound, and so should be approximately equal across species. Hence, steady state tissue concentrations should be about equal across species, in spite of the mouse's relatively high breathing rate (which only affects the speed with which equilibration is reached). Given equal substrate concentrations, the rate of metabolism of the compound in metabolizing tissues (in amount per liter of tissue per minute) conventionally scales in proportion to the animal's surface area to volume ratio. Hence, mice have relatively fast metabolism compared to humans, and are expected to metabolize about 13 times more compound per liter of tissue per minute at steady state.

In sum, mice have a 13-fold higher administered dose, and they are expected (on the basis of the above argument from general principles) to metabolize 13-fold more compound than humans. Thus, the fraction of their administered dose that is metabolized is expected to be about equal

to that in humans. Of course, the conventional allometric scaling of breathing rate and of metabolism of the compound, as well as the steady state assumptions, need not be adhered to in a particular case. The above argument provides a point of departure, based on the conventional and accepted allometric differences among differently sized species, against which to judge the actual pharmacokinetic results. In a compound with "typical" pharmacokinetics, the metabolized dose would be a constant fraction of the administered dose, and the calculations based on delivered dose would give the same risk extrapolation as those based on administered dose.

In the case of dichloromethane, the results of the Reitz-Andersen pharmacokinetic model show that at similar air concentrations, humans and mice do indeed metabolize about the same fraction of their administered dose, as expected. But owing to changes in the relative activity of the GST and MFO metabolic pathways at high and low doses (arising from the saturation of MFO at high exposures) the proportion of a dose metabolized by the GST pathway is lower at low doses than at high ones, in both mice and humans. As a result, the low-dose human tissue exposures to the presumed proximate carcinogen are a few fold (but not hundreds of times) lower than one would expect from the above scaling argument. Accordingly, the EPA document advocates lowering the inhalation unit risk by 8.8-fold, to allow for the fact that slightly less of the administered dose is metabolically activated in humans at low doses than in mice at the bioassay levels of exposure. The difference is mostly due to the high-to-low-dose component and not to species differences in metabolism, although for other compounds this could be different.

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