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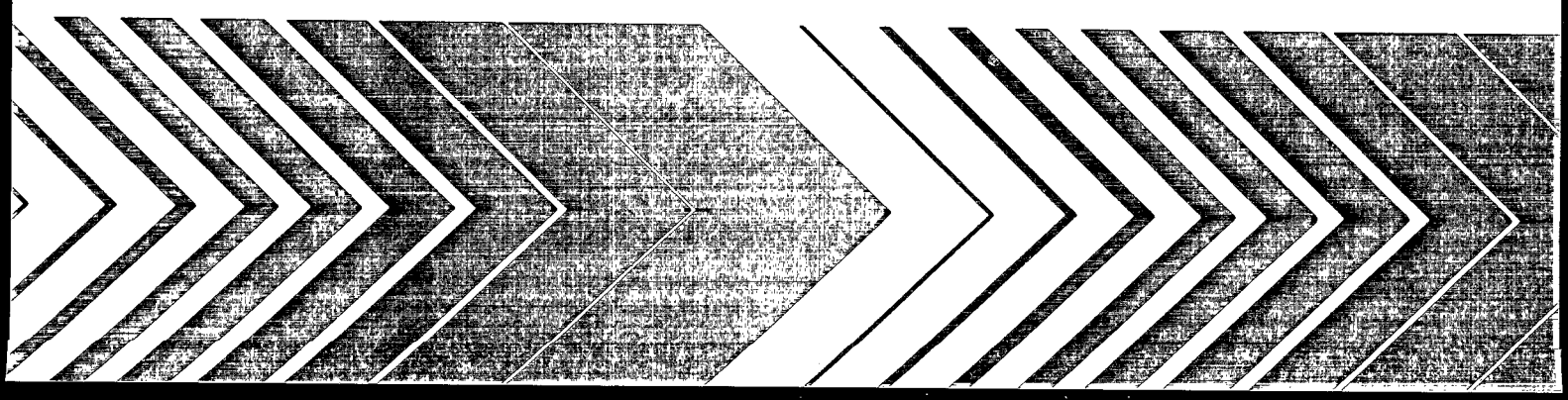
Biological Data for Pharmacokinetic Modeling and Risk Assessment

Report of a Workshop Convened by the U.S. Environmental Protection Agency and ILSI Risk Science Institute



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BIOLOGICAL DATA FOR PHARMACOKINETIC MODELING AND RISK ASSESSMENT

Report of a Workshop Convened by the U.S. Environmental Protection Agency and ILSI Risk Science Institute

Asheville, North Carolina
May 23-25, 1988

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PREFACE

On May 23-25, 1988, the U.S. Environmental Protection Agency (EPA) and the ILSI Risk Science Institute held a workshop in Asheville, North Carolina, titled "Biological Data for Pharmacokinetic Modeling and Risk Assessment." The goal of this workshop was to encourage a better understanding between the communities in toxicology, risk assessment, biological modeling, and pharmacokinetics. The workshop was divided into five sessions, each covering a specific topic related to pharmacokinetics. Within each session, speakers from academia, industry, and government presented their expertise. At the conclusion of the workshop, participants identified research and data needs for pharmacokinetic models in risk assessment. This report summarizes the proceedings of the workshop.

ACKNOWLEDGEMENTS

This document was prepared by Eastern Research Group, Inc., Arlington, Massachusetts, based on submissions by speakers, commentors, and rapporteurs of the workshop. It was reviewed by Lawrence Fishbein and Carol J. Henry of the Risk Science Institute and all workshop chairpeople and rapporteurs. Their time and contributions are gratefully acknowledged.

ABSTRACT

This report summarizes the information presented at the "Workshop on Biological Data for Pharmacokinetic Modeling and Risk Assessment" held by EPA and the Risk Science Institute on May 23-25, 1988, in Asheville, North Carolina. This report provides a general background of risk assessment and reviews how biological data are used in risk assessment and the ways pharmacokinetic modeling can reduce the uncertainties in risk assessment. Different biological models and their value are described, and the differences between predictive and descriptive models are explained. The report reviews the development and history of pharmacokinetic models, the strengths and limitations of the models, the types of biological data necessary to use pharmacokinetic models, and the role of sensitivity analysis in incorporating pharmacokinetic data into risk assessment. Hepatic metabolism data is used as an example of incorporating in vitro and in vivo data into pharmacokinetic models. Two approaches used for cross-species scaling are included, as well as biotransformation and the correlation of in vitro and in vivo data. Actual case studies showing pharmacokinetic modeling used in risk assessment are also described, and research needs are identified.

INTRODUCTION

The Workshop on Biological Data for Pharmacokinetic Modeling and Risk Assessment was held May 23-25, 1988, in Asheville, North Carolina. The overall goal of the workshop was to encourage a better understanding among the research communities in toxicology, risk assessment, biological modeling, and pharmacokinetics and to encourage a greater understanding of the needs of each group.

Most research communities recognize that there are a number of critical uncertainties inherent in the major assumptions underlying current risk assessments that utilize modeling techniques based on animal bioassays. The principal uncertainties relate to the necessity of extrapolating experimental results across species from rats or mice to humans, from high dose regions (laboratory animal exposure) to low dose regions (human exposure in the environment), and across routes of administration. Also, current risk assessment methodology provides no insight for determining which dosage or active material should be modeled. In addition, risk assessors are not assured that modeling administered dosage data has any direct relationship to delivered or target dose either for the test compound or an active metabolite. Hence, there is an overwhelming need for an evaluation of the scientific base for the assumptions (and underlying uncertainties as described above) used in the risk assessment process. Pharmacokinetics provides such a tool.

The goals of the Workshop on Biological Data for Pharmacokinetic Modeling and Risk Assessment were:

To communicate to biologists the value of pharmacokinetic models and the kinds of data needed.

To provide insight and guidance to risk assessors with respect to the rigor of analysis.

To focus on making the uncertainties in modeling more explicit.

To identify research/data needs for pharmacokinetic models in risk assessment.

The workshop focused on five themes. The first session, "Overview of Risk Assessment," provided a general background of risk assessment and reviewed how biological data are used in risk assessment and the ways pharmacokinetic modeling can reduce the uncertainties in risk assessment. The second session, "Modeling Biological Phenomena," was designed to acquaint biologists, biochemists, pharmacologists, and toxicologists with different biological models and their value. The use of models in biology and the differences between the predictive and descriptive models were explained. Session Three, "Overview of Pharmacokinetic Modeling," described the development and history of pharmacokinetic models and included discussions on the strengths and limitations of pharmacokinetic models, the types of biological

data necessary for these models, and the role of sensitivity analysis in incorporating pharmacokinetic data into the risk assessment process. The fourth session, "The Use of Experimental Data in Pharmacokinetic Modeling," used hepatic metabolism data as an example of incorporating in vitro and in vivo data into pharmacokinetic models. Two approaches used for cross-species scaling were reviewed, as well as biotransformation and the correlation of in vitro and in vivo data. The final presentation in this session was a review of molecular dosimetry.

The last session presented actual case studies showing how pharmacokinetic modeling has been used in risk assessment. These case studies described modeling information on three chemicals shown to be carcinogenic in rodents: benzene (also a known human leukemogen), butadiene, and methylene chloride. Both an inhalation model and an ingestion model were described for methylene chloride. These presentations were followed by explanations of how such data have been used in risk assessments. Benzene risk assessments used data from oral carcinogenicity studies conducted by the National Toxicology Program. The butadiene presentation provided a modification of the 1985 U.S. Environmental Protection Agency (EPA) risk assessment for butadiene, which incorporates new experimental and epidemiological data. The presentation on methylene chloride described the impact of pharmacokinetic models on risk assessment.

This document contains summaries of each workshop session that provide comprehensive overviews of the capabilities and problems of using physiologically based pharmacokinetic (PBPK) modeling at the present time. The details of how to actually do this are given in the abstracts and the listed references. Reports from two rapporteurs focus on the chemical-specific needs for PBPK models and the application of pharmacokinetic data in resolving or reducing the uncertainty in risk assessment.

The concluding section of the document outlines research needs for pharmacokinetic modeling identified by the participants that will help decrease the scientific uncertainties in risk assessment.

The workshop was organized and supported by a cooperative agreement between the ILSI Risk Science Institute and the EPA. The Workshop Planning Committee consisted of Dr. Kenneth B. Bischoff, University of Delaware (Workshop Chairperson); Dr. Linda Birnbaum, National Institute for Environmental Health Sciences; Dr. Richard D'Souza, The Procter and Gamble Company; Dr. Irene B. Glowinski, ILSI Risk Science Institute; Dr. Dale Hattis, Massachusetts Institute of Technology; Dr. Stan Lindstedt, University of Wyoming; Dr. Hugh Spitzer, American Petroleum Institute; and Dr. Catherine St. Hilaire, ENVIRON Corporation.

We wish to gratefully acknowledge additional financial support from the following organizations which made possible the attendance of selected presenters and attendees: The Dow Chemical Company, American Industrial Health Council, American Petroleum Institute, Mobil Oil Company, The Procter and Gamble Company, and Shell Oil Company.

SESSION SUMMARIES

SESSION I: OVERVIEW OF RISK ASSESSMENT

William Farland, Session Chair
Hugh Spitzer, Session Summary

Dr. William Farland opened this session by reviewing the current risk assessment methodology used by the U.S. Environmental Protection Agency (EPA) and by explaining how the physiologically based pharmacokinetic (PBPK) modeling can fit into this methodology. He pointed out that there is an increasing interest in developing better biologically based models for assessing risk in an effort to address many of the assumptions inherent in the mathematical models. PBPK models are critical in this effort since they provide the methodology to assess metabolite distribution, differences in metabolism rates between organs and across species, and better estimates of dose-response. In addition, the use of PBPK in risk assessment can have a significant impact on exposure assessment and dose-to-target issues. Dr. Farland noted that EPA has already used PBPK data in assessing risk for some halogenated compounds, thus demonstrating a willingness to use such information in the risk assessment process.

Dr. Nicholas Ashford reviewed the risk assessment process within a legal/political context. He reflected on the risk assessor's obligation to the risk manager, stressing that hidden values that can possibly govern the selection of the type of models and data sets used in assessing a particular chemical. Because of these hidden values, the bottom line in risk assessment should not be a number but a distribution of possibilities. Dr. Ashford noted that, for high exposures to a substance, the decisions and methods for control are relatively simple. However, for low levels of exposure, the risk assessment must take into account a great many uncertainties and thus becomes very complex. The risk assessor should give the risk manager a sense of the uncertainties and explain the impact of the uncertainties on the final estimates.

Dr. Ashford elaborated further on the risk assessment process, stating the need for a uniform intellectual approach with its demand for rigor, not a uniform set of formulae. He also emphasized the need for balance, objectivity, and a professional review process, i.e., a review conducted by various types of experts. In closing, Dr. Ashford touched on the social issues involved in the regulatory process and discussed how society must deal with uncertainty. He pointed out that the key question for the risk manager is: "How much can I afford to be wrong?"

Dr. Joseph Rodricks discussed the practice of risk assessment. He pointed out that, in the absence of perfect science, efforts must be made to improve the methodologies. Without perfect data or a "best"

answer, science policy guidance must use all available data. Dr. Rodricks stressed that the risk assessor has an obligation to use all information *and* to clearly communicate the uncertainties.

Dr. Rodricks then reviewed current risk assessment practices, pointing out their advantages and disadvantages. The current practices suggest the use of: (1) a generic method for all chemicals; (2) a compound-specific approach; or (3) a combination of the generic and compound-specific methodologies. Because of the large number of chemicals to assess, the generic approach has the advantages of avoiding complexity and promoting consistency. However, this simplification discourages the use of chemical-specific data as well as the development of novel or alternative assessment methodologies. Perhaps more important, however, the generic approach tends to obscure the uncertainties in the analysis.

Dr. Rodricks then turned his attention to new approaches and the burden these new or alternative approaches place on the risk assessor. He pointed out the need to keep pressing the research community for new and better data for risk assessment. He also stressed that the risk assessor had to more clearly explain to the research community the assumption/hypothesis to be addressed by the research and the type of data needed. In using alternative approaches, the risk assessor must understand that the selection of assumptions and models, i.e., particular data sets, or the decision to use or not use information on mechanism of action, is not based on science but on science policy. Thus, as the risk assessor uses particular pieces of information, he or she must describe the reasons for the selection and the uncertainties that accompany it. The analysis, therefore, can be very complex for certain chemicals. In using this approach, the risk assessor puts an enormous burden on the risk manager. Because of the uncertainties involved, the risk manager must decide how much uncertainty is tolerable in any given risk assessment.

Dr. Rodricks ended his talk by pointing out the three critical uncertainties/limitations in the use of PBPK modeling that the risk assessor must address. First, the risk assessor must determine if the correct metabolite or surrogate was selected for the chemical in the risk assessment. Second, the risk assessor must recognize the limitations on the use of the data caused by the differences in dosing pattern (e.g., acute exposure for PBPK modeling vs. chronic dosing in the bioassay). Finally, the risk assessor must determine if the appropriate interspecies scaling factors (translation from one species to another) were used in the extrapolations.

Dr. Portier ended the session by reviewing the mathematical models used in carcinogenic risk assessment and the uncertainties of each model. He pointed out that currently there are three levels of modeling: (1) data interpretation; (2) tumor incidence/dose-response; and (3) species conversion. He

discussed some of the questions the biostatistician addresses in the analysis of data, describing the various uncertainties and how each can be approached mathematically. He then turned his attention to the use of PBPK modeling to improve the accuracy and, therefore, reduce the uncertainty of the risk questions regarding variability. He explained that first, the investigator must learn how to deal with individual vs. population variability; and second, the investigator must be aware that since dose is not known without error, variability must be estimated as a parameter. Dr. Portier ended his talk by pointing out the number of assumptions and, therefore, sources of variability in the EPA's use of PBPK models using the Andersen approach to assessing methylene chloride.

In the discussion that followed the formal talks, Dr. Richard D'Souza asked the panel to discuss the issue of uncertainty vs. variability. The panel members agreed that it was an issue of concern and that as uncertainty decreased, variability would increase.

SESSION II: MODELING BIOLOGICAL PHENOMENA

Kenneth B. Bischoff, Session Chair and Session Summary

Dr. Mary Davis presented an introduction to the uses of modeling in biology to: (1) summarize data; and (2) attempt to relate that data to the theoretical processes proposed in the model (i.e., elimination by renal clearance vs. hepatic metabolism). Models can be quantitative or qualitative, and can include physical, chemical, and biological processes.

Pharmacokinetics uses two main classes of models: predictive and descriptive. The latter are classical compartmental models and are primarily associated with blood or plasma concentrations and elimination. In these models, several compartments (one of which includes plasma) are postulated, and the compartment volumes (of distribution) and intercompartment fluxes are determined to fit the observed (plasma) data. Parameter estimation techniques are important in defining and using these models. A descriptive model is able to show the effects of different dosage regimens, steady-state levels, etc.; to relate elimination information; and to detect differences in the handling of compounds. However, the model is limited because the compartments have no physiologic meaning, and the kinetic constants are often hybrids of several processes. Therefore, it is difficult to extrapolate classical compartmental models across species, sex, or age.

Predictive models are physiologically based pharmacokinetic (PBPK) models that utilize organ volumes to establish compartments, blood flows and clearance rates, protein binding, diffusion and permeabilities, and enzyme kinetics. Certain organs are also utilized because they are sites of toxicity, metabolism, elimination, or storage. From this information, mass balance equations are derived and simultaneously solved.

Extending the PBPK philosophy to pharmacodynamics (drug effects) can account for biological responses such as DNA repair and macromolecular turnover. Some parameter estimation is involved, but if a PBPK model does not describe the data well, it must be refined by adding parameters that were presumably left out. Predictive models are able to scale between species; to extrapolate to low or high doses and different routes of exposure; and to accommodate differences in organ function and size, and metabolic and clearance processes from disease or growth. The main disadvantages of this model type are that a large variety of data and knowledge of the underlying processes are needed, and the models usually must be solved numerically using computers.

It is useful to realize that models can be used in an "investigative mode," as well as a "simulation mode." The latter mode uses model solutions to generate predicted tissue drug levels as a function of exposure and then uses these levels to manipulate dosage regimens to achieve some desired result. This mode is the most common interpretation of "using a model." The investigative mode uses the model to quantitatively analyze the data in terms of the proposed processes and focuses on better understanding those processes. In this method, a whole-body model may not even be necessary. Examples of both modes can be found in the literature (see Appendix B).

SESSION III: OVERVIEW OF PHARMACOKINETIC MODELS

Robert L. Dedrick, Session Chair
Kenneth B. Bischoff, Session Summary

Dr. Kenneth Bischoff began this session by reviewing the development and history of pharmacokinetic models. The purpose of pharmacokinetic models is to quantitate by mathematical models absorption, disposition, metabolism, excretion, and biological responses to drugs and chemical agents for clinical applications and toxicology and risk assessment. Dr. Bischoff presented examples of classical compartmental models as well as the more recent, physiologically based pharmacokinetic (PBPK) models.

Early work on pharmacokinetic models focused on the use of anesthetic agents to predict brain levels. Ethanol also was studied by using Michaelis-Menten kinetics for hepatic metabolism. The concepts of clearance and volume of distribution in pharmacokinetics were defined and applied. The term "pharmacokinetics" is derived from "pharmacokinetik," a term (in German) apparently coined by F. Dost (1953).

The PBPK approach attempts to base the models on real biological information concerning the physicochemical, physiological, and pharmacological properties of the drug, rather than using, for example, abstract definitions of central and peripheral compartments. Organ blood flow rates, solubilities, protein binding, and local membrane permeability are all specifically incorporated into the models. This information permits the model to translate results between animal species and predict effects of different routes of administration and different doses on the tissue levels of the chemical.

Dr. Bischoff described several specific examples of the application of this approach to thiopental, methotrexate, and other drugs and provided references to both the history and review articles on PBPK.

Dr. Robert Dedrick reviewed the strengths and limitations of pharmacokinetic models. Many forms of pharmacokinetic models have been extensively used in pharmacology for the design and analysis of preclinical and clinical trials, as well as for guidance of therapy. Although classical compartmental, physiological, and specific model-independent approaches all have important applications, Dr. Dedrick focused on the mechanistic insight that can be obtained from the physiologic models.

Concerning in vivo-in vitro correlations, the benefit of using systems ranging from isolated enzymes to perfused organs is the uncoupling of the local effects from the complexities of chemical and metabolite distribution and interactions in the intact animal. Although published examples are supportive of the concept and predictability of the events of interest, no comprehensive methodology exists.

The extensive literature on animal anatomy and physiology can be used as a basis for physiologic pharmacokinetic models. This information provides many model parameter values (e.g., organ blood flow) in different species, often through empirical allometric correlations with body weight. However, other aspects, such as xenobiotic metabolism, can have large and unpredictable variations between species, and experimental data will be required for a particular chemical and animal.

The use of pharmacokinetic models for determining bioavailability and dosing schedules is well established. Application of the design to regional drug administration has occurred to some extent, focusing on obtaining some pharmacokinetic advantage over systemic administration. Diffusion, convection, and mixing often need to be considered in more detail than usual to truly optimize the advantages.

Pharmacodynamics (drug effects) as well as pharmacokinetics must be considered in order to associate drug concentrations with a biological effect. The paucity of biologically based pharmacodynamic models limits their use in quantitative risk assessments since pharmacokinetics alone tells nothing about the probability of a chemical causing, for example, cancer in humans, or even about the appropriate dose metric necessary for predicting human cancer risk based on animal experiments.

Dr. Harvey Clewell's presentation focused on data needs for modeling. The purpose of using models in risk assessment is to increase the scientific basis and to reduce or better quantify the levels of uncertainty. Models are helpful for designing bioassays and should be used in initial experiments. Qualitative information (e.g., mechanism of action) must be gathered to define the model structure (i.e., the physiological and biochemical basis for defining the quantitative relations), and quantitative data are needed to define the model parameters.

When using biologically based mathematical models in risk assessment, pharmacokinetics needs to be coupled with pharmacodynamics (toxic endpoints, e.g., carcinogenesis). For the latter, information such as cell turnover rates, mutation, and DNA repair rates must be known in humans and in animals. There are seven aspects required for developing the risk assessment: (1) classification of the chemical as

a carcinogen; (2) mechanism of carcinogenesis; (3) nature of the proximate carcinogen; (4) model structure; (5) model parameters; (6) species differences; and (7) extrapolation to low doses. Only the model parameters, and to some extent, the model structure have been extensively treated. The first four aspects of risk assessment often are decided on rather ill-defined bases; the extrapolation to low doses is, by U.S. Environmental Protection Agency policy, handled by the linearized multistage model, and species differences are a refuge for empirical "safety factors."

There are two extremes in risk assessment: (1) the "safe" risk, which avoids underestimating by using conservative methods that do not rapidly change over a period of years; and (2) the "accurate" risk which avoids erroneous risk management decisions by using new techniques, avoiding conservative safety factors, etc. These two approaches need to be brought together.

Dr. Clewell recommended that: (1) models should be used in the development of bioassays; (2) legislative agencies should have the support of modeling groups; (3) basic data should be collected and organized; and (4) formal methodologies should be used to incorporate uncertainty into the quantitative outcome.

Dr. Murray Cohn focused on one role that sensitivity analysis can play when incorporating pharmacokinetic data into the risk assessment process. The well-studied example of methylene chloride can exemplify this role. Major uncertainties concern high-to-low dose extrapolation and species-to-species extrapolation. Some specifics include assumptions about mechanism of action, e.g., the roles of the parent compound and the possible reactive metabolic intermediates. Other problems can be with possible nonunique parameter estimates for the models and the effect on predictions from animal data. Another issue in the risk assessment process can be the methods for scaling to humans, especially for the metabolic parameters.

Some uncertainty is reduced or better defined through incorporating pharmacokinetic considerations into dose-response analysis. Also, the sensitivity analysis can provide a basis for corrections to the above results. It is not clear how the uncertainties can be reduced compared to the traditional methods utilizing extrapolation factors of "mg/kg/day" or "mg/m^2/day." Finally, the ill-defined question "What is the "target dose?" also is an issue in the risk assessment process.

SESSION IV: THE USE OF EXPERIMENTAL DATA IN PHARMACOKINETIC MODELING

Richard W. D'Souza, Session Chair and Session Summary

Dr. Marilyn Morris presented an overview of hepatic metabolism. Enzyme activity (V_{max}, K_m) in the metabolizing organ is an important determinant of the rate of metabolism of a compound. In addition to enzyme activity, a number of factors influence metabolism rates including blood flow, cofactor availability (e.g., thiol or sulfate), enzyme distribution patterns, and diffusion rate limitations. The relative importance of these factors changes with the dose level of the compound, the extraction ratio, and other variables.

Most physiological pharmacokinetic models assume a spatially uniform model for hepatic concentration and metabolism. Other models used are the parallel-tube model (sinusoidal model), dispersion model, and distributed model. Although the spatially uniform model is an oversimplification and is conceptually incompatible with our knowledge of liver anatomy and physiology, it has worked well in describing the kinetics of several drugs, for example, lidocaine, phenytoin, and propranolol. It is important to consider these different metabolism models when developing physiological pharmacokinetic models, to select the most appropriate.

Enzyme activity parameters obtained in vitro may be misleading, as they may not be sensitive to the same factors as those obtained in vivo. Some in vivo validation, therefore, is necessary.

One in vitro system, the intact perfused-liver preparation, may offer definite advantages over other in vitro systems (e.g., microsomal preparations) as it maintains liver architecture and can control perfusion rate to simulate in vivo blood flow rate. Also, the effects of various factors mentioned above can be studied by appropriately perturbing the system.

Dr. Joyce Mordenti reviewed two approaches to cross-species scaling (translation from one species to another): the allometric approach and the physiological pharmacokinetic approach. Both approaches have some advantages and some limitations. The allometric approach is a body-weight power relationship that typically uses data from the literature, making this approach quick and inexpensive. Because of the ease and simplicity of use, this model is valuable and often recommended for a first approximation. The allometric approach is well established for scaling anatomic/physiological parameters (e.g., volumes, flow rates) and also for scaling physiological processes (e.g., renal clearance). This approach has had some success in scaling first-order metabolism rates, except for oxidation (e.g.,

antipyrene), where humans may metabolize more slowly than was predicted from a single-term (body-weight) power function. In this case, a two-variable power function, for example, body-weight and brain-weight, can be used. The allometric approach is empirical and not well understood, so there is little recourse when it fails. It is recommended that pharmacokinetic data from different species first be plotted on a log-log grid before proceeding to other methods.

Physiological scaling is accomplished by adjusting for important differences in various model parameters between species. It is, therefore, more realistic biologically than allometry and, thus, is the recommended method. However, it can be time consuming and expensive when obtaining or validating parameters experimentally. Hybrid allometric-physiological scaling can be performed and has had both successes (e.g., with phenobarbital, phenytoin) and failures (diazepam).

Dr. Glenn Sipes discussed biotransformation and the development of in vitro systems to predict in vivo events. In vitro systems provide the opportunity to study metabolism of xenobiotics in humans. Several systems of varying scope and complexity have been used including subcellular fractions, cell suspensions, and precision-cut liver slices.

In vitro precision-cut liver slices may have the potential to mimic in vivo metabolism. This system has been tested for viability with protein synthesis and glucose release and can perform integrated Phase I (e.g., oxidation, de-ethylation) and Phase II (e.g., glucuronidation, sulfation) metabolism. Data from this system have also been consistent with in vivo literature data (e.g., sulfate conjugation in the rat, slow and fast human acetylators, etc.) and have demonstrated potential for studying enzyme inhibition, cellular damage, and other processes. Due to a lack of commercially available instrumentation, only one laboratory currently has the capability to perform these studies. The system is still in the developmental stage and other factors such as glutathione conjugation will be studied in the near future.

Data from the various in vitro systems are not expected to give exactly the same results (e.g., for kinetic constants), nor are they expected to have a one-to-one quantitative relationship with in vivo data. One in vitro-to-in vivo correlation compares the results of different in vitro studies with in vivo data in animals. Once a data base is broadly established, it may be possible to correctly predict in vivo metabolism rate constants in humans from in vitro data in humans. Furthermore, metabolism and toxicity parameters could be directly scaled from animal to human, based solely on in vitro data. Such a data base would be very useful for developing physiological models.

The final presentation in this session was a review of molecular dosimetry by Dr. George Lucier. Dr. Lucier explained that molecular dosimetry (using DNA and other adducts) can be used effectively with physiological pharmacokinetic models to assemble a more comprehensive picture of the events taking place from a carcinogen at the site of absorption to the toxic/carcinogenic response.

DNA adducts must not be studied in isolation; the complex processes that include metabolic activation/deactivation, DNA repair and persistence, and the concentration of chemicals at the receptors must be understood to correctly interpret DNA adduct data. Besides specific organs and tissues, specific cell types should be studied for DNA adduct formation, repair, etc. In humans, adducts can be studied in accessible fluids to gain information on precarcinogenic events at nonaccessible sites. For example, hemoglobin and lymphocyte adducts can be quantitated. Several methods can be used for measuring adducts, including immunoassays on monoclonal and polyclonal antibodies, ^{32}P post-labeling, and in vitro lymphocyte uptake.

SESSION V: THE USE OF PHARMACOKINETIC MODELING IN RISK ASSESSMENT: CASE STUDIES

Linda Birnbaum, Session Chair and Session Summary

Development of pharmacokinetic models can lead to improved dose-setting and study design. Some model predictions can also lead to new questions. An important use of pharmacokinetic models is in the area of risk assessment, where such models can be used to extrapolate between doses, sexes, and even species of animals. The focus of this session was to show how pharmacokinetic models (in this case all physiological models) could be used in the assessment of risk. Three chemicals of major industrial importance, all of which have been shown to be carcinogenic in rodents, were examined: benzene, butadiene, and methylene chloride. The physiological model for benzene and one of the models for methylene chloride were based upon the model for styrene developed by M. Anderson and coworkers. The models for butadiene and one for methylene chloride were developed independently of the "Anderson" paradigm.

Benzene

Benzene has been known for many years to cause cancer in humans. Only recently, however, has it been shown to be carcinogenic in rodents. Dr. Michelle Medinsky presented a pharmacokinetic model describing the metabolism of benzene in rats and mice. Her model, based on extensive oral and inhalation metabolism data, indicated that differences between the species were observed in the pathways for the metabolism of benzene. She concluded that after both oral and inhalation exposures, mice form relatively more of the hydroquinone and muconic acid metabolites - markers for the putative toxic pathways of metabolism - than do rats. In contrast, rats form relatively more of the metabolites that serve as markers of the detoxification pathways, phenyl and mercapturic acid conjugates, than do mice. At higher exposure concentrations, due to the lower K_m for the toxic metabolite pathways, metabolism shifts more toward detoxification. The relatively greater production of toxic metabolites at low doses suggests that linear extrapolation seen at high exposure concentrations might obscure the potential toxicity occurring at low exposure concentrations. This is a good example of the utility of a pharmacokinetic model that incorporates physiological, chemical, and metabolite parameters. Another good example is the demonstration by simulation that the species differences in metabolite production are due to differences in the metabolism rates, not differences in physiological parameters such as alveolar ventilation rate.

Dr. John Bailer presented a risk assessment of benzene using different dosing metrics with the data generated from the oral carcinogenicity studies conducted by the National Toxicology Program (NTP). He computed the internal dose (ID) received by the rodents as a function of the administered dose (AD), using the information available in the literature about levels of benzene metabolites. The relationship of internal to AD was clearly nonlinear because there was a nonlinear increase in toxic metabolites as a function of AD. The model used for the relationship between the ID and AD was a Michaelis-Menten function, in which:

$$ID = \frac{\alpha AD}{\beta + AD}$$

Using the multistage model of Crump and Allen, Dr. Bailer's risk assessment, derived from the NTP gavage carcinogenicity studies, was based upon both the ID predicted from the Michaelis-Menten function and the AD; the ID led to a safe dose less than one order of magnitude smaller than a safe dose based upon the AD. However, the species differences were highlighted using ID as a measure of exposure. In rats, the ID was roughly linear relative to the AD; hence, a safe dose derived from a risk assessment based upon either metric should be roughly the same. In contrast, in mice the nonlinear relationship between ID and AD resulted in a lower safe dose being estimated when ID was used. Of course, measures of ID other than total metabolites could be used, one of these being the concentration of toxic metabolites associated with some AD. If the relationship between ID and AD proved to be the same in humans as in mice, or conversely in rats, a risk assessment for humans could be done using ID as the metric. The estimate of risk in this case would be similar to that made using several human epidemiological studies.

Dr. Richard Irons commented on the use of models in risk assessment of benzene. He focused on the assumptions generally made in pharmacokinetic modeling and how they are used to extrapolate to a given endpoint, i.e., toxicity or cancer. In his opinion, experimental data suggest that, for benzene, the assumption that exposure is cumulative with risk is not valid, in part because the metabolic and interactive pathways used by that chemical are complete. Another point that should be incorporated into modeling for benzene is the cell-cycle dependence of the toxic response: benzene affects only dividing cells. Thus, continuous administration of a carcinogen is less effective at evoking a response than is intermittent exposure. The recent discoveries that, at a given time, only one (or at most a few) stem cell is populating the entire hematopoietic system mean that modeling leukemogenesis requires attention to a very small target. The initial event in leukemogenesis may not involve mutation of a single stem cell but may involve expansion of the number of stem cells at risk via physiological

mechanisms such as altered growth factor responses or differentiation. Such a model would require the incorporation of more than just metabolite levels in the target tissues or single-hit models leading to carcinogenesis.

Butadiene

Butadiene is a potent carcinogen in mice, inducing tumors at multiple sites, especially lymphomas, hemangiosarcomas of the heart, and lung adenomas/carcinomas, all within 1 year of exposure. Although oncogenic in rats as well, the response is much weaker than in mice, and the neoplastic tissues are mainly endocrine. Using a readily available computer software package, Dr. Dale Hattis developed a physiological model to describe the pharmacokinetics of butadiene. His objective was to insert more causal mechanistic information into the mathematical models used for risk assessment. He proposed that the development of a physiological model would uncover anomalies between the experimental data and the theory, leading to the development of additional data, followed by modification of the theory. In fact, said Dr. Hattis, modeling often raises as many questions as it answers. In his pharmacokinetic model, he attempted to reinterpret the dosages of active metabolites actually delivered to the rats and mice during a 2-year bioassay. Given data on metabolism in humans, as well as in rats and mice, the model allowed better predictions to be made about the dosages across species. For butadiene, the difference in effect is nearly an order of magnitude. There was also an appreciable difference in the risk projections derived from the experiment in rats and mice, which may have related to the role of an endogenous retrovirus in the mice. Dr. Hattis stressed the need to test the sensitivity of the model conclusions using reasonable alternative estimates for the parameters. He also called on experimentalists to produce the data that the modelers need, for example, multiple time points in disposition experiments.

Dr. Steven Bayard presented a modification of the 1985 U.S. Environmental Protection Agency risk assessment of butadiene; his modification incorporated new experimental as well as new epidemiology data. He focused on the mouse-to-human extrapolation model and the comparison of cancers predicted from this model with those actually observed in humans. Recent studies, indicating increases in hematopoietic system cancers in humans, are consistent with the extreme sensitivity of mice to the development of lymphomas following exposure to relatively low concentrations of butadiene. The levels of two active butadiene metabolites, epoxide and diepoxide, which have been measured in animal models, were incorporated into the risk assessment models. However, the lack of human metabolism data makes this exercise quite speculative.

Dr. Irons commented on the use of pharmacokinetic data in the risk assessment of butadiene carcinogenicity. He felt that the differences in rat/mouse carcinogenicity by butadiene are difficult to explain by metabolite differences alone since the spectrum of tumors varies, as does the incidence. The qualitative metabolite profiles are also the same in both species, although there are quantitative differences. The potent leukemogenic activity of butadiene in mice may, in fact, be related to the presence of an endogenous retrovirus, since mice that do not have this virus are much less susceptible to butadiene-induced lymphomas (all are T-cell in origin while spontaneous lymphomas in the mice are of B-cell origin). In fact, butadiene treatment has been shown to specifically activate a single endogenous retrovirus. The parallels to human T-cell leukemia viruses are obvious. Those mice that do not have the retrovirus do exhibit the typical butadiene-induced bone-marrow toxicity of the virus-carrying strain. Therefore, while bone-marrow toxicity may be necessary for the carcinogenic response, it may not be sufficient. Thus, modeling carcinogenesis based on toxicity may not be appropriate for this compound.

Methylene Chloride

Methylene chloride has recently been shown to be a carcinogen in rodents following inhalation exposure. However, exposure to the chemical in drinking water did not result in tumors. Several such issues, including effect-of-exposure route and dose-to-target tissues, have received a great deal of attention. Extrapolation of the rodent data to humans has been quite controversial. Dr. Richard Reitz described a physiological model which calculates the "delivered dose" to the target organs, liver and lungs. The model incorporated two metabolic pathways: a high-affinity, low-capacity pathway involving mixed function oxidation and a low-affinity, high-capacity pathway involving glutathione conjugation. Enzyme levels measured in various species were incorporated into the model, which also accounted for different routes of exposure, both oral and inhalation. A parallelogram approach involving in vivo and in vitro rodent (rat, mouse, hamster) results was used to extrapolate from in vitro human data for both enzymatic pathways to the in vivo situation. The predictions of the model explained the negative carcinogenicity results of the drinking water study as compared to the positive tumor response observed after inhalation of methylene chloride. The model predicts that dose effects will be nonlinear and, in this case, that extrapolation from high dose to low dose may result in overestimating risk. Development of this physiological model, which was validated by extrapolating from one route of exposure to another and by determining that the experimental data fit the predictions, provides a perspective for risk analysis since it allows calculation of the dose delivered to the target tissues. It does not describe the mechanism of toxicity for methylene chloride. Dr. Reitz stressed that a quantitative risk assessment cannot provide precise estimates of risk, but rather can put a plausible upper bound on risk. The real risk remains unknown and, in fact, may even be zero. The usefulness of quantitating risk is to allow rank ordering of

hazards. Incorporation of a physiological model for dose extrapolation in risk assessment may obviate the need for interspecies scaling.

A different physiological model for the pharmacokinetic behavior of methylene chloride was presented by Dr. Michael Angelo. The objectives of this model were to compare ADs between bolus gavage treatment and drinking water exposure. Pharmacokinetic information was needed to understand the different internal disposition patterns that resulted from two different oral dosing regimens. Data were obtained by varying the dose, route, dosing vehicle, and frequency of administration. Dose-dependent metabolism from methylene chloride was observed, in agreement with the enzymatic data presented by Dr. Reitz. Metabolism did not appear to be affected by repeated dosing. However, there was an apparent species difference between rats and mice in eliminating methylene chloride from blood, which may reflect the difference in the levels of the two metabolic pathways. The liver concentration profile of methylene chloride was also not reflected in the blood levels, emphasizing that pharmacokinetic data for blood may not be truly representative of distribution in a specific tissue. This may be explained by the occurrence of membrane-limited transport between the liver and blood compartments. The model developed was flow limited and predicted that a vehicle such as corn oil would slow the absorption and intestinal transport of methylene chloride and thus affect absorption. Model simulations demonstrated that a corn oil carrier had a very large effect on the distribution and metabolism patterns of methylene chloride following high dose administration as compared to the pharmacokinetic profile resulting from dosing in water. Thus, use of a pharmacokinetic model can help to predict how a controllable factor, such as choice of dosing vehicle, can influence the disposition characteristics of a compound. Using the model, oral and inhalation exposures were simulated using equivalent "internal" doses based on the AUC (area under the blood level curve, related to the total fraction of the dose that was absorbed) for a given tissue or total metabolites formed by either the monoxygenase or glutathione pathways. Which of the two measures of ID is used, however, cannot be decided by the model but must be determined by the fit of the data. Dr. Angelo stressed that physiological models can help to explain physiological behaviors that are influenced by such factors as dosing vehicle and dose level. Tissue data are essential to ensure that the model represents true pharmacokinetic behavior.

The impact of pharmacokinetic models on the risk assessment of methylene chloride was discussed by Drs. Jerry Blancato and Lorenz Rhomberg. They stressed that pharmacokinetic models cannot replace dose-response models. Instead, pharmacokinetic information can be viewed as a dose assessment tool. In the case of methylene chloride, the pharmacokinetic models can predict the delivered dose, which can then be related to risk. However, allometric scaling continues to be incorporated into risk

assessments and surface-area scaling is used to account for the different lifespans of rodents and man. The question of equivalent target tissue dose for animals and humans, given their different lifespans, still requires attention.

Dr. Rory Conolly concluded by commenting on the two different physiological models and risk assessments presented. He stressed that a major issue in risk assessment is the measure of dose, and one of the major uses of physiological models is to estimate the ID received by the target tissue. He stressed that optimum models will contain as few parameters (experimentally measurable variables) as possible. A question he asked was, if additional structures are added to the model, do they describe real compartments? While it is easy to extrapolate from high to low doses and from route to route using a properly constructed model, it is more difficult to extrapolate between species because it is frequently impossible to measure some of the parameters in humans. In such cases, allometric scaling can be used, with a certain degree of caution. The innate variability of living creatures introduces a degree of variability into any of the models which would be improved by incorporating a distribution of parameter values, rather than single point measurements. Methods to incorporate distributions are available. Overall, it is clear that validated models will improve our ability to make quantitative comparisons.

CASE STUDY COMMENTS

BENZENE CASE STUDY

Dr. Richard Irons: Commentor

I think it is important that we consider what assumptions must be made in the process of introducing extrapolation of risk. There are two issues on which my comments will focus: (1) what assumptions are made about pharmacokinetic modeling and its application to benzene toxicity; and (2) what assumptions are made about the mechanisms of carcinogenesis. Unlike most of the compounds discussed at this conference, benzene is an accepted and well-known *human* leukemogen. It is associated with an increased risk of Acute Myelogenous Leukemia. In that context, modeling represents an opportunity to test the relevance of animal data to the situation in man. So far, we have assumed that, in terms of dosimetry, we are focusing on a stationary target. I suggest that in the case of benzene, we are not dealing with a stationary target. I also suggest that we may not, as previously assumed, completely or, perhaps, even remotely understand what the metabolites are doing. And I also suggest that the assumption that a single phenomenon (i.e., a direct structural relationship such as an adduct) is, with respect to either dosimetry or mechanism, somehow related to cancer may not be true for benzene.

There are at least three different levels of metabolism that are involved in benzene-induced toxicity to the bone marrow. Primary metabolism of benzene occurs predominantly in the liver and is a mixed function oxidase-dependent activity. Although it has been argued that a ring-opened product may occur, it is clear that the principal product is phenol. Secondary phenolic metabolism also occurs within the liver to produce polyphenolic metabolites; this also appears to be a cytochrome-dependent process. Hydroquinone and catechol are relatively stable and are transported to the bone marrow, where they accumulate independent of metabolism for reasons which are not understood. The accumulation of these metabolites does not correlate with the tissue partition coefficients. It appears that tertiary metabolism at the level of hydroquinone in the bone marrow correlates very well with toxicity produced by dibenzoquinone. This involves monoperoxidase activity which is concentrated in the bone marrow. Although we and others have provided some evidence of a capability for direct metabolism of benzene by bone marrow, it is extremely limited and dwarfed by the ability of the liver with respect to primary metabolism.

One of the basic assumptions for risk assessment and modeling is that some product of dose x duration is equivalent or at least proportional to risk. A corollary assumption, predicted, I think, primarily on its mathematical simplicity, is that exposure is cumulative with respect to risk. For benzene we have data that, at an acute and chronic level, may not be consistent with this particular assumption.

The first issue that can be addressed is the potential complexity of benzene metabolism. We know that if we administer hydroquinone and measure a crude endpoint of acute toxicity or subacute toxicity (bone marrow cellularity), we can produce a transient suppression of bone marrow. With continued exposure, a refractory response is observed. We have learned that if phenol is administered alone, no toxicity is seen; but if phenol and hydroquinone are administered concomitantly, a marked increase in toxicity associated with the administration of hydroquinone is seen. This appears to correlate with an enhancement of monoperoxidase-mediated metabolism of hydroquinone in the presence of phenol. This is a possibility that should be considered when metabolites influence the subsequent bioactivation or metabolism of other metabolites. A complex situation arises that can't be related simply to primary metabolism of the parent compound.

Even with concomitant administration of phenol and hydroquinone, and although the magnitude of toxicity is greater and of longer duration, toxicity is only transient, and with continued treatment on a daily basis, eventually a refractory response in bone marrow is observed. One explanation for this is that benzene is known to be a cycle-specific agent. When we talk about bone marrow, we are talking about a highly proliferative tissue, producing about a kilogram of tissue or more per day in an average adult. Thus, issues such as regimen-dependence become very important in understanding benzene toxicity. If we look at the tissue kinetics in rapidly and asynchronously dividing cells, such as bone marrow, there will be a proportion of cells in each phase of the cycle at any given time. If, in fact, toxicity is phase-specific, then at any given time there will only be a fixed proportion of the cells that are susceptible to toxicity.

Bone marrow diffusion cultures performed by John Marsh at Yale, which enable a direct comparison of a variety of different tissues in mouse, dog, and human bone marrow, illustrate this point. Adriamycin yields a typical log dose-response curve in an acute experiment where an increasing dose of adriamycin increases cytotoxicity. In contrast, increasing the dose of methotrexate, a cycle-specific agent exhibiting schedule-dependent effects, will not increase cytotoxicity. After a given point, toxicity will not increase because the cell population will become synchronized and will be protected from the effects of the agent. With respect to the toxicity of benzene metabolism, when the same doses of hydroquinone and phenol are administered repeatedly 3 days a week with a 4-day interval in between, no refractory response is observed. Instead, increased toxicity and the development of a very severe bone marrow aplasia is seen. Thus, cell-cycle dependence appears to be a fairly important issue with respect to modeling acute benzene toxicity, at least as far as its metabolites are concerned.

How does this relate to long-term or chronic exposure and the issue of leukemogenesis? For years the lack of an experimental model for leukemogenesis associated with benzene has been a limiting factor. This may be as much a consequence of experimental design as it is of species differences. A number of inhalation studies with benzene have been conducted in rats and mice. In CD-1, B6C3F$_1$, and C57BL/6 mice, where animals were exposed to benzene concentrations between 300 and 3,000 ppm for 2 years or a lifetime, the data with respect to leukemia were either negative or inconclusive. Occasionally a leukemia will show up in a study with a large number of animals, but not enough to convince anybody.

Following initial regimen-dependence studies, Cronkite (1987) decided to look at a limited duration exposure of 100 to 300 ppm in C57BL/6 and CBA/Ca mice. When mice were exposed to benzene for 8 weeks or 16 weeks and held for a lifetime, a very efficient leukemia response was observed. However, if animals were exposed to 3,000 ppm benzene for 8 days as opposed to 80 days (the same product in terms of dose duration), no cancer of any sort was observed over a lifetime. In the C57BL/6 mouse, the 12-week exposure to the same concentration of benzene, 3 or 6 days per week, is very efficient in inducing lymphoid leukemia or myelodysplastic changes in the spleen. These lesions do not appear immediately after cessation of exposure but appear if the animals are then held for a lifetime. Thus, the lifetime maximum tolerated dose does not appear to produce an efficient or measurably convincing leukemogenic response whereas a high exposure of more limited duration does.

Now I'd like to briefly address the developing area of experimental leukemogenesis. In general, leukemogenesis is often thought to be a more complicated process than carcinogenesis and is widely held to be a multifactorial process. Whether leukemogenesis and carcinogenesis in solid tissues are similar, I think we know more about leukemogenesis at this stage than about other types of cancer. In addition, some of the simplifying assumptions we apply to solid tissue cancers cannot be applied to leukemogenesis.

Leukemogenesis is a multifactorial process involving critical events in more than one compartment. Virtually all leukemias, with very few exceptions, originate in stem cells, independent of the particular cell line in which the leukemia is expressed. Multipotent and certainly committed stem cells are a very rapidly proliferating cell population. Although it has been known for a long time that the number of pluripotent stem cells that are active at any one time is minute, it is not known how small that number is. In the last year, the ability to insert a foreign gene into individual stem cells has allowed the tracking of the activation and regulation of individual stem cells (Lemischka et al., 1986; Snodgrass and Keller, 1987). In mouse studies conducted at the Whitehead Institute and in Europe, it has become evident that the number of pluripotential stem cells giving rise to all the cells of the entire hematopoietic system

can be as few as one. Conceptually, this can only mean that nature places a very high priority on protecting these cells at all costs, keeping them at rest while virtually all proliferative activity takes place at a very fast rate. Why is this important? It is important because the vast majority of leukemias are known to originate in cells at different levels within the stem cell compartment. These cells may all be aptotic. In contrast, cells in the rapidly proliferating precursor cell population appear to be aptotic (i.e., irreversibly committed to terminal differentiation). As such, they are sensitive targets for cytotoxic agents but are incapable of neoplastic transformation. When we measure the effects of toxicity on these cells and attempt to draw relevance with respect to leukemogenesis, at best we're looking at an indirect population, and at worst, the results may have no relevance at all.

The implication for benzene toxicity is two-fold. First, if one assumes the same type of phenomenon as a single hit to define increased risk or the initiation of cancer, there is a problem: What is the target cell? Second, all secondary and tertiary benzene metabolites recapitulate alpha- and beta- unsaturated diketone groups that react almost selectively with sulfhydryl groups. They only reluctantly react with nitrogens, carbons, or other potential target molecules, and there has been no definitive demonstration of a DNA adduct of benzene in vivo. Whether or not this occurs to any appreciable extent remains to be seen. It now is clear that if a DNA adduct can be identified in vivo, it will be at doses that are several-fold in excess of the minimum carcinogenic dose. In addition, there is considerable evidence suggesting that benzene alters the differentiation of stem cell populations in response to the damage occurring in the cycling population. I would like to suggest a hypothesis that is consistent with paradigms currently being explored in experimental leukemogenesis. The initial event that may predispose a subject to increased risk of leukemia may simply involve a shift in the relative numbers of stem cells from the resting pluripotential stem cell compartment to the multipotent or committed stem cell compartment. Cytotoxic agents and benzene in particular are known to increase proliferation in the stem cell compartment and to deplete the number of pluripotent stem cells. The initiation event may, in fact, occur second or third in the process of the progression of events that leads to leukemogenesis.

I have explored this hypothesis because we are all familiar with either a single- or two-hit model involving initiation already. It is, however, worthwhile to consider that there are potentially different mechanisms other than a one-hit model to explain various types of carcinogenesis.

I'd like to conclude by suggesting that the biological complexity of various animal model systems, as well as the differences in metabolism, pharmacokinetics, distribution, and the interspecies differences in tumorigenesis may require us to consider creating composite models in which we carefully construct

what, in fact, attorneys refer to as "the reasonable man" from bits and pieces of various models from other species. No one simple paradigm is likely to adequately reflect the real situation in humans.

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BUTADIENE CASE STUDY

Dr. Richard Irons: Commentor

One of the principal issues with respect to butadiene carcinogenicity and toxicity is the dramatic species differences. Multiple mechanisms may be involved in explaining carcinogenesis, and this may be of some importance in understanding the relevance of butadiene as a potential hazard for man. Although we have just begun to study butadiene carcinogenesis in experimental animals, it is an area that illustrates the potential for chemical-biological interactions that are relatively unexplored in modern toxicology. It also illustrates some of the limitations of pharmacokinetic modeling as it exists today.

As has been discussed, there are differences in metabolism, differences in disposition, and differences in carcinogenicity with respect to butadiene across species. I would differ somewhat from Dr. Bayard in that I am not convinced that the metabolic changes between mice and rats can account for the marked species differences, certainly in leukemogenesis. If one looks at the high dose required for carcinogenesis in rats versus the low dose effective in mice, metabolite concentrations are almost the same. And you still have marked differences in the pattern of carcinogenicity.

The extensive disposition data that has been collected in butadiene metabolism studies do not appear to correlate with the established major target organs. In fact, using different genetic models, acute target organ toxicity does not correlate with carcinogenesis, in this case, leukemia.

Many of the recent butadiene studies have shown marked species differences as shown by Owen (1981) and Huff et al. (1985). Butadiene is a very potent leukemogen; it is probably the most potent mouse leukemogen that has been studied in the context of an environmental or occupational hazard. In expanding Dr. Bayard's comments, all of the tumors associated with butadiene exposure are T-cell lymphomas. They are either leukemias or lymphomas in the mouse. They are all derived from the thymus, but, as is the case for this lesion in the mouse, the bone marrow is the principal target organ. Therefore, if it fits the paradigm for all leukemias, bone marrow toxicity would be expected. I don't think it is biologically correct to compare the background incidence for lymphomas in the $B6C3F_1$ mouse with butadiene-induced lymphomas that are T-cell in origin. Background lymphomas in the $B6C3F_1$ mice are of B-cell origin. They can be considered two separate diseases. Thus, butadiene-induced lymphoma in the $B6C3F_1$ mouse is a very significant lesion with an incidence of 60 percent.

One of the problems with the $B6C3F_1$ mouse, and virtually all laboratory strains of mice with one or two exceptions, is that they carry retroviruses that are associated with leukemia, under a variety of conditions. In order to explain the potential species differences, we decided to examine whether one could model butadiene on the basis of what is known about viral-induced leukemias in the mouse. It was found that leukemia in these $B6C3F_1$ mice is preceded over a 20-week period by a marked activation of the specific retrovirus that has been implicated as playing a role in leukemogenesis in mouse models. This is not an all-or-none situation, and it is very complex.

This type of activation is very intriguing because this has not been described previously for agents such as butadiene. There are many endogenous retroviruses in the mouse, and butadiene appears to be turning on only one. However, it is one that has been implicated in the past in leukemia. Retroviral production is increased approximately 10,000-fold as a function of exposure to butadiene.

In order to obtain an indication of whether or not the virus was playing a role, we decided to do comparative studies of leukemogenesis in $B6C3F_1$ and NIH Swiss mice. The NIH Swiss mouse is a mouse in which we know the endogenous ecotropic retrovirus associated with playing a role in leukemogenesis in murine strains is truncated and not expressed. A large, but not absolute, difference in the incidence of leukemogenesis in these two strains was observed. The $B6C3F_1$ mouse exhibits the typical 60 percent incidence of lymphoma, while the NIH Swiss mouse has an incidence of 13 to 14 percent. Thus, the rate of leukemogenesis in these two strains differs by four- to five-fold. The incidence of leukemia in the NIH Swiss mouse cannot, presumably, be related to the retrovirus. Since we could not rely on pharmacokinetics or disposition to help us at that point, we looked at the bone marrow which is the target organ. When micronuclei production, cellularity, and chromosomal aberrations were examined, the data in the $B6C3F_1$ and the NIH Swiss mice were superimposable. Quantitatively and qualitatively, the target organ toxicity was the same. This, along with the viral activation, suggests that, in fact, there is some, albeit presumptive, role for a retrovirus in this model system. It also suggests that typical biomarkers associated with toxicity that might also relate to leukemogenesis do not correlate with leukemogenesis in this mouse model.

This leukemia type is preceded by a marked selective activation of an ecotropic retrovirus. We characterized the most likely potential gene interactions in the mouse that could be predicted to lead to specific activation of retrovirus. These would not be extrapolatable to any other species because there are unique specific transactivating genes that regulate virus expression in the mouse. We found that they are not altered by butadiene. The mechanism of activation thus appears to be the de novo activation of ecotropic retroviral sequences and not an indirect mechanism involving alteration in mouse host

resistance or virus restriction. This type of direct activation is the only potential mechanism that might possibly relate to activation of retrovirus in other species such as man. Thus, to date, we have not been able to exclude the mouse as a model for retroviral activation independent of its relevance as a model for leukemogenesis.

It is simply too early to say whether the mouse model is relevant. However, we are pursuing the issue of whether it is possible for butadiene or its metabolites to alter the latency of biology of human retroviruses as well.

A number of additional studies need to be performed before we can provide any definitive conclusion as to whether or not the lymphoma seen in the mouse is directly related to a retrovirus. At this point, our paradigm seems to be reduced to two likely scenarios, and there are in vitro models for both. Given the possibility that retroviruses and butadiene are independently leukemogenic, we are looking at additive mechanisms. On the other hand, either butadiene alters or influences leukemogenicity associated with a mutagen, the retrovirus, or the retrovirus is acting in a cocarcinogenic manner associated with butadiene exposure.

From the standpoint of biology, what is the relevance, or the potential relevance, of understanding these models? For leukemogenesis associated with butadiene, it is possible that the $B6C3F_1$ mouse may not be the most appropriate model for extrapolation to the general population. If retroviral genes are implicitly related to mechanisms underlying the high incidence of lymphoma, then they are probably not the most reliable indicators of the potential for butadiene to cause leukemia in man. In addition, one must evaluate the potential relevance of the mouse model for risk associated with a *defined* population in man that may be carrying specific retroviruses. In this population, the compound might affect the latency of behavior of those viruses. Thus, this model raises several issues with respect to potential health risks in man and the process of evaluating hazard in general.

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METHYLENE CHLORIDE CASE STUDY

Dr. Rory B. Conolly: Commentator

Introduction

A major issue in carcinogen risk assessment is the measure of dose. For example, oral gavage dose and exposure concentration have been commonly used (though the latter is not a measure of dose per se). There may be significant nonlinearities, however, between these external doses and internal surrogates, e.g., the concentration of parent compound in target tissue. Furthermore, within the target tissue, saturable and/or competing pathways for metabolism of the proximate and ultimate carcinogens may introduce additional nonlinearities. Internal dose surrogates that reflect these nonlinearities can be a meaningful basis for risk assessments of chemical carcinogens. This discussion will address the use of physiologically based pharmacokinetic (PBPK) models for computer simulation of dose surrogates. After some introductory comments on the PBPK approach, the PBPK models described by Drs. Angelo and Reitz for simulation of methylene chloride (MC) dose surrogates will be discussed.

PBPK Models

PBPK models contain mathematical descriptions of the target species physiology and carcinogen chemistry which determine pharmacokinetic behavior. The optimum models are parsimonious, i.e., they contain no more structural detail than is needed. Digital computers are used to exercise these models to obtain simulations of the internal dose surrogates. These simulations are accurate when: (1) model structure is representative of the essential elements of the actual, real-world structure; and (2) the parameter values used are accurate. (It should be emphasized that in PBPK models, parameters correspond to measurable entities in the target species.) When these conditions are met, the model is "valid" and can be used predictively.

Interspecies Extrapolation

When PBPK models are validated with data obtained from laboratory animals, simulation of dose surrogates for humans requires scaling of the model across species. The physiological and biochemical structure encoded in a PBPK model can be adjusted for changes in body size using allometric relationships. It is always preferable, however, to actually measure parameter values in the different species. This, of course, is not always possible. Allometric scaling is valid to the degree that: (1) the

experimental species are physiologically similar to humans; and (2) the allometric relationships are properly defined.

Dose Extrapolation

It is often necessary to simulate pharmacokinetic behavior for doses well below the range over which experimental data are available. If model structure is correct and has been properly validated, there is no dose constraint on the accuracy of such low dose simulations. By its nature, a physiologically based model contains the information needed for simulation of carcinogen pharmacokinetics at low doses as well as high. It is possible, therefore, to use a valid PBPK model to simulate internal dose surrogates for any exposure scenario.

Variability

Concern was expressed during the workshop over the variability of model predictions. This variability arises because the models are physiologically, or more generally speaking, biologically structured. Parameters in such models do not have single correct values simply because the real world correlates of the parameters are also variable. A group of 10 people or mice, for example, will typically have 10 different cardiac outputs, liver volumes, and so on. In the mathematical terms of the PBPK model, it is most useful to think of a range of possible values for any given parameter with a distribution of values around the mean. The shape(s) of the distribution for parameters commonly used in PBPK models is unknown and this problem needs investigation. Still, model output is a function of the particular set of parameter values used for a given simulation and naturally varies as different values are used.

Concern that this aspect of PBPK modeling is a failing is unfounded. Variability arises because PBPK models are analogues of real-world systems such as mice, rats, humans, etc. These analogues enable us to examine the variability inherent in responses of these systems to toxic chemicals, which is an advantage. Methods for incorporating distributions of parameter values into PBPK models are available. Drs. Portier and Reitz both presented Monte Carlo approaches to obtaining distributions of model behaviors. This type of work is innovative and more is needed. Of particular concern are assumptions about the shape of the distribution of parameter values and estimates of parameter variance.

PBPK Models and MC

The PBPK models for MC described by Drs. Reitz and Angelo have been used to simulate internal dose surrogates. The questions raised by their presentations are addressed below using the overall paradigm for PBPK modeling outlined above.

In the model described by Dr. Angelo, body organs have two subcompartments, a blood-flow-limited vascular region and a diffusion-limited extravascular region. This structure allows accurate simulations of MC blood and tissue concentration data and was developed after preliminary versions of the model, lacking the subcompartment structure, produced inaccurate simulations.

The use of subcompartments is a significant complication of model structure and must be evaluated against the need for parsimony noted above. Could some other less drastic change in the model suffice? In our laboratory (NSI Inc., Dayton, Ohio), a similar blood-tissue concentration discrepancy was found with chloropentafluorbenzene (CPFB). In this case, simulation accuracy was improved by "unlumping" the rapidly perfused compartment. Explicit descriptions for kidney, lung, testes, and brain were used and the respective partition coefficients obtained. These were quite different from each other and the "unlumped" model then accurately simulated blood and tissue levels of CPFB. Related to the question of parsimony in the Angelo model is the issue of model structure. Is the two-subcompartment structure real in the sense that it is isomorphic with target species for MC, or is it a nonphysiological modification? If it is the latter, then the model cannot be used with confidence for the interspecies and high dose to low dose extrapolations which are the great strength of PBPK models.

It is a misconception to speak of PBPK models for particular routes of exposure. A properly structured PBPK model should work for any and all routes of exposure. Of course, an appropriate description must be included in the model for each route. A model that accurately simulates MC pharmacokinetics after intravenous dosing will work for oral dosing, provided that the rate of oral absorption and any vehicle effects are properly described. A PBPK model that works for one route of exposure but not another suffers from either a structural defect or from a validation failure.

Dr. Reitz described a PBPK model in which MC metabolism by the glutathione S-transferase (GST) pathway in the lung was used as the dose surrogate. This work represents the state of the art in the use of PBPK models and computer simulation for estimation of dose surrogates. As such, it also serves to identify the current limits of the approach. It was necessary to estimate in vivo GST activity toward MC for the human lung. This was accomplished by measuring human GST activity towards MC in vitro and

extrapolating to an in vivo value based on an in vivo/in vitro ratio obtained from animal experiments. This approach is reasonable, but much more data are needed on these types of correlations. A mechanistic explanation of in vivo/in vitro differences in enzyme activity would also be useful. A better data base and an understanding of mechanism would together help to identify when the type of correlation used by Reitz is acceptable and when it is not. This particular problem is just part of the larger issue of how physiological parameters scale across species. The use of PBPK models in risk assessment is creating a growing need for experimental work specifically directed to this question.

The simulations developed by Anderson et al. (1987) show a good correlation of the GST pathway dose surrogate with MC carcinogenesis. These data are only correlational, however. Mechanistic studies illustrating the role of the GST pathway in MC tumorigenesis are needed.

Finally, the issue of interspecies dose scaling for MC must be addressed. As noted above, there is little doubt that MC carcinogenicity is due to bioactivation, and there is good evidence suggesting that glutathione conjugation is the activation step. Scaling of the physiological parameters of the model was according to well-established allometric relationships which are adequately supported by experimental data. Also, Andersen et al. (1987) estimated the rates of the relevant metabolic processes in several different species. The addition of interspecies dose correction factors over and above the scaling rules built into the PBPK model ignores the logic of the PBPK approach. Moreover, it decreases the accuracy of the dose surrogate simulation.

There are, of course, uncertainties in the experimental estimation of parameter values, in the specification of allometric relationships, and also with the accuracy of model structure. These uncertainties should be addressed by inclusion of a safety factor applied to the simulation of the dose surrogate for the human case.

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RAPPORTEUR REPORTS

APPLIED RESEARCH NEEDS

Angelo Turturro

Introduction

The major focus of my comments will be on the application of pharmacokinetic data to the problem of limiting uncertainty in a risk assessment. Murray Cohn brought up a number of cautions in using this information, to which I will add a few of my own. However, use of information such as this is part of the evolving science of risk assessment, and the question is not whether to use this information, but how to use it.

As an aside, this workshop included many participants trained in the discipline of engineering, and the overall discussion has been well served by their presence. People trained in engineering especially understand why conservatism is built into risk assessment because the engineer is responsible if a chemical reactor vessel collapses or a process does not work. In a sense, risk managers in a regulatory agency are in the same position. If people are hurt, or even if the public *thinks* that people will be hurt, risk managers are held accountable. Thus, there is an inherent conservatism in risk assessment, which I think is necessary and practical. When one is protecting the public health, one has to be especially careful about the consequences of one's actions.

General Rules

One effort that would especially help in risk estimation is the definition of some general rules for addressing questions about toxicity. Efforts in cross-species scaling and physiologically based models reported in this workshop are attempting to define these rules. I know this is anathema to most of us who spend our research time looking at a particular compound, but it would be useful to define some general way a regulatory agency can estimate toxicity in order to avoid being overwhelmed by the number of chemicals that must be addressed. And this number is growing. Because of the great diversity of compounds, I assume that there will not be a general rule for all chemicals, however some chemical class-specific rules or guidelines related to different mechanisms of toxic action may be possible. For instance, one "class" that could be addressed is chemicals that undergo minimal metabolism and do not bind well to macromolecules. The pharmacokinetics of chemicals such as these may be relatively straightforward, e.g., may be estimated by simple clearance models. These models may allow fairly

simple extrapolation across species of target dose to a cell. Obviously, all chemicals would not fit into this class. But if one can define the target dose and limits of the class, regulation would become easier.

An important part of the data base for this task will be derived from the tremendous amount of study done on the metabolism of proprietary drugs. Pharmaceutical companies have provided information on pharmacokinetics, both for defining drug efficacy and toxicity, as well as in the development of drugs by increasing delivered dose. This information, especially pharmacokinetic data involving toxicity estimates among different species, can be crucial for making general risk estimation rules.

Risk Assessment

One question that can be asked is "Why do risk assessment at all?" Risk assessment is a critical part of the risk management process. The key factor is to practice appropriate risk management. In 3500 B.C., risk managers, such as high priests, estimated risk of public actions by looking at animal entrails. Today, with the scientific method, we still look at entrails, but with more detailed analysis.

Unfortunately, there is a good deal of uncertainty in the process of assessing risk to humans. If we decrease this uncertainty, we can be less conservative in our estimates, reducing the "insurance" we presently use. Series of conservatisms are built into models basically to protect us from our own ignorance; we want to eliminate that ignorance without endangering public health.

Much of the discussion at this workshop centered around cancer risks because that is the area that risk assessments have focused on in the past. It is useful to emphasize that there can be risk assessments for a number of toxic endpoints besides tumors, such as reproductive and neurotoxic endpoints, that are important to public health and well-being. These risk assessments share many of the same uncertainties in pharmacokinetics and pharmacodynamics as cancer estimations and can also benefit from pharmacokinetic analysis. For instance, as was discussed in the workshop presentation describing the relationship between benzene exposure and aplastic anemia, a good pharmacokinetic model for the anemia may be much more helpful for evaluating the effect of benzene on public health and its risk to the public than a cancer model. An example of an area that needs exploration is the effects of methotrexate (used in chemotherapy) on tumor cells, a subject that even experts who use the compound are unclear about. We have to understand that pharmacokinetics are not simply for cancer, and the pharmacodynamic patterns we should be concerned about are not strictly those for tumorigenesis.

Risk assessment is usually separated into four parts:

(1) Hazard identification, which identifies the potential human adverse effect

(2) Exposure assessment, which estimates human exposure

(3) Dose-response assessment, which develops a dose-response curve for the adverse effect

(4) Risk characterization, which applies all of the above information to make an estimate of human risk.

I will use this framework of risk assessment to structure this discussion.

Hazard Identification. The key to using pharmacokinetics in hazard identification is to clearly identify the toxic agent or agents. Developing pharmacokinetic data for this step is not very useful unless one can truly demonstrate that the chemical species being investigated is key to the toxic effect. This demonstration must be convincing to everyone. If this is demonstrated, and if it is shown that the active species is not produced in humans, then the results of an animal test need not be extrapolated to humans. Thus, pharmacokinetics can tremendously reduce risk. Alternatively, it could indicate that humans are more sensitive to the effects of an agent.

One important consideration, especially for chronic endpoints, is that agents may have multiple effects. For instance, for a genetic toxin that is also cytotoxic, there is no reason to believe, a priori, that only one metabolite causes both the genetic damage and the cytotoxicity. A single agent may thus impact both initiation and promotion with different pharmacokinetics. If one is studying cancer, one has to consider possibilities such as metabolites which increase proliferation or impair the immune function system.

Another significant aspect to emphasize is that the sum of activation, deactivation or detoxification, and reactivation is important, especially in carcinogenesis. An example of this is the toxicity of N-hydroxyarylamine conjugates (Young and Kadlubar, 1982). In this case, the agent was metabolized and the conjugate stored in the bladder prior to voiding. This conjugate was reactivated by urine pH and time of retention in the bladder, parameters that can be correlated with the species susceptibility to carcinogenicity. Pharmacokinetics could have been determined with every possible blood-borne agent metabolite, yet would have been of very little significance. Situations such as these are reminders that information on mechanisms is important in order to understand the significance of data on disposition.

And finally, the physiology or the metabolism of the animal can be changed by a toxic compound. An example of this is the effect on an animal's consumption of calories. In some studies, because of factors such as agent palatability, animals will consume fewer calories. As little as a 20 percent reduction in calories compared to the control will eliminate the effects of a high fat diet in promoting carcinogenesis (Boissoneault et al., 1986). With caloric restriction, the animal's whole body physiology is changed (Turturro and Hart, 1989). Thus, using standard protocols (for instance, measuring bolus injections in a normally fed animal) may not be relevant to the physiological state of the animal in which tumors develop. At the higher doses, the alteration of animal physiology, chemistry, or metabolism should be prevented. This is the type of issue that will cause a regulatory agency to be concerned and that our research should address.

Exposure Assessment. Exposure assessment was not discussed much in this workshop. However, this is the part of risk assessment in which most resources are used and major uncertainties exist. It is very difficult to accurately estimate, for instance, what the time course of concentration is in a single place if there are fifteen point sources producing Chemical A. Compounding this by the variability in human activities leads to tremendous uncertainty in the estimate of exposure. For instance, try to determine your exposure to 1-nitropyrene (in air and food) this morning or over the last year and then try to apply this analysis to a population. One way to address this problem is to use biomarkers of exposure. These markers, such as certain DNA damages, may not be directly toxic. If a target agent and/or a target dose cannot be adequately defined, other information, like total metabolites, agent incorporated, etc., can be used to help define the relationship of administered to applied dose. This will be a valuable area of research if simple useful exposure markers can be developed to deal with the uncertainty in estimating dose.

Dose-Response Assessment. Estimating the dose-response relationship describes the heart of risk assessment, i.e., assessing risk under practical conditions of use and exposure. The variability of a population response is critical, both as a function of metabolism and susceptibility to the effects of the agent. Seven of ten humans may be similar to the average inbred rat we use in estimating metabolism. However, and this was brought home by George Lucier, there may be a substantial number of people who can either metabolize more efficiently or not at all. The ratio of the mean metabolism of these subpopulations, therefore, may be orders of magnitude and there may be a subset of people who are at risk. Given the bias toward public health, in order to show that a chemical will not be a danger, the regulatory agency must be convinced that a sensitive subpopulation either does not exist, or is so small

that any risk to them can be managed. Even the perception that such a population could exist would be a problem. Research in this area is based on the basic issue of extrapolation of animal data to humans.

Another issue is enzyme induction. Although a number of toxic agents are used to produce specific cellular fractions with induced enzymes, it is often not appreciated that many compounds will induce the enzymes that metabolize them. These will change the pharmacokinetics, suggesting that chronic effects may vary significantly from acute ones.

The central problem in many situations is extrapolation from high dose to low dose. When a high dose is given, sometimes effects are induced that are different than at the low dose. Since, unfortunately, it is necessary to extrapolate to a region on the dose-response curve that is not observable, one really has to make a case that the high dose and low dose mechanisms are similar to use information from one range to the other. Saturation, cytotoxicity, hormone imbalance, and accumulation of toxic products are all possibilities that may distort the dose-response relationship, especially after chronic administration.

Finally, there was a comment previously that once one has a few interacting parameters, the statistics become formidable. Fortunately, there is a new area of mathematics that is addressing problems such as these. It is called sensitive dependence on an initial condition and known popularly as "chaos" (Tsonis and Elsner, 1989). "Chaos" provides methods to calculate some of the recurrent factors that arise in these seemingly chaotic systems. It could be interesting to apply chaos mathematics that have been devised for problems such as weather prediction and other very complex systems to the biology of multiple parameters interacting in a statistical sense.

Risk Characterization. It should never be forgotten that risk assessment will be applied to people where the interaction between factors such as common dietary agents, environmental agents, and physiological condition must be considered. For example, if we know that certain ingested foods induce the aryl hydrocarbon hydroxylase and a certain compound uses that enzyme for its metabolism, we should take this into account when applying the results of the risk assessment to the real world. Interaction with diet as well as genetic variability can produce the real world responses to chemical compounds. The same is true about common environmental agents. For instance, it would be of limited value to discuss the risk associated with a phototoxic agent applied to the skin without also discussing exposure to sunlight. Physiological state is also a factor. Pregnancy is sometimes addressed, but old age and diseased states are almost never considered, despite the known effects that disease, such as renal pathology, can have on pharmacokinetics.

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CHEMICAL-SPECIFIC NEEDS FOR PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

Hugh L. Spitzer

Introduction

I would like to start my comments by acknowledging the key role Richard Reitz from Dow Chemical Company has played in legitimatizing the use of physiologically based pharmacokinetics in risk assessment. I think, more than anything else, it was his willingness to have frank and open discussions with scientists from the regulatory agencies, be available to discuss and test the model, and go back into the laboratory and do additional experiments that resulted in the use of physiologically based pharmacokinetic (PBPK) models in the methylene chloride risk assessment. Dr. Reitz might not be the father of PBPK, but he certainly is the doctor of record that brought it into the regulatory process.

I think you are going to be disappointed if you think the effort behind the use of pharmacokinetics will result in greater exposure without increased risk. If the discussion over the last few days is a reasonable indication, PBPK can clearly result in either an increase or decrease in the risk associated with a given exposure. This is a healthy situation because it demonstrates that the approach is neutral.

Overview

Traditionally, for the purpose of risk estimation, the U.S. Environmental Protection Agency makes a direct extrapolation of tumor incidence from rodents exposed to high concentrations of a chemical to humans exposed to low concentrations. These extrapolations are based on a series of conservative assumptions that incorporate only limited consideration of some physiological parameters in risk estimation.

The PBPK model offers an exciting new opportunity to reduce the uncertainties in high to low dose and cross-species extrapolation. The PBPK model, unlike classic compartmental models, gives a more realistic representation of the behavior of chemicals in biological systems. The PBPK model differs from the conventional compartmental analysis in that the actual physiology of the animal serves as the basis of a compartmental description. Thus, the physiological model represents the mammalian system in terms of specific organs or groups of organs/tissues based on common characteristics. This allows each organ or group of organisms to be considered with respect to intrinsic volumes, blood flow rates, partition

coefficients, and biochemical constants specific to the species and chemical under investigation. The value of this approach is three-fold. First, the model can utilize a wide array of data not previously considered, but which could significantly impact extrapolation from species to species. Second, the model has the ability to provide a better estimate of dose to target tissue. Finally, the model's predictive power is not restricted to the range of conditions or animal species for which the experimental observation exists. Thus, this process, if used correctly, could reduce the uncertainty for estimating dose to target tissue.

The model is usually pictured as a flow chart of blood through the various organ systems. These systems may be identified specifically, as is usually done with liver and lung, or as general groups such as fat, muscle, or gut. Absorption and desorption in the tissues can be modeled by assuming attainment of equilibrium between blood and organ, as zero, or first order kinetic processes (ed: or any other rate form). If equilibrium is assumed, the important parameters used in estimating transfer of the chemical are: organ volume, blood/organ partition coefficient, and blood flow through the organ. Metabolism is measured in various ways (e.g., vapor uptake, metabolite production), or estimated by choosing values which provide the best agreement with experimental data on disposition of the parent compound. Metabolic kinetics may be first-order, but Michaelis-Menten kinetics are usually required to fit the data.

The simulated processes assigned to each organ system are incorporated into a computer program that provides estimates of chemical or metabolite concentrations in all of the organ systems of the model for any input parameter (inhalation concentration, oral, intravenous, or intraperitoneal dose). Known or postulated species differences in chemical metabolism or elimination may be incorporated in the model. Because one PBPK model is assumed to be valid for all mammalian systems, extrapolation across species is easily accomplished by substitution of those physiological constants characteristic of the species of interest. Extrapolation from high to low doses is obtained directly from the model.

The goal of PBPK is to predict the concentrations of the active metabolite of a toxic or carcinogenic chemical in the target organ as exposure doses, exposure regimens, and routes of exposure vary. Using the list of parameters summarized in Table 1, it is possible to assess which critical elements/assumptions necessary for PBPK modeling have been addressed. This type of analysis will become even more important as investigators begin to develop organ-specific metabolic data and genotoxic data and begin to understand the transfer of metabolites between organs. Thus, ultimately, the goal of PBPK modeling must be to significantly reduce the uncertainties involving the potential risk associated with human exposure to a chemical. If successful, we will then be able to better explain the

public risk associated with a given exposure and, with some confidence, hopefully reduce the need to express risk estimates using 95 percent upper-bound confidence limits.

Chemical-Specific Needs

Part of this workshop has been devoted to a discussion of the use of PBPK modeling on selected chemicals. This discussion is useful in that we are now beginning to better understand how PBPK fits into risk assessment and the data required for its acceptance. I think it is important to understand that the criteria for acceptance will change as we gain confidence in the process. This, to a large degree, is the nature of consensus building in the scientific community.

The use of PBPK can impact two elements of a risk assessment, exposure and dose-response. It is important to understand that for each element, the data required will differ. Over the past few days, workshop presentations describing the use of PBPK for methylene chloride demonstrated how data on metabolism impact dose-response. Also, PBPK for 1,3-butadiene provided for adjustments in exposure parameters. Lastly, presentations were given on the risk associated with exposure to benzene. These presentations were particularly interesting because they demonstrate how one needs to consider both route of exposure and differences in species.

The presentations in total show how the use of increased/decreased assumptions can impact PBPK in risk assessment. Indeed, the concern voiced by Chris Portier should be kept in mind as we go from very simple to complex systems: are we creating greater uncertainties? An example of the problem we face can be demonstrated from the presentations on benzene. While the use of metabolic data clearly advance the assessment, the need to assume that liver metabolism reflects the risk of processes going on in bone marrow may not be appropriate.

I had hoped that this workshop would provide some guidance on minimal data needs for pharmacokinetics. After listening to the presentations and discussions over the last three days, I am not sure that the request for guidance is a wise one at this time. Right now, we appear to be unconstrained in our approach, which is healthy. In time, I think scientific discussion will begin to point us in a direction which will allow us to define the limitations on the use of PBPK in risk assessment.

TABLE 1

PARAMETERS AFFECTING THE PHYSIOLOGICALLY BASED PHARMACOKINETIC ASSESSMENT OF CHEMICALS

PARAMETER	CONSIDERATION
Uptake	How does concentration (exposure) affect uptake?
First pass effect	Is metabolism during exposure of primary concern?
Tissue equilibrium	Does the partition into lipid-rich compartments effectively sequester the chemical?
Biological half-life of parent compound and "active" metabolites	How long is post exposure in the animal or person at risk?
Transport of "active" metabolites between compartments and to target tissue(s)	Does the amount metabolized affect transport? How does excretion affect amount transported via the blood?
Specificity of enzymes and enzyme induction	What consideration must be made for species differences? Do endogenous substrates affect rates of metabolism?
Carcinogenic potential	Does an unmetabolized chemical play a role in the process? Is there an unidentified metabolite of concern?

CONCLUSION

BIOLOGICAL DATA FOR PHARMACOKINETIC MODELING AND RISK ASSESSMENT: RESEARCH NEEDS

Kenneth B. Bischoff and Irene B. Glowinski

One of the goals of the workshop was to identify research or data needs for pharmacokinetic models in risk assessment. The following list of research and data needs, compiled from the workshop transcript, summarizes comments made by workshop participants during the conference summation and the final overview and wrap-up sessions.

Suggestions given by workshop participants are grouped into several broad categories, beginning with pharmacokinetic models, which was the focus of the workshop. Research that defines pharmacodynamic models, especially with a stronger basis in biology, is the topic of the next two categories and possibly constitutes a greater need than pharmacokinetic models. The fourth group of research needs, model parameters, is divided into physiological and physiochemical parameters, and metabolic parameters which are required for both pharmacokinetic and pharmacodynamic models.

Research Needs

1. Pharmacokinetic Models

The basis for and the necessary detail required in any pharmacokinetic model, as well as the uncertainties, must be better defined.

More care is needed in delineating assumptions used in a particular model.

There is a need to define the most critical assumptions and whether these can be validated.

Sophisticated statistical techniques should be applied to physiologically based pharmacokinetic models in addition to classical pharmacokinetic models.

Physiologically based pharmacokinetic models must be developed for toxic compounds with very long lives, such as heavy metals.

Pharmacokinetic models must be developed at the intracellular level.

2. Basis for Pharmacodynamic Effects

The basis for appropriate definition of metabolic or other biological markers as surrogates for a toxic agent must be better understood.

The relationship between the delivered dose to a tissue and the biologically effective dose for the endpoint in question must be better defined. For example, researchers should determine whether their conclusions are species-dependent.

A more fundamental basis is needed for choosing the appropriate dose-metric as the proper measure of toxicity. Examples of the dose-metric could be dose rate (mg/kg/day), a peak concentration (mg/L), a lifetime dose (mg/kg/lifetime), or, for carcinogenesis, related to the cell cycle time. Also, it must be determined if the dose-metric is species-dependent.

There is a need to analyze the assumptions involved when using a marker of toxicity that may not correctly identify other organ systems. For example, DNA adducts may not adequately predict effects on the immune system.

Known human carcinogens must be studied in animals and the results of these studies should be correlated.

3. Pharmacodynamic Models

Biologically motivated pharmacodynamic models must be developed using the same philosophy for physiologically based pharmacokinetic models, i.e., the use of biochemical and toxicological concepts in formulating pharmacodynamic models must increase.

Biologically based pharmacodynamic models must be developed that account for possible changes in the model and associated parameters during different biological states. Also, carcinogenesis models other than the two-stage model should be considered.

It must be realized that there are endpoints other than cancer for which models must be developed.

4. Model Parameters

Both pharmacokinetic and pharmacodynamic models require various types of parameters that need to be numerically identified from experimental measurements. Some of these parameters can often be obtained from literature, but others are more substance- and/or species-specific and must be directly measured.

More studies should examine the effect of modifying factors on pharmacokinetic and pharmacodynamic parameters such as sex, strain, age, dietary factors, environmental factors, and other changes in physiological conditions.

Modern sophisticated, noninvasive techniques such as NMR and PET scanning should be used for measurement of pharmacokinetic parameters.

Methods of model reduction and any subsequent loss of information should be studied.

Model predictive uncertainty (range of outputs) should be examined in more depth using statistical simulation methods such as the Monte Carlo method.

A. Physiological and Physiochemical Parameters

More accurate values of anatomical-physiological parameters, such as blood flow rates and tissue volumes in several species, must be developed.

A *consensus* must be reached for the most representative anatomical and physiological parameters for different species.

In order to have a model adequately predict desired tissue levels, researchers need to understand if there is a need to account for minute-to-minute changes in organ blood flow and volumes.

Existing partition coefficients and binding data should be evaluated for accuracy.

Standardized measurement techniques must be developed for physiological and physicochemical parameters.

B. Metabolic Parameters

With respect to Michaelis-Menten kinetics, the relationship between V_{max} and K_m values in various systems must be understood, ranging from isolated enzymes up to whole organs and animals.

There is a need to understand how to assemble the kinetics of isolated enzymes into networks of enzyme reactions.

The translation of V_{max} and K_m values between species should be better understood.

Rate equations other than those of the Michaelis-Menten form must be considered.

More information on the distribution of enzymes in different species should be obtained.

Enzyme induction must be further defined, especially concerning acute vs. chronic toxicity and high vs. low dose.

5. Other Sources of Information

Valuable data exist in other fields that could be utilized in toxicology and risk assessment. Detailed techniques that take advantage of existing data in these fields (e.g., radiation biology, cell kinetics, and toxic effects of drugs) should be explored.

6. Glossary

In order to promote cross-disciplinary research in pharmacokinetics and pharmacodynamics, a glossary of modeling terms with appropriate examples should be developed.

APPENDIX A

AGENDA

WORKSHOP ON BIOLOGICAL DATA FOR PHARMACOKINETIC MODELING AND RISK ASSESSMENT
May 23-25, 1988

Sponsored by:
ILSI Risk Science Institute
U.S. Environmental Protection Agency
The Dow Chemical Company
American Industrial Health Council
American Petroleum Institute
Mobil Oil Corporation
The Procter & Gamble Company
Shell Oil Company

WORKSHOP AGENDA

Monday, May 23, 1988

8:30am Welcome
Kenneth B. Bischoff

8:45am I. Overview of Risk Assessment
Chairperson - William Farland
Overview/Introduction

Panel Presentations

8:55am (1) Concepts - academic or theoretical discussion of risk assessment.
Nicholas A. Ashford
Discussion

9:30am (2) Practices - how data are used in a risk assessment.
Joseph V. Rodricks
Discussion

10:05am (3) Assumptions - highlight and identify where pharmacokinetic modeling can reduce the uncertainty.
Christopher Portier
Discussion

10:40am COFFEE BREAK

10:55am General Discussion

11:25am II. Modeling Biological Phenomena
Chairperson - Kenneth B. Bischoff
Overview/Introduction

11:40am Introduction to Modeling
Mary E. Davis

12:10pm General Discussion

12:30pm LUNCH BREAK

1:45pm III. Overview of Pharmacokinetics Models
Chairperson - Robert L. Dedrick
Overview/Introduction

Panel Presentations

1:55pm (1) Development and history of pharmacokinetic modeling.
Kenneth B. Bischoff
Discussion

2:30pm (2) Purposes: What models can do and their limitations.
Robert L. Dedrick
Discussion

3:05pm COFFEE BREAK

3:20pm (3) Data needs for modeling.
Harvey Clewell III
Discussion

3:55pm (4) Major uncertainties in pharmacokinetic modeling and sensitivity analysis.
Murray S. Cohn
Discussion

4:30pm General Discussion

5:00pm ADJOURN

Tuesday, May 24, 1988

8:45am IV. The Use of Experimental Data in Pharmacokinetic Modeling
Chairperson - Richard W. D'Souza
Introduction

Panel Presentations

9:00am (1) Hepatic metabolism (rates/pathways).
Marilyn E. Morris
Discussion

9:35am (2) Cross-species scaling.
Joyce Mordenti
Discussion

10:10am COFFEE BREAK

10:25am (3) Correlation of in vitro and in vivo data.
I. Glenn Sipes
Discussion

11:00am (4) Target tissue/cell dose/chemical carcinogens.
George Lucier, Steve Belinsky, and
Claudia Thompson
Discussion

11:35am General Discussion

12:00pm LUNCH BREAK

1:30pm V. The Use of Pharmacokinetic Modeling in Risk Assessment/Case Studies
Chairperson - Linda S. Birnbaum
Introduction

Analysis of Different Models and Compounds

1:40pm (1) Benzene
Model - Michèle A. Medinsky
Risk Assessment - A. John Bailer
Comment - Richard Irons

2:50pm COFFEE BREAK

3:05pm (2) Butadiene
Model - Dale Hattis
Risk Assessment - Steven Bayard
Comment - Richard Irons

4:30pm ADJOURN

Wednesday, May 25, 1988

8:45am VI. The Use of Pharmacokinetic Modeling in Risk Assessment/Case Studies
(continued)
Introduction

Analysis of Different Models and Compounds (cont'd.)

8:55am (3) Methylene Chloride
Model - Inhalation Data - Richard H. Reitz
Model - Ingestion Data - Michael J. Angelo
Risk Assessment - Jerry Blancato and
Lorenz Rhomberg
Comment - Rory B. Conolly

10:35am COFFEE BREAK

10:50am General Discussion

11:20am VIII. Conference Summation
Chairperson - Kenneth B. Bischoff
Introduction

(Reports of Rapporteurs)

11:30am (1) Basic biological research needs.
Alan Wilson

11:50am (2) Chemical-specific needs.
Hugh Spitzer

12:10pm (3) Applied research needs.
Angelo Turturro

12:30pm OVERVIEW AND WRAP-UP
Kenneth B. Bischoff

12:45pm ADJOURN

APPENDIX B

ABSTRACTS OF PRESENTATIONS

SESSION I: OVERVIEW OF RISK ASSESSMENT

CONCEPTS: ACADEMIC OR THEORETICAL DISCUSSION OF RISK ASSESSMENT

Nicholas A. Ashford

This presentation provides a framework for considering values in the use of science in the regulatory process. The science in question includes both the assessment of technological risk and the assessment of technological options to reduce those risks. The focus of the inquiry is on the role of the scientist and engineer as analysts or assessors. The difficulties in separating facts and values are addressed by focusing on the central question: What level of evidence is sufficient to trigger a requirement for regulatory action? For the purposes of this discussion, the regulatory process will include notification of risks to interested parties, control of technological hazards, and compensation for harm caused by technology. The discussion addresses the problems in achieving both a fair outcome and a fair process in the regulatory use of science.

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SESSION I: OVERVIEW OF RISK ASSESSMENT

USE OF DATA IN RISK ASSESSMENT

Joseph V. Rodricks

At our present stage of understanding, the completion of risk assessment requires not only the use of traditional forms of scientific data, but also the imposition of a number of assumptions and models that have not been subjected to thorough empirical verification. The risk assessor is forced to adopt such assumptions and models because no direct methods are available to measure many small, or even moderately large, risks that have proven to be of sufficient social concern to have prompted the enactment of regulatory laws.

To achieve consistency in the absence of scientific certainty, regulatory agencies have typically adopted assumptions and models that are applied generically to all agents. It is becoming clear, however, that increased understanding of the chemical and biological behavior underlying the production of toxicity can lead to the substitution of agent-specific information for generic models and assumptions. Nowhere is the potential for using such information to improve risk assessment greater than in the area of pharmacokinetics.

Because no data concerning underlying mechanisms of toxicity (in which I include pharmacokinetic and metabolism data) can be expected to answer all questions, the use of such data in risk assessments will necessarily introduce uncertainties. Regulators will be reluctant to use such information if the risks of specific agents are revealed to be less than those predicted under the use of generic models (i.e., because there is uncertainty associated with the lower risks, the fear arises that risks have been underestimated). Of course, as long as regulatory agencies reject the use of data on mechanisms of toxicity because they do not answer all questions, research scientists will have little incentive to develop such data. This unhealthy situation can be remedied if risk assessors strive to use all available data on mechanisms in their assessments and then exhaustively describe for decision-makers the relative degrees of scientific support merited by results based on the use of generic models and those based on the use of mechanistic information. Decision-makers are then free to choose from among several possible risk estimates, based on the scientists' best judgment regarding their relative merits.

If research scientists ask that risk assessors adopt such an approach, the research scientists should be sensitive to the types of dilemmas that the use of mechanistic information creates for the risk

assessor. In the area of pharmacokinetics, three major questions are typically encountered in the use of data in risk assessment:

1. How can we be certain that the metabolites whose pharmacokinetic behavior we are measuring or modeling are responsible for the observed toxicity?

2. How can we be certain that pharmacokinetic behavior observed over a relatively short period of dosing can be expected to hold over periods of long-term exposure?

3. How can we be certain that pharmacokinetic behavior observed in experimental animals closely resembles behavior in humans?

Perhaps we shall find answers to these questions at this workshop.

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SESSION I: OVERVIEW OF RISK ASSESSMENT

THE IMPLICATIONS OF USING MORE COMPLICATED MODELS FOR RISK ESTIMATION IN CARCINOGENESIS

C.J. Portier and N.L. Kaplan

Quantitative risk assessment of mathematical models that have biologically interpretable parameters is becoming more common. The current practice is to obtain estimates of these parameters and ignore their intrapopulation variability when estimating safe-dose levels. If some of the model parameters have large intrapopulation variability (e.g., metabolic parameters in humans), then this procedure is likely to substantially underestimate the population variability of the safe dose.

The purpose of this investigation is to describe a method based upon Monte Carlo resampling (Efron, 1982; Portier and Bailer, 1987) for including the intrapopulation variability of biologically interpretable parameters in the risk estimation process. As an example, the physiologically based pharmacokinetic model used by Anderson et al. (1987) for estimating a safe-exposure dose for methylene chloride was reexamined. Their model included 23 biologically interpretable parameters consisting of tissue weights, blood flow rates, partition coefficients, and metabolic constants. The results indicate that the intrapopulation variability of the model parameters can have a substantial effect on the distribution of the safe doses, by broadening the range of possible values and thus increasing the standard deviation and lowering the fifth percentile. Where the intrapopulation variability was assumed to be small in both animals and humans, the standard deviation of the safe dose more than doubled, and the fifth percentile decreased by a factor of 1.5. Where the metabolic parameters in humans were assumed to have a high degree of variability, the standard deviation of the safe dose increased by a factor of more than 10, and the fifth percentile decreased by a factor of more than 7, actually falling below the corresponding EPA lower bound (U.S. EPA, 1985).

Variability analysis is an important tool in the estimation of risk from exposure to chemical carcinogens. The methods described here can be used to study the variability of safe-dose estimates for mathematical models with biologically interpretable parameters estimated from different studies. As our understanding of the carcinogenic process improves, the models used for quantitative risk assessment will become more complex and there will be an even greater need for the numerical methods described here.

The results of our study suggest that intrapopulation variability of the model parameters can increase the variability of safe dose estimates an appreciable amount. This increased variability represents a better estimate of the overall uncertainty in the risk assessment process.

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SESSION II: MODELING BIOLOGICAL PHENOMENA

INTRODUCTION TO MODELING

Mary E. Davis

Mathematical or quantitative models are used in our attempts to understand nature, to summarize data, and to relate that data to the processes or systems included in the models. Mathematical or quantitative models are rules describing how the component parts of a system interact with each other, often in response to external forces or perturbations; it is the interaction between these component parts that is of interest. Models are formulated from data that characterize a system. If the model predicts the actual behavior of that system then there is some confidence that the relationship among the components of the model is similar to their relationship in reality. This belief is strengthened if the model can predict behavior beyond the extremes of data used to formulate the relationship the model describes.

Classical, descriptive models are appealing because they require relatively modest amounts of data and yet reveal much understanding of how a compound is handled. To develop a compartmental model for absorption, distribution, and elimination of a compound, the data required are derived from the concentration of that compound in plasma or its excretion. A model of one or more compartments is then fit to the data, allowing the number and size of the compartments and the transfer between compartments to vary in order to fit the data more closely. This type of model describes the time-course-of-exposure and steady-state conditions well and can detect differences in the handling of a compound between experimental groups. However, because the compartments have no physiologic meaning, and the kinetic constants are hybrids reflecting several processes, one cannot determine what changes have occurred to cause experimental groups to differ. And because the constants are not related to distinct biological processes, it is not possible to extrapolate beyond the conditions of measurement (that is, across species or sexes, or to where animal growth is occurring).

In contrast to classical pharmacokinetic models are physiologically based pharmacokinetic models. The two types differ in several important respects: (1) the input data that drive the model; (2) the amount of prior knowledge needed to develop a good model; and (3) the type of information the model produces and how the model, with its information, can be used. In essence, physiologically based pharmacokinetic models describe the disposition of the compound of interest as it actually occurs in the organism. Instead of using amorphous compartments of variable size, the compartments used have an

anatomical basis, representing individual organs, with their respective volumes. Rather than considering single rates of elimination, movement of the compound into and out of the organ is taken into account, e.g., blood flow to the organ, as well as the compound's intrinsic ability to cross the membranes involved. Several organs can be grouped together if the organs handle the compound similarly and do not need to be modeled separately for other reasons (e.g., a target organ of a site of metabolism or elimination). The detail of the model depends upon the compound being modeled and the model's uses. For some chemicals, it is important to include the turnover of macromolecules involved in defending against toxicity (e.g., glutathione, binding proteins), and for carcinogenesis, the rates of DNA repair and cell replication are needed to accurately describe the response. Physiologically based pharmacokinetic models require more and different types of information, including physiological and biochemical data (e.g., organ volumes, blood flow, rates of metabolism and excretion), physical data (e.g., diffusion, permeability, protein binding), and an understanding of the biology of the risk being modeled.

After the data are collected, the model is formulated and compared to actual observations. If the predictions differ from the observed values, then the model is refined, adding the processes that were omitted but that are important to the compound's disposition. Thus, physiologically based pharmacokinetic models help guide experimental design. Because the constants used relate to physiological entities that can be measured in other animal species, physiologically based pharmacokinetic models can predict behavior of a compound in other species as well, and do particularly well if there is information on the metabolism of those species. In vitro experiments can provide much of the needed metabolic information, so physiologically based pharmacokinetic models are attractive for extrapolating from experimental animals to humans. Similarly, the metabolic and clearance parameters can deal with saturation of those processes, and thus physiologically based pharmacokinetic models are amenable to extrapolating beyond the exposures actually used, or into populations with impaired metabolic functions such as the young and old, and other sensitive populations. Exposure to chemical mixtures is another area in which physiologically based pharmacokinetic models can help guide research efforts because the interactions between many chemicals are due to competition for metabolism or elimination, or other changes that alter distribution.

For physiologically based pharmacokinetics models to achieve full potential in risk assessment there needs to be a better understanding of the biological processes underlying risks. Often only a small proportion of an exposed population develops tumors; the mechanisms for individual differences in susceptibility are not understood, yet are important for minimizing societal risk. Physiologically based pharmacokinetics has the potential to make great contributions in applying our understanding of species

differences in reproduction and development, for example, to protecting developing organisms while administering beneficial therapies to the parent.

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SESSION III: OVERVIEW OF PHARMACOKINETIC MODELS

DEVELOPMENT AND HISTORY OF PHARMACOKINETIC MODELS

Kenneth B. Bischoff

This presentation will describe the origins of pharmacokinetics and the reasons why pharmacokinetics has been found useful in research and applications of pharmacology and toxicology. The main purpose is to quantitate the results and to formulate predictive mathematical models for absorption, disposition, metabolism, excretion, and biological response for clinical applications and risk assessment.

A brief review of the history of pharmacokinetics will be given, focusing on the introduction of key concepts. (A comprehensive history is given by J. Wagner (1981).) Then the more recent development of physiologically based pharmacokinetic (PBPK) models will be described, again focusing on introducing key ideas.

Early work, beginning around the turn of the century, was concerned with anesthetic agents, since knowledge of specific brain concentrations seemed important. Ethanol and salicylate were also studied, and Michaelis-Menten kinetics and renal clearance were defined. The one-compartment open model was developed by E. Widmark and J. Tandberg (1924), and R. Dominquez (1934) studied creatinine and defined volume of distribution. F. Dost (1953) apparently coined the term "pharmacokinetik" (in German). During the 50s and 60s, many researchers were involved: B. Brodie, S. Riegelman, E. Nelson, J. Wagner, E. Garrett, P. Wiegand, E. Kruger-Thiemer, G. Levy, J. Gillette, M. Gibaldi, M. Rowland, L. Benet, and R. Jelliffe. (See the excellent text by Gibaldi and Perrier (1982).)

Physiological pharmacokinetics attempts to base models on real biological data concerning the physicochemical, physiological, and pharmacological properties of a drug, rather than using abstract definitions of, for example, central and peripheral compartments. Thus, organ blood flow rates, liquid solubility, protein binding, and local membrane permeability are all specifically incorporated into the models, and this level of detail permits translation of results between animal species and prediction of effects from different routes of administration and different doses.

This concept was actually proposed 50 years ago by T. Teorell (1937), whose model consisted of the circulatory system, drug depot, fluid volume, kidney elimination, and tissue inactivation. However, he

could mathematically solve only a greatly simplified form of model. J. Jacquez, R. Bellman, and R. Kalaba (1960) considered a two-region model with six compartments, but as computers became readily available, K. Bischoff and R. Brown (1966) developed a multiregion computer model based on chemical-engineering mass-balance concepts. Adding Michaelis-Menten kinetics and nonlinear Langmuir protein binding, K. Bischoff and R. Dedrick (1968) formulated a model for thiopental, and then in 1971, they studied methotrexate pharmacokinetics in several species. R. Dedrick (1973) specifically discussed interspecies scaling and first used the term "physiological pharmacokinetics." Since then, many investigators have applied these concepts to pharmacology and toxicology. Several reviews are provided below.

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SESSION III: OVERVIEW OF PHARMACOKINETIC MODELS

WHAT MODELS CAN DO AND THEIR LIMITATIONS

Robert L. Dedrick

Pharmacokinetics has been used extensively in pharmacology for the design and analysis of preclinical and clinical studies and the guidance of therapy. Classical compartmental, physiologic, and model-independent approaches have been used. All have important applications depending on the purpose of the investigation. This presentation emphasizes physiologic models because these generally provide greater mechanistic insight than other commonly used approaches. Several topics are included.

A. In Vitro-In Vivo Correlations

Xenobiotic metabolism is frequently studied in model systems such as extracted enzymes, tissue homogenates, cell suspensions, tissue slices, and isolated perfused organs. These systems permit investigator control of the reaction conditions and uncouple the metabolism from the complexities of chemical and metabolite distribution in the whole animal. Data that predict rates of metabolism in the body must be used correctly to account properly for the actual conditions that exist at the site(s) of metabolism with respect to such effects as blood flow, membrane transport, and binding. Published literature has been generally supportive of the concept that reasonable predictability may be obtained, but the literature is still limited.

B. Species Similarities and Differences

An extensive literature exists on the anatomy and physiology of animals. Some of this can be adapted to physiologic pharmacokinetic models directly; alternatively, established correlations can be used to derive plausible estimates of relevant parameters. Capillary permeability, for example, appears quite similar among mammals for a particular capillary or tissue type. Empirical allometric correlations have successfully displayed the orderly interspecies variation with body weight of a variety of organ sizes and physiologic functions; however, intraspecies correlations of this type may differ or be unsuccessful. Xenobiotic metabolism often shows large and unpredictable variations among species; experimental data generally will be required for each particular chemical and animal.

C. Dose Schedule and Bioavailability

Pharmacokinetic theory for determining bioavailability is well established. Also, methodology exists for predicting plasma concentrations as a function of dose schedule. A single bolus dose, for example, can be used to characterize drug pharmacokinetics in a particular subject and the parameters derived used to calculate an infusion schedule with a particular concentration objective. Errors can be made if nonlinearities are not correctly described or if pharmacokinetic parameters change with time as a result of drug effects, growth of the animal, or other causes.

D. Regional Drug Administration

Drugs have been applied to the skin, infused into arteries and tissues, instilled in a variety of body cavities, and inhaled into the lungs. Some interesting and important considerations relate to the distributed character of regional drug administration because the compartments are not well mixed. Diffusion, convection, and mixing are involved, and these processes are less well understood than the basic idea of pharmacokinetic advantage between compartments with uniform concentrations.

E. Biological Response Models

Pharmacokinetics is limited to describing and predicting the physical and chemical aspects of the distribution of drugs and other chemicals in the body. Pharmacodynamic information is required to associate drug concentrations (or concentration history) with a biological effect. The paucity of adequate biological models is a major impediment to the rational use of pharmacokinetic theory in risk assessment. Current pharmacokinetic theory can provide much information and often substantial predictive power concerning questions of dose-to-dose, route-to-route, and species-to-species pharmacokinetics. Pharmacokinetics by itself tells us nothing about the probability of a chemical causing cancer in humans or even the appropriate dose metric to be used for predicting human cancer risk from studies on experimental animals.

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SESSION III: OVERVIEW OF PHARMACOKINETICS MODELS

DATA NEEDS FOR PHARMACOKINETIC MODELING IN RISK ASSESSMENT

Harvey Clewell, III

The purpose of applying pharmacokinetic models to chemical risk assessment is to increase the scientific basis of the process and to reduce the level of uncertainty in the result. Pharmacokinetic models can aid in the experimental design of bioassays. They also permit the incorporation of biological information into quantitative risk estimates, serving not only to document the approach taken by the risk assessor but also to highlight those areas where the approach is most sensitive to error. The best time to develop a model is in parallel with initial data collection, so that the data collection can be directed by the model and discrepancies between the data and the model can be verified and used to refine the model. A considerable body of data is needed to develop a physiologically based pharmacokinetic model. Qualitative information on the toxic effects of the chemical, its metabolism, and the mechanism of action is needed to define the model structure. Quantitative information must also be collected both to determine the parameters of the model and to check the model's validity.

The next logical step in the application of biologically motivated mathematic models for risk assessment is the linking of pharmacokinetic models to models of carcinogenesis. Crucial to this next step is the development of a body of data on typical values of the parameters used in these models. These needed data include cell turnover rates, mutation rates, and DNA repair rates in both humans and rodents, as well as measures of the impact of specific chemicals on these rates. The result will be a quantitative risk assessment that incorporates not only the uptake, distribution, and metabolism of a chemical but also the specific mechanism by which it leads to tumor formation.

There are at least seven levels of uncertainty in quantitative chemical risk assessment. They are:

1. Classification of the chemical as a carcinogen
2. The mechanism of carcinogenesis
3. The nature of the proximate carcinogen
4. The structure of the PK model
5. The parameters in the PK model

6. Species differences in sensitivity

7. Extrapolation to ultra-low doses

Of these, only the fifth has been treated in any quantitative fashion to date. The seventh is, by policy, handled by the linearized multistage model. The first four are generally treated simply as decisions to be made, and there are not as yet any accepted methodologies for reflecting the uncertainty in these decisions in the final outcome. The sixth has become the refuge for a safety factor that was originally derived inappropriately from the toxicity across species of a particular class of chemicals. As PK models are extended into the process of carcinogenesis, all of these uncertainties will become quantifiable, at least to some extent.

There are several related issues that tend to divide the risk assessment community. On the one extreme are the risk-averse, those who are concerned primarily with estimating "safe" absolute risk estimates on chemicals. Given the greater perceived cost of underestimating risk as opposed to overestimating it, these individuals tend to compound conservative assumptions and resist any changes that would lower the risk estimate because of their fear that the residual uncertainty in the process could led to an overall underestimate of risk. On the other extreme are the risk-tolerant, those who are concerned primarily with accurate relative risk estimates. Motivated by a fear that inaccurate information could lead to erroneous risk-management decisions, these individuals tend to favor the rapid incorporation of new techniques in the risk assessment process and are opposed to redundant safety factors and risk assessment calculations that ignore chemical differences. Since the true risk for most regulated carcinogens may never be known, it is imperative that these two communities work together to reduce or deal with uncertainties in a mutually satisfactory manner.

There are four areas in particular that could greatly improve the process of chemical risk assessment:

> The usefulness of carcinogenic bioassays would be greatly improved if PK models were used in the design of the studies and if animals were included in the study specifically for the assessment of changes in PK parameters throughout the entire duration of the study. In addition, time-to-tumor and time-to-death data should always be recorded.
>
> The legislative agencies charged with performing chemical risk assessments should have the support of PK research groups possessing both modeling expertise and PK data acquisition capability.
>
> Basic data are critically needed on parameters required for biologically motivated cancer models, particularly stem cell population data, cell turnover rates, and mutation/repair rates in rodents and man.

Formal methods are needed for incorporating the uncertainty in qualitative risk assessment decisions into the quantitative outcome.

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SESSION III: OVERVIEW OF PHARMACOKINETIC MODELS

MAJOR UNCERTAINTIES IN PHARMACOKINETIC MODELING AND SENSITIVITY ANALYSIS

Murray S. Cohn

Pharmacokinetics is useful in the risk assessment process, provided that data exist for extrapolations between routes -- from one exposure scenario to another, from high to low dose, and from species to species. It is important to realize the uncertainties inherent in the use of pharmacokinetic information; some of these uncertainties are examined in the referenced works. The most important research, however, will concern the use of pharmacokinetic information for species-to-species extrapolation in the carcinogenic risk assessment process. At this time, it is my opinion that pharmacokinetics can be used to describe the "target site" dose in various species, but that the relative effects of such doses, i.e., tissue sensitivity in various species (pharmacodynamics), are unknown. This gap precludes using such information for risk assessment purposes.

Additional research is needed in two areas. The first is examination of pharmacodynamic processes, either through empirical examination or by modeling, to try to ascertain the relationship between target-site dose and effect. This approach can be very complex; research areas include DNA repair, cell cloning rates (of transformed cells), cell death rates, normal cell turnover rates, immunological responses to transformed cells, number of cells at risk etc., all of which obviously depend upon a thorough understanding of the mechanism of carcinogenic transformation in all species being considered. Although this approach should be pursued, any meaningful output is years away.

The second approach may have more immediate use. There are a number of known human carcinogens, all of which are carcinogenic in animals when appropriately tested. If proper pharmacokinetic studies were to be done for humans and all relevant animal species tested with these various carcinogens, one might begin to understand the magnitude of pharmacodynamic effects, since both total effects (responses to applied dose in humans and animals) and pharmacokinetic effects will have been determined or estimated. We may find that pharmacodynamic effects are fairly constant for various species pairs of a given general mechanistic type (e.g., initiation, cytotoxicity). This approach requires pharmacokinetic research and modeling of the type described at this workshop, and a careful analysis of the correlations that may become apparent. Once we know the approximate magnitudes of such effects, it may be easier to plan the studies needed to conduct the necessary experimentation via the first approach described above.

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SESSION IV: THE USE OF EXPERIMENTAL DATA IN PHARMACOKINETIC MODELING

HEPATIC METABOLISM

Marilyn E. Morris

In risk assessment there is a need to evaluate the hepatic elimination of toxic compounds or the formation of toxic metabolites at varying doses. It is important to realize, though, that the Michaelis-Menten parameters, which are determined in most in vitro studies, can be used to predict hepatic metabolism in vivo only after considering the physiological determinants of hepatic metabolism and what factor is rate limiting for the metabolism of a compound. In risk assessment one also needs to extrapolate that information on hepatic metabolism from animals to humans. These two areas represent current research needs with respect to the assessment of hepatic metabolism in risk assessment.

A. Determinants of Hepatic Metabolism

Insight into hepatic elimination requires an understanding of the physiological determinants of the process and how these relate to each other in a quantitative manner. Determinants of hepatic metabolism include the following:

1. Organ bloodflow (transit time)
2. Drug binding
3. Enzymatic activities: K_m and V_{max} for formation of metabolites
4. Cosubstrate availability
5. Diffusional barrier
6. Localization of enzyme systems
7. Competing metabolic pathways

An important consideration is that the rate-limiting step for the metabolism of a compound may change with changing dose.

B. Use of the Perfused-liver Preparation in the Study of Hepatic Metabolism

Numerous in vitro techniques including perfusion systems, liver homogenate, liver slices, isolated hepatocytes, and subcellular fractions, as well as purified enzyme preparations, have been widely used in the examination of hepatic metabolism. We have used predominantly the perfused-liver preparation since, in contrast to other in vitro preparations, organ structure, spatial heterogeneity, and architecture are preserved with this technique. Biliary excretion of drugs and metabolites also may be quantitated. Liver perfusion techniques have been applied to many animal species and have been used extensively in studies of drug metabolism and drug toxicity.

C. Assessment of Metabolite Formation Rates and Pathways with the Model Substrate, Gentisamide

We have used the perfused-rat-liver preparation in the assessment of the pathways and rates of metabolism of the model substrate, gentisamide (2,5-dihydroxybenzamide, GAM). Gentisamide-5-sulfate (GAM-5S) and gentisamide-5-glucuronide (GAM-5G) are major metabolites, and gentisamide-2-sulfate (GAM-2S) is a minor metabolite. Single-pass rat-liver perfusions were used to examine the effect of stepwise increases or decreases of input GAM concentration (C_{in}) on the extraction ratio of GAM and steady-state formation rates of metabolites. Steady-state metabolite-formation rates were determined by the sum of the steady-state efflux in perfusate and bile. Fitting the steady-state metabolite formation rates and the logarithmic mean drug concentration at the various C_{in}'s to the Michaelis-Menten equation furnished parameter estimates for the three metabolic pathways.

D. Prediction of Hepatic Metabolism

Hepatic clearance models have been derived to allow a method for the prediction of, first, hepatic clearance of drugs, and second, the effect of perturbations in the physiological determinants of elimination on clearance and blood-concentration time profiles. A number of models of hepatic clearance have been proposed that have been shown to be compatible with experimental data. These models generally use the parameters hepatic blood flow, free fraction of drug in blood, and hepatic intrinsic clearance (the intrinsic ability of the liver to eliminate the drug). The models include:

1. Venous Equilibrium (Well-Stirred) Model

The liver is assumed to be a single well-stirred compartment with the concentration of unbound drug in effluent blood in equilibrium with that in the liver, expressed as follows:

$$Cl_H = \frac{Q\ f_B\ Cl_{u,int}}{Q + f_B\ Cl_{u,int}}$$

where Cl_H is hepatic clearance, Q is hepatic blood flow, f_B is the free fraction in blood, and $Cl_{u,int}$ is the intrinsic clearance of free drug.

2. Sinusoidal Perfusion (Parallel-Tube) Model

The parallel-tube model assumes that the liver is composed of a large number of identical tubes or sinusoids, arranged in parallel, with enzymes uniformly distributed in parenchymal cells surrounding the cylinder. Tube concentrations decline in an exponential manner as drug is eliminated so that the mean hepatocyte concentration is taken as the logarithmic mean of the unbound input and output concentrations. Modifications of this model include a statistical distribution of sinusoid lengths (distributed model) and heterogeneous enzyme distribution. The basic model is configured as follows:

$$Cl_H = Q\ (1 - e^{-f_B Cl_{u,int}/Q})$$

3. Dispersion Model

The dispersion model was so named because of its analogy to nonideal flow in a packed-bed chemical reactor. The model is characterized by two main parameters: the efficiency number (R_N), which describes the efficiency of drug removal by the liver, and an axial dispersion number (D_N). The D_N is a measure of the dispersion or spread in residence times of drug molecules moving through the liver. As D_N approaches infinity, Cl_H is identical to the result predicted by the well-stirred model; as D_N approaches O, Cl_H is the same as that predicted by the undistributed sinusoidal perfusion model.

Since the relationships between Q, f_B, and $Cl_{u,int}$ vary, the prediction of Cl_H will vary with the different models of hepatic clearance. However, a change in any determinant has the same general effect, regardless of the model used. Significant differences between models occur only when drugs with high total intrinsic clearance are examined.

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SESSION IV: THE USE OF EXPERIMENTAL DATA IN PHARMACOKINETIC MODELING

CROSS-SPECIES SCALING

Joyce Mordenti

I. Allometric Approach

A. Equations
1. One independent variable: $Y = aW^b$, where W = body weight
2. Two independent variables: $Y = cW^d(BW)^e$, where BW = brain weight

B. Data Requirements
1. Enough data are available to obtain statistical significance.
2. If protein binding is greater than 80%, it is essential that the percentage of protein binding is similar in all species and linear over the concentration range of interest. When in doubt, scale unbound drug concentrations.
3. Physical methods of elimination (renal, biliary, pulmonary) are preferred for one independent variable; oxidative metabolism scales with two independent variables.

C. Uses
1. Estimation of pharmacokinetic parameters (clearance, volume of distribution, half-life, etc.) (Figure 1)
2. Estimation of entire pharmacokinetic profiles (Figure 2)
3. Estimation of toxicity endpoints (Figure 3)

D. Advantages: Easy and fast

E. Limitations
1. Empirical
2. No attempt is made to give physiological meaning to the pharmacokinetic parameters.
3. May not work well for metabolized compounds, although there are data to support allometric approach for some metabolized compounds.

II. Physiologic Approach

A. Equations: Series of differential equations that are solved simultaneously

B. Data Requirements for Model (Figure 4)
1. Need anatomical, physiological, biochemical, and binding data for drug disposition, such as:
    a. Blood flow to eliminating organs
    b. Tissue and fluid volumes
    c. Blood-to-plasma and tissue-to-plasma drug concentration ratios
    d. Drug protein binding
    e. Enzyme kinetics
2. Linear or nonlinear pharmacokinetic data
3. Linear or nonlinear protein binding

C. Uses
   1. Estimates of drug disposition in other animal species are obtained once the model is described in detail for one species (Figure 5)
   2. Estimation of toxicity endpoints
   3. Low-dose to high-dose extrapolation (and vice versa)

D. Advantages
   1. Pharmacokinetic parameters have physiological meaning
   2. Method of choice for metabolized compounds
   3. The parameters in the model can be modified to make a priori predictions of pharmacokinetic changes associated with disease states, age, pregnancy, or drug-drug interactions (Figure 6).

E. Limitations
   1. Requires sophisticated computer programs, mathematical prowess, lots of experimental time, and money
   2. Data in humans may not be available; therefore, may need to rely on in vitro experiments, allometric estimates, or may need to assume same value as animals.

III. Recommendations: Develop a set of criteria for deciding when the physiologically based pharmacokinetic model is needed and when simpler models will suffice.

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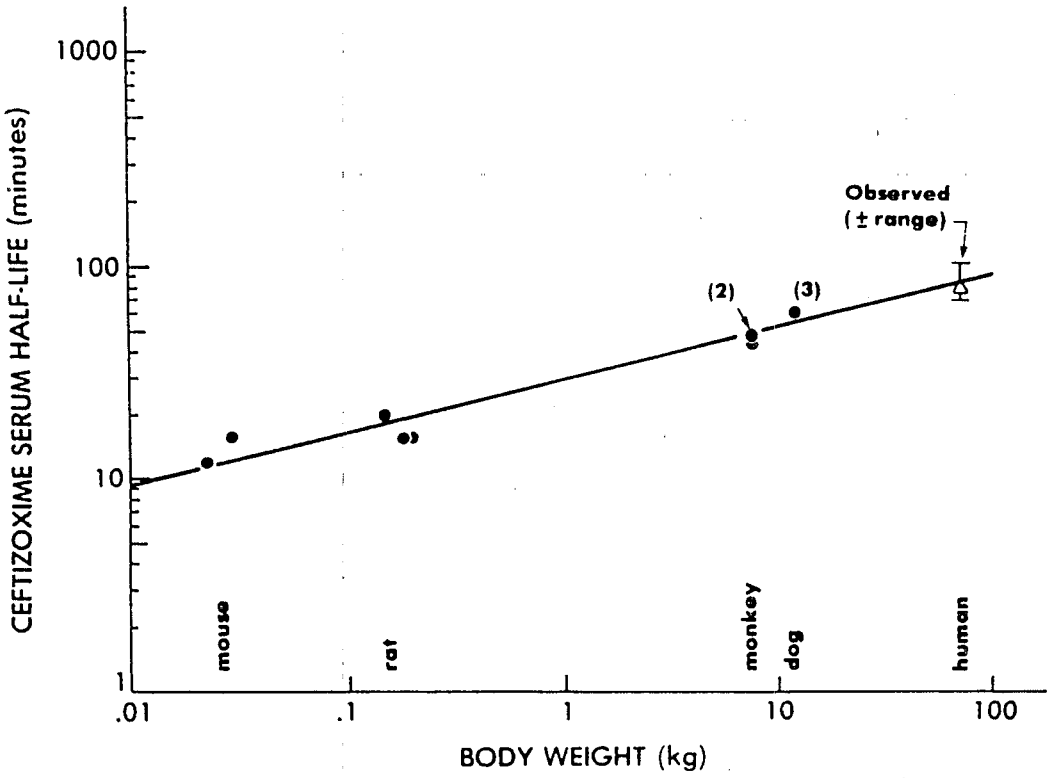

Figure 1. Log-log plot of ceftizoxime half-life versus body weight. The solid circles represent the half-life of ceftizoxime in various animal species. The solid line is the least squares linear regression line. The prediction of ceftizoxime half-life in humans is read off the regression line at 70 kg. The triangle represents the reported ceftizoxime half-life in humans (mode), and the bars represent the range of values from the literature. Numbers in parentheses indicate numbers of data points. Source: Mordenti, J., 1985. "Forecasting cephalosporin and monobactam antibiotic half-life in humans from data collected in laboratory animals." Antimicrob. Agents Chemother. 27:887-891.

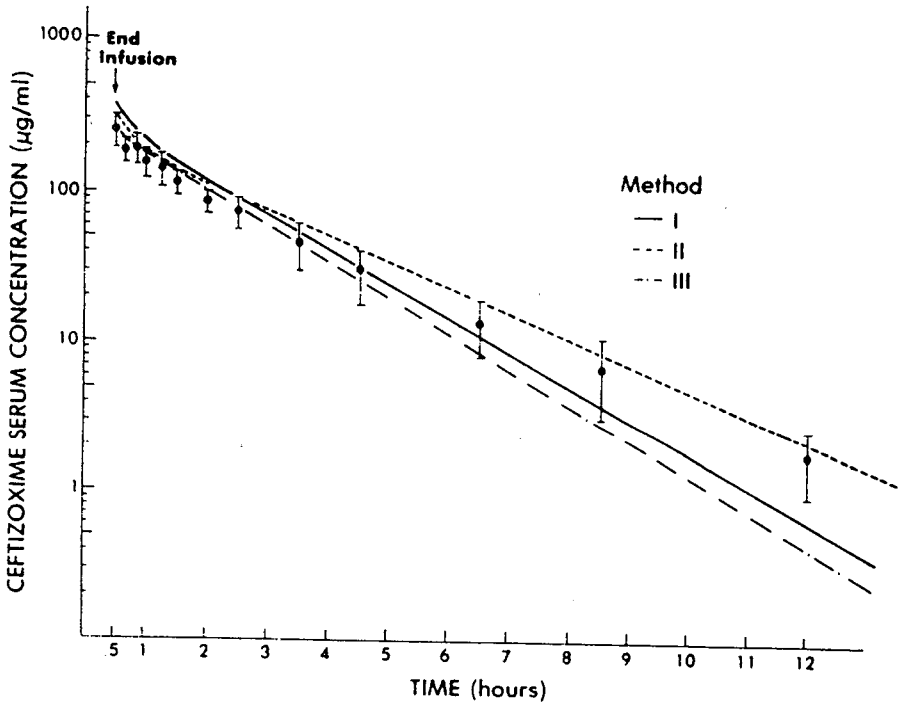

Figure 2. Comparison of the predicted and experimental ceftizoxime concentrations in human serum after a 4-gram, 30-minute iv infusion. KEY: solid circles represent ceftizoxime serum concentrations; the solid and dashed lines represent three prediction methods. Source: Mordenti, J., 1985. "Pharmacokinetic scale-up: accurate prediction of human pharmacokinetic profiles from animal data." J. Pharm. Sci. 74:1097-1099.

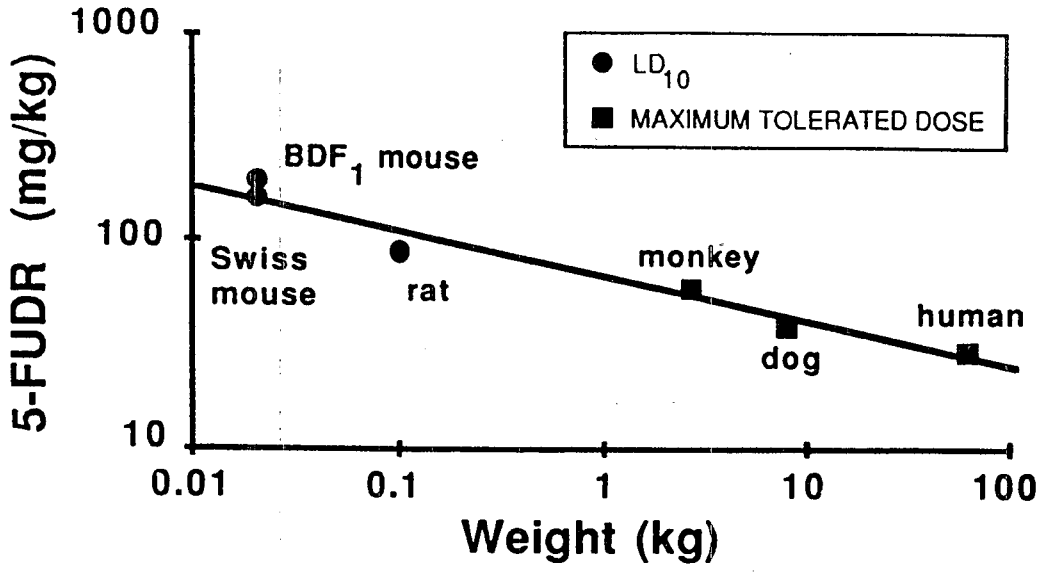

Figure 3. Log-log plot of toxic doses versus body weight data for 5-FUDR (floxuridine). The solid line is from an equation fitted using the method of least squares on unweighted logarithmically transformed data. Source: Mordenti, J., 1986. "Dosage regimen design for pharmaceutical studies conducted in animals." J. Pharm. Sci. 75:852-857.

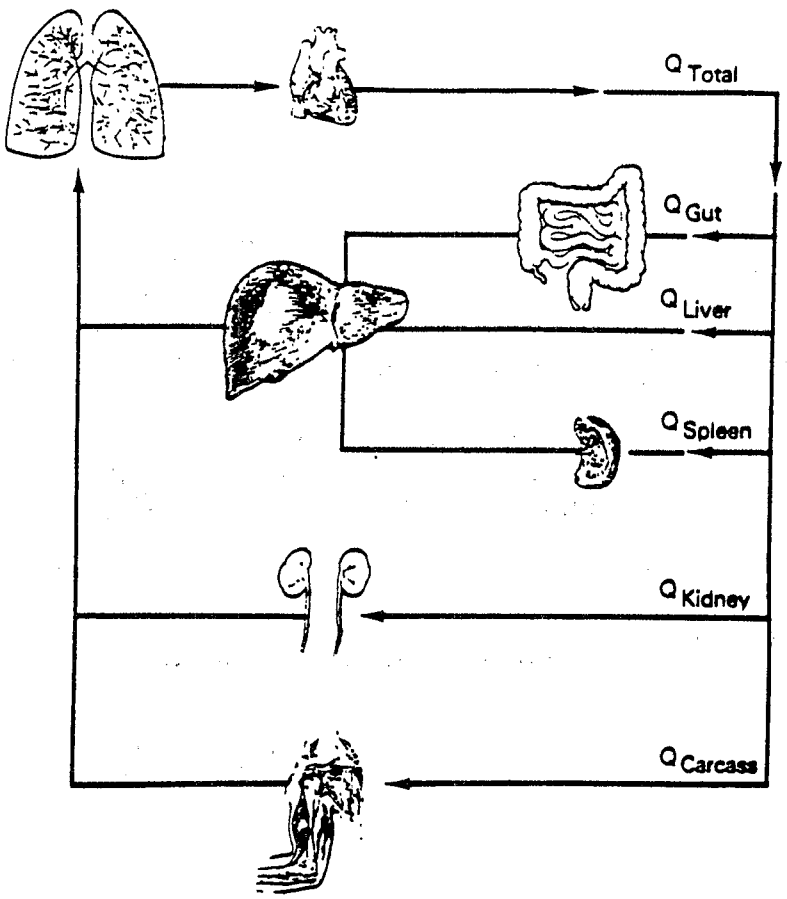

Figure 4. Compartmental model for IgG pharmacokinetics. Antibody is distributed to each organ according to arterial plasma flow to that organ (Q_i). Source: Covell, D. et al., 1986. "Pharmacokinetics of monoclonal immunoglobulin G_1, $F(ab')_2$, and Fab' in mice." Cancer Res. 46:3969-3978.

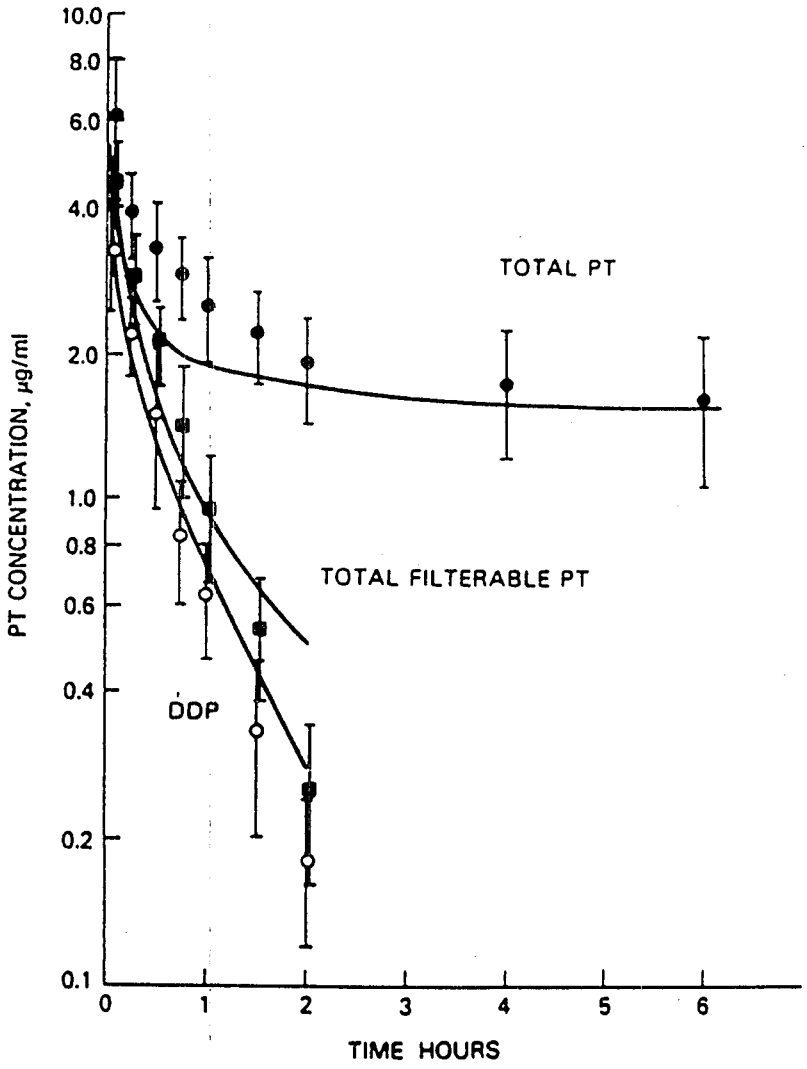

Figure 5. Physiologic model predictions of platinum (Pt) containing species in human plasma following an iv dose of 100 mg/m^2. Each point represents the mean $\pm$ SD for 5-6 patients for total Pt, total filterable Pt, and *cis*-dichlorodiammineplatinum (DDP). Source: King, F., Dedrick, R., and Farris, F. 1986. "Physiologic pharmacokinetic modeling of *cis*-dichlorodiammineplatinum in several species." J. Pharmacokinet. Biopharm. 14:131-156.

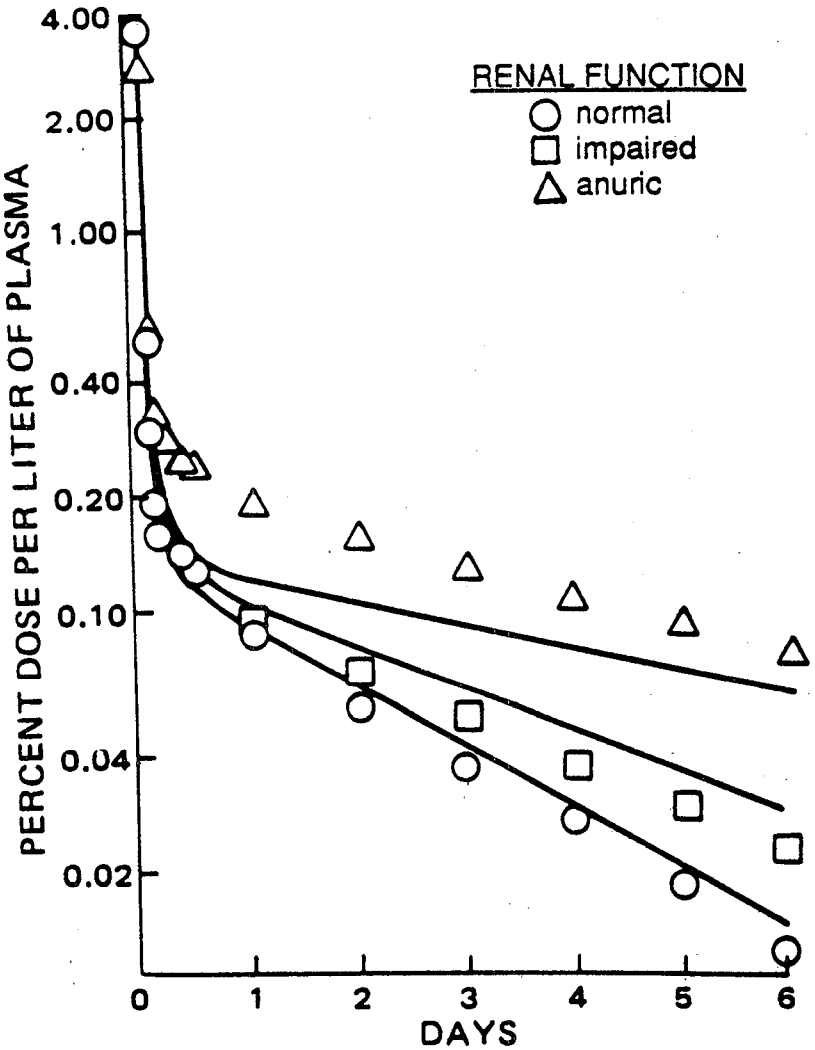

Figure 6. Predicted and observed plasma digoxin concentrations in patients with normal renal function, in patients with moderate renal impairment, and in patients with severe renal failure. Predictions based on scale-up of a dog physiologic model. Source: Harrison, L. and Gibaldi, M., 1977. "Physiologically based pharmacokinetic model for digoxin disposition in dogs and its preliminary application to humans." J. Pharm. Sci. 66:1679-1683.

SESSION IV: THE USE OF EXPERIMENTAL DATA IN PHARMACOKINETIC MODELING

BIOTRANSFORMATION OF ENVIRONMENTAL CHEMICALS: DEVELOPMENT OF IN VITRO SYSTEMS TO PREDICT IN VIVO EVENTS

Glenn Sipes

Differences in rates and/or routes of biotransformation of chemicals often explain species differences in the pharmacologic/toxicologic responses to the chemicals. Therefore, knowledge of the metabolism of a chemical is critical in the early phases of its toxicity testing. Obtaining such data is relatively straightforward for laboratory animals. However, with respect to humans, such data are difficult to obtain, particularly for chemicals that are not to be used therapeutically and that have toxic potential (i.e., pesticides, solvents, chemical intermediates, etc.). In estimating the human risk as a result of exposure to environmental chemicals, knowledge of human metabolism is also needed. Therefore, in vitro systems are needed that can determine how humans metabolize particular chemicals. These in vitro systems can then be used to compare directly how animals and humans biotransform chemicals. From in vitro findings it will be possible to make pharmacokinetic and limited toxicological predictions. These predictions can then be tested in laboratory animals. If the in vitro findings accurately predict in vivo findings in animals, then the limited in vitro data obtained with human metabolism preparations can be given more weight in estimating human risk from an exposure to a particular chemical.

A number of in vitro preparations can be used to obtain metabolic data. These include purified or partially purified enzymes, subcellular preparations (i.e., mitochondria, microsomes, cytosol, etc.) or tissue homogenates, slices, or wedges. The type of preparation used depends on the availability of tissue (a problem with humans) or the question being asked.

Over the past few years, my colleagues and I have used subcellular preparation of human liver, particularly microsomal preparations, to determine if humans can metabolize particular polychlorinated biphenyls. Data from human hepatic microsomal preparations were then compared to data obtained from animal preparations. These simple metabolic systems showed that humans cannot readily metabolize 2,2',4,4',5,5'-hexachlorobiphenyl (2,4,5-HCB). This inability to metabolize 2,4,5-HCB explains why this compound accumulates in human adipose tissue. Human hepatic microsomes were able to metabolize 2,2',3,3',6,6'-hexachlorobiphenyl and 4,4'-dichlorobiphenyl, two biphenyls that are known to be eliminated readily by rats, dogs, and monkeys and probably by humans. As a sidelight, the in vitro studies explained why dogs can metabolize and more readily eliminate 2,4,5-HCB. Dog microsomes

contain a particular cytochrome P-450 with high catalytic activity (as compared to other species) towards this environmental contaminant (Duignan et al., 1987).

Although subcellular preparations are useful to answer certain questions (the intrinsic ability to metabolize a particular chemical), they do not provide data on the nature of metabolism that occurs in intact cellular systems. Under these conditions coupled and competing pathways are operative and will influence the rates and routes of metabolism. We refer to this as integrated biotransformation.

We have recently developed a dynamic liver culture system to study the integrated biotransformation and potential toxicity of chemicals. The system requires the use of a mechanical slicer to produce slices from sections of liver bathed in oxygenated buffer. The precision-cut slices that are produced are thin (250 μm) and of uniform size and weight (25 mg/slice). For incubation the slices are placed on a wire mesh screen, which is inserted into an incubation vial. The vials are then incubated on a temperature-controlled roller system, so that the slices rotate in and out of the culture media at a preset rate and are maintained at 37° C. This dynamic culture system enhances oxygenation of cells throughout the slice. These innovations have resulted in slices that are viable for over 20 hours, when assessed for maintenance of protein synthesis, potassium, and ATP content and leakage of lactic acid dehydrogenase into the culture medium. Chemical-induced changes in these indices of viability also are useful markers of chemical-induced toxicity (Sipes et al., 1987).

Human and rat slices prepared as described and incubated in various buffers can biotransform a number of xenobiotics. Both the cytochrome P-450 system and various conjugating enzymes are stable for 12-20 hours of incubation (depending on the particular liver). Substrates tested to date include 7-ethoxycoumarin, biphenyl, chlorobenzene, 1,2-, 1,3- and 1,4-dichlorobenzenes, and phenacetin for P-450 mediated reactions, and the hydroxylated metabolites of several of these for assessment of glucuronidation and sulfation capacity. These slices can also acetylate a number of aromatic amines and be used to phenotype humans as fast or slow acetylators (Gunawardhana et al., 1988). When allyl alcohol, bromobenzene, or the isomers of dichlorobenzene were incubated with rat liver slices, dose-dependent toxicity was observed as assessed by changes in protein synthesis, slice K^+ and ATP content, and LDH leakage (Sipes et al., 1987). Factors that modify the toxicity of these compounds in vivo resulted in similar alterations in the hepatotoxicity produced by these chemicals in this in vitro system. Human liver slices also revealed dose-dependent toxicity for those chemicals tested.

Clearly, further refinement and testing of this dynamic liver slice system are necessary. However, it should become a useful tool to help elucidate mechanisms of toxicant-induced liver injury. In addition,

it will aid in the extrapolation of in vitro data to the in vivo situation, as well as in the extrapolation of animal data to humans. Such systems are particularly needed for the study of environmental chemicals, since ethical considerations preclude their administration to humans.

Particular research needs are four-fold: to assess the in vitro metabolism of more chemicals by in vitro human preparations; to develop appropriate kinetic parameters in vitro that can be used to predict in vivo clearance; to test these predictions in vivo (in laboratory animals); and to develop methods to cryopreserve human tissues so that they can be used as needed to assess human chemical metabolism. (Supported by NIEHS-N01-ES-55112).

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SESSION IV: THE USE OF EXPERIMENTAL DATA IN PHARMACOKINETIC MODELING

TARGET TISSUE/CELL DOSE OF CHEMICAL CARCINOGENS

George Lucier, Steven Belinsky, and Claudia Thompson

There is a great deal of uncertainty regarding estimation of human risks from low dose exposure to chemical carcinogens when using high dose animal data as the basis for extrapolation. It is becoming increasingly evident that molecular approaches can contribute a great deal to reducing the uncertainty that is inherent in the risk assessment process when gross biological endpoints such as tumors are used. For example, macromolecular interactions such as DNA adducts can be detected at doses much lower than those needed to produce significant increases in tumors.

There are several issues that affect the use of DNA adducts as a molecular dosimeter. These include:

1. Adduct heterogeneity - Different adducts have markedly different capacities to produce genetic damage. Therefore, measuring total DNA adducts can produce misleading data.

2. Cell specificity - Different cell types have different capacities to form and repair DNA adducts. Therefore, measuring DNA adducts in whole-organ preparations can produce misleading data.

3. Surrogate markers - In molecular epidemiologic studies, it is customary to quantify DNA adducts in lymphocytes. In some cases, lymphocytes will be good surrogate markers for adduct concentrations in target cells and in other cases they will not.

4. Indirect adduction - Some chemicals, such as estrogens, form no DNA adducts or only small amounts, but they do dramatically influence the DNA adduction of dietary constituents or other endogenous compounds. In other words, a carcinogen that does not bind to DNA directly is not always a nongenotoxic carcinogen.

5. Adduct detection - The two most sensitive methods for detecting DNA adducts are antibody techniques and ^{32}P-postlabeling methods. In general, ^{32}P methods are best for bulky aromatic adducts, whereas antibody methods are more suitable for alkylated adducts.

DNA adducts have been used as a molecular dosimeter in some cases. One of the best examples is NNK (a carcinogenic metabolite of nicotine). The promutagenic adduct of NNK, O^6-methylguanine, is formed more efficiently at low doses than high doses in lungs; this finding is consistent with dose-response relationships for carcinogenicity. Moreover, the Clara cell has considerably more O^6-methylguanine than other lung cell types, and this cell is considered to be the progenitor cell in NNK-induced lung cancer. In other studies, DNA adducts have been detected in human lymphocytes by

^{32}P-postlabeling, but adduct profiles were not different between smokers and nonsmokers. A great deal of interindividual variation exists in adduct concentrations, and this variation may reflect differences in metabolic activation/deactivation reactions, including the presence of a glutathione transferase isozyme that is polymorphic in human populations.

A recent NTP study revealed significant discordance between short-term tests for genetic toxicity and in vivo carcinogenicity (lifetime exposures). These data suggest that up to 40% of the chemicals that are positive for carcinogenicity in the NTP bioassay are acting through nongenotoxic mechanisms. The implication is that effects on signal transduction pathways, receptor-mediated proliferative responses, and cell cycle control are involved in the mechanism of action of many carcinogens, including dioxin. Therefore, one research need is to evaluate the quantitative relationships between biochemical events involved in tumor promotion and carcinogenic incidence. Another need is for a better characterization of structure-activity relationships. A central underlying need in molecular dosimetry studies is increased knowledge of the diverse mechanisms of chemical carcinogens.

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SESSION V: THE USE OF PHARMACOKINETIC MODELING IN RISK ASSESSMENT: CASE STUDIES

BENZENE PHARMACOKINETIC MODEL: CASE STUDY

Michele A. Medinsky

Studies on the chronic toxicity of benzene conducted by the National Toxicology Program indicated that B6C3F$_1$ mice were more sensitive to the carcinogenic effects of benzene than were F344 rats. A physiological model was developed to describe the uptake and metabolism of benzene in rats and mice. Our objective was to determine if differences in toxic effects could be explained by differences in pathways for benzene metabolism or by differences in total uptake of benzene. Compartments incorporated into the model included liver, fat, a poorly perfused tissue group, a richly perfused tissue group, an alveolar or lung compartment, and blood (Figure 1). Metabolism of benzene was assumed to take place only in the liver and to proceed by four major competing pathways (Figure 2). These include formation of hydroquinone conjugates (HQC), formation of phenyl conjugates (PHC), ring-breakage and formation of muconic acid (MUC), and conjugation with glutathione with subsequent mercapturic acid (PMA) formation. Values for parameters such as alveolar ventilation, cardiac output, organ volumes, blood flow, and partition coefficients were taken from the literature (Table 1). Metabolic rate constants (V_{max} and K_m) were determined experimentally.

Model simulations confirmed that during and after 6-hour inhalation exposures, mice metabolized more benzene, on a μmole/kg body weight basis, than did rats. After oral exposure, rats metabolized more benzene at doses above 50 mg/kg than did mice; this was due to more rapid absorption of benzene by mice, resulting in more benzene being exhaled unmetabolized. Model simulations for PHC and PMA, generally considered to be detoxification metabolites, were similar in shape and dose-response to those for total metabolism. However, simulations for the metabolites representative of the putative toxication pathways, HQC and MUC, indicated that after both oral and inhalation exposures at all concentrations, mice would produce more of these metabolites than would rats. This trend was especially apparent for HQC (Figure 3). Differences were due to the greater rates of metabolism in mice. Increased metabolism of benzene via the HQC and MUC pathways in mice is consistent with the observed susceptibility of this species to benzene toxicity.

The key gaps in our understanding of benzene metabolism include lack of information on the concentrations of the unconjugated metabolites in liver, blood, and target tissues and insufficient

understanding of the capacity of human tissue to metabolize benzene. In particular, the concentrations of hydroquinone and phenol in bone marrow appear to be critical to the production of the reactive metabolite, benzoquinone. Models that describe the flux of unconjugated hydroquinone and phenol through the target organs and incorporate metabolism by human tissues will generally help us estimate health risks of exposure to benzene.

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TABLE 1
PARAMETERS USED IN THE PHYSIOLOGICAL SIMULATION MODEL FOR BENZENE METABOLISM IN F344 RATS AND B6C3F$_1$ MICE

PARAMETER	VALUE	
	RATS	MICE
Physiological		
Alveolar ventilation (L/hr.kg)	32.4	55.3
Cardiac output (L/hr.kg)	19.4	34.8
Gastrointestinal transfer (fraction/hr)	0.25	3.0
Body weight (kg)	0.288	0.030
Blood flow (fraction of cardiac output)		
To liver	0.25	0.25
To fat	0.09	0.09
To poorly perfused tissues	0.15	0.15
To richly perfused tissues	0.51	0.51
Organ volumes (fraction of body weight)		
Blood	0.042	0.042
Liver	0.03	0.03
Fat	0.11	0.11
Poorly perfused tissues	0.66	0.66
Richly perfused tissues	0.076	0.076
Other (not perfused)	0.082	0.082
Chemical		
Molecular weight (g/mol)	78.2	78.2
Partition coefficients		
Liver/blood	1.0	1.0
Fat/blood	28.0	28.0
Poorly perfused tissues/blood	0.6	0.6
Richly perfused/blood	1.0	1.0
Blood/air	18.0	18.0
Biochemical parameters		
V_{max} (μmoles/hr.kg)	122	200
K_m (μmoles/L)	40	1.0
$V_{max,phc}$ (μmoles/hr.kg)	174	333
$K_{m,phc}$ (μmoles/L)	5	3.0
$V_{max,pmc}$ (μmole/hr.kg)	104	90
$K_{m,pmc}$ (μmoles/L)	15	4.0
$V_{max,hqc}$ (μmoles/hr.kg)	1.7	27
$K_{m,hqc}$ (μmoles/L)	0.5	0.1
$V_{max,muc}$ (μmoles/hr.kg)	3.5	12
$K_{m,muc}$ (μmoles/L)	0.5	0.1

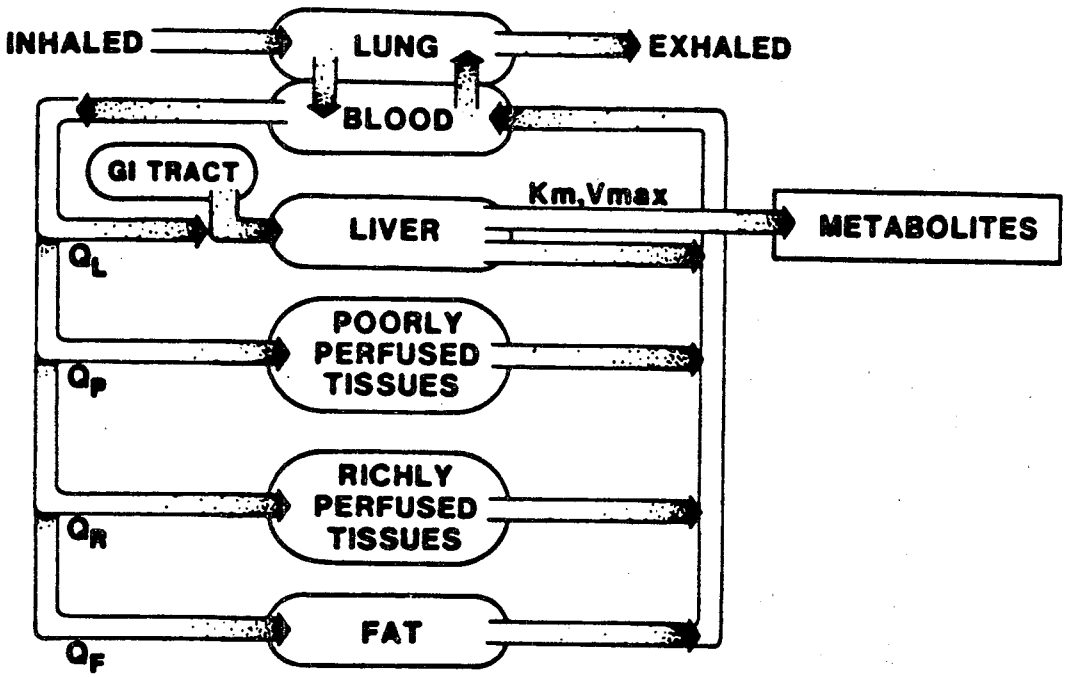

Figure 1. Physiological model of benzene metabolism. Metabolism of benzene was presumed to take place in the liver compartment. Pathways for benzene metabolism incorporated into the model are shown in Figure 2.

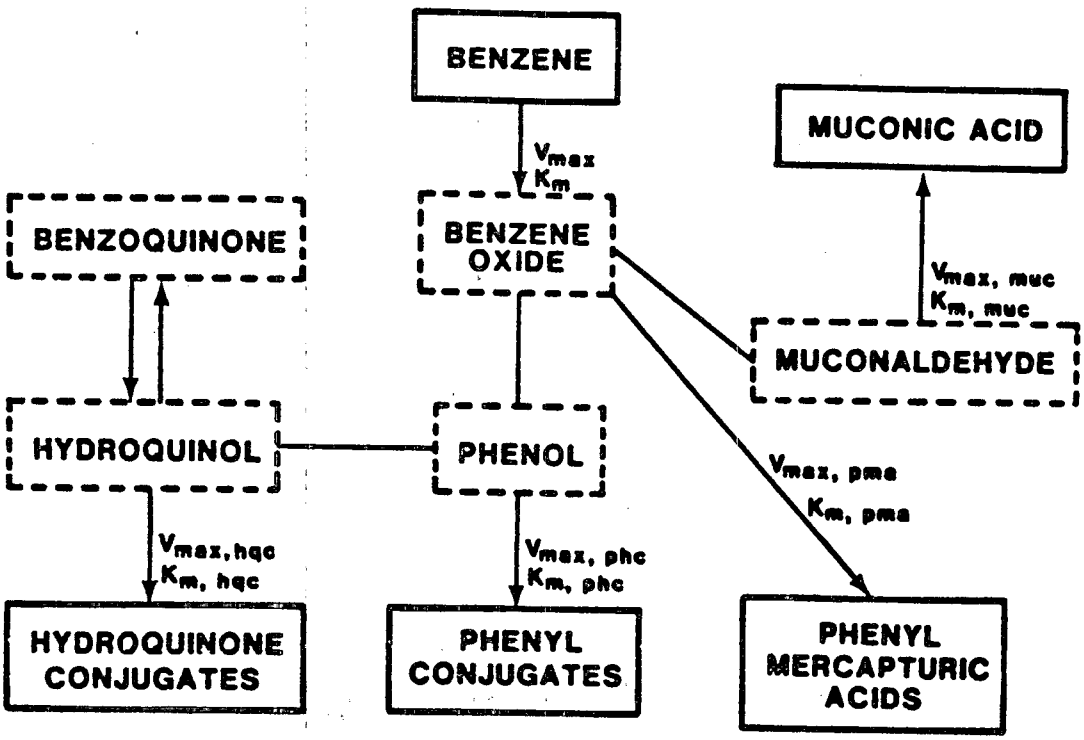

Figure 2. Scheme for metabolism of benzene. Biochemical rate constants are outlined in Table 1. This scheme for metabolism of benzene takes place in the liver compartment described in Figure 1. The solid boxes represent metabolites of benzene that were measured and used in model simulations. The dotted boxes represent immediate metabolites that were not incorporated into the model.

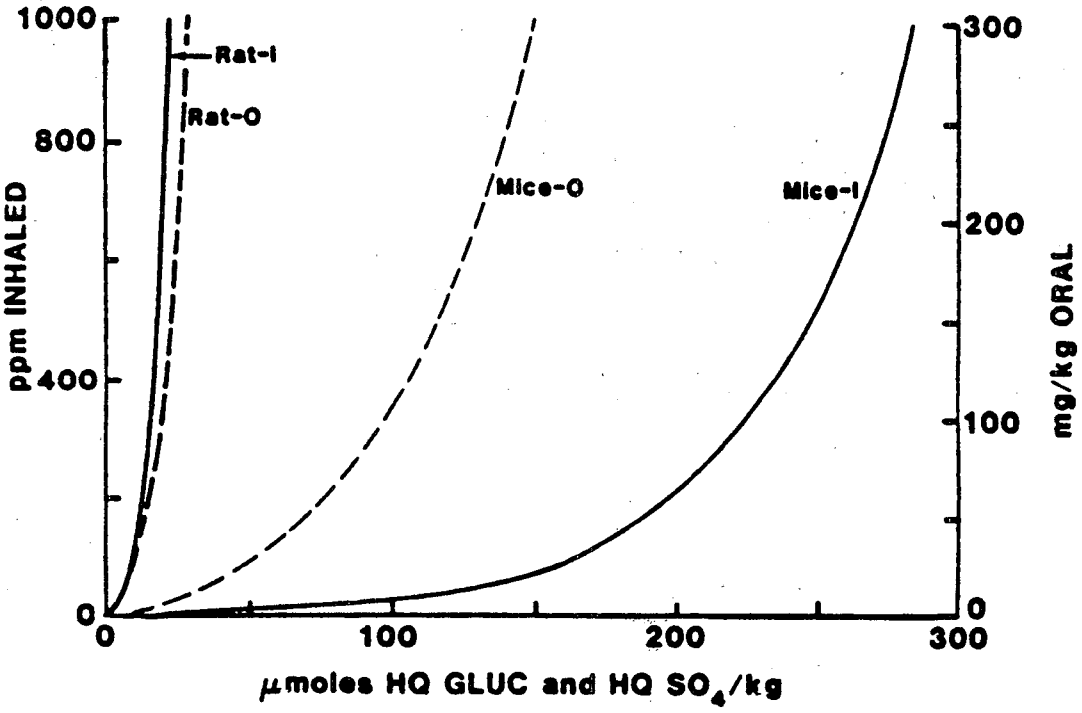

Figure 3. Hydroquinone conjugates excreted by rats and mice compared after single oral administration (O) or a single 6-hour inhalation exposure (I) to benzene. Lines represent results of model simulations for rats and mice for μmoles of hydroquinone conjugates (glucuronide and sulfate) per kg of body weight.

SESSION V: THE USE OF PHARMACOKINETIC MODELING IN RISK ASSESSMENT: CASE STUDIES

IMPLICATIONS OF PHARMACOKINETIC DATA AND MODELS IN A RISK ASSESSMENT OF BENZENE

A. John Bailer

Risk assessments of benzene have been based upon both human and animal studies. In this report, metabolite information is used to construct an internal dose (a surrogate of the biologically effective dose) for a given administered dose. The relationship between the administered dose and the internal dose is nonlinear and is well described by a Michaelis-Menten function. The administered doses from the National Toxicology Program's rodent carcinogenicity study of benzene are transformed into internal doses, and the internal doses are used in conjunction with a multistage model to perform a comparison of previous estimated "virtually safe doses" (VSDs) associated with small added health risks. The ratio of VSD for the administered-dose risk assessment to VSD for the internal-dose risk assessment was approximately 1.0 for F344/N rats and ranged from 2.5 to 5.0 for B6C3F$_1$ mice in the National Toxicology Program study. Given an occupational exposure of 1 ppm, a risk estimate of 0.7 excess cancers/1,000 exposed with an upper bound of 3.5/1,000 was obtained. Risk estimates based upon internal doses constructed from levels of toxic metabolites of benzene are in the same range as the total metabolite-based risk estimates. A better characterization of the dose-response function for benzene may be provided by obtaining data describing the molecular dosimeters of benzene. These molecular dosimeters ideally will be a better surrogate for the target tissue dose.

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SESSION V: THE USE OF PHARMACOKINETIC MODELING IN RISK ASSESSMENT: CASE STUDIES

THE USE OF PHARMACOKINETIC ANALYSIS IN RISK ASSESSMENT - THE CASE OF BUTADIENE

Dale Hattis

Introduction

A trend to put more causal mechanism information into the mathematical models used for risk assessment has been gathering momentum for some time. This trend allows greater input from basic experimental science into the risk assessment/policy formulation process, and it can provide a framework for asking interesting and relevant questions within the framework for basic experimental science itself.

A philosophy of science issue results from this trend. Experimental scientists in Baconian tradition are reluctant to build elaborate mathematical models; more mathematical/statistical workers who have largely been in control of risk assessment procedures often do not have the detailed familiarity with causal mechanisms to feel comfortable building realistic mechanism-based representations of complex biological processes. In any event, doing so would complicate the use of their usual black-box curve-fitting approaches to analysis. I want to advance today what may be a startling proposition to the experimentalists among you - that by uncovering anomalies in the fit between data and theory, analysis can be as fruitful in producing new "knowledge," in some cases, as additional data-gathering activities and can properly be thought of as a complementary and synergistic enterprise in science.

The particular efforts I will discuss today take the form of using pharmacokinetic analysis to:

1. Reinterpret the dosages of active metabolites actually delivered in animals in the course of chronic 2-year bioassays.
2. Improve the projection of dosage across species using data on metabolism in humans, rats, and mice.

Models of this type will also be of importance for appropriately interpreting information on newer biological markers for steps in the carcinogenic process, such as DNA adduct formation (dynamic processes of formation and repair of adducts).

Overview of Lessons

Before getting immersed in the details of butadiene, I should foreshadow just what broad "lessons" I think you should draw from them:

1. Pharmacokinetic analysis is nobody's unambiguous quick-and-dirty cookie-cutter solution to the problem of uncertainty in carcinogenic risk analysis. Each of the models I have developed to date:

   a. Needed to undergo serious structural modification in the light of the data available for the specific case. The process of doing those modifications is a developing art, requiring liberal doses of judgment rather than cookbook formulas.

   b. Raised as many questions as it answered -- often revealing unsuspected sources of uncertainty, and nonobvious difficulties in fairly assessing the extent of the uncertainty.

2. As often as not, at the end of the day, the pharmacokinetic analysis doesn't make a major difference in the final numerical projection of risks (particularly with ethylene oxide). The exception is butadiene, where I got nearly an order-of-magnitude effect.

3. Nevertheless, in the long run, pharmacokinetic analysis can make it easier to ask "better"/more relevant experimental scientific questions. It can also help make risk assessment models somewhat better by incorporating realistic and experimentally testable information about the causal processes underlying both carcinogenesis and other adverse health effects.

The Framework for Modeling

The work reported here has a number of features that distinguish it from other current efforts to build pharmacokinetic analysis into quantitative risk assessments:

Our models are implemented in an easy-to-use Apple MacIntosh microcomputer-based systems dynamics modeling system (STELLA). Because the system makes extensive use of graphics to represent models and quickly display results, it is straightforward for use by people with little experience in programming, and it helps them understand the effects of changes in model structure and parameters.

Our human models incorporate a realistic diurnal pattern of change in breathing rates and blood flows to different tissues. Different assumptions can be built in for the level of activity during waking hours and the timing of exposure relative to activity and sleep.

Our tissue partition coefficients were determined by regression analysis of data from other compounds in relation to oil/air and water/air partition coefficients. Those oil/air and water air partition coefficients were estimated from butadiene's structure using the methods and data of Leo. Because the partition coefficients were estimated, it is particularly important to test the sensitivity of the model conclusions to reasonable alternative estimates.

We produce "best estimate" as well as "plausible upper limit" projections of human risk.

Butadiene

Past studies use net absorption of data of Bond et al. (1986) to determine "dose." PLEA TO EXPERIMENTALISTS: DO MORE THAN ONE TIME PERIOD OF INHALATION EXPOSURE FOR TOTAL DISPOSITION EXPERIMENTS.

Other Topics Discussed:

Implications of the work of Kreiling et al. (1986a,b) for model structure.

Comparison of model outputs with Bond and Kreiling experimental data, and methods for setting the adjustable parameters.

Revisions of delivered dose estimates:

- Humans: indicated five-fold less absorption than estimated by the U.S. Environmental Protection Agency (EPA) and ENVIRON.

- Animals: indicated two- to five-fold greater absorption and metabolism than estimated by EPA and ENVIRON.

Risk projections from different data sets, using different model variants (differing blood/air partition coefficients).

Model parameters that do not make much difference (less than 10% in ultimate geometric mean risk estimates).

Risk projections from experiments in rats and mice differ appreciably, even after correction for the initial step in butadiene metabolism (possible viral difference, inability to predict which will be the better model for humans).

Problems with low dose nonlinearity for the male rat data.

Comparison with the results of nonpharmacokinetic-based risk assessments (EPA and ENVIRON, Table 1).

Tentative resolution of the low-dose nonlinearity/background interaction problem.

The use of human epidemiological data.

- IISRP claims based on Matanoski data (humans 100- to 1,000-fold less sensitive than mice).

- "All Tumors" risk projection implicitly is assumed to be totally contained within the subcategory of nonleukemia lymphopoietic cancers.

-- Healthy worker effect.

-- Implicit assumption that in 18 years of follow-up, 18/50 of expected lifetime cancers will appear.

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TABLE 1

COMPARISON OF 95% UPPER CONFIDENCE LIMIT PROJECTIONS FROM OUR PHARMACOKINETIC-BASED ANALYSIS WITH THOSE DERIVED FROM EPA (1985) AND ENVIRON (1986) (45-YEAR, 8 HOUR/DAY, EXPOSURE TO 1 PPM)

DATA SET	EPA (1985)[a]	ENVIRON (1986)[b]	THIS STUDY (BEST ESTIMATE MODEL)
Male Rats	6.43E-4	7.98E-4	1.55E-4
Female Rats	0.00853	9.29E-4	0.00119
Male Mice	0.131	0.00644[c]	0.0325
Female Mice	0.0665	---	0.0243

[a]Derived from EPA's upper 95% q_1^* coefficients after correcting for 45-year, 8 hours/day, 5 days/week exposures.

[b]ENVIRON'S projections have been multiplied by (45/40) and (52/50) to place them on a comparable basis with our assumed 45-year, 52-week exposures.

[c]These calculations excluded malignant lymphomas. Some of the rest of the difference in this line may be accounted for by mg/kg interspecies projection, in contrast to $mg/kg^{2/3}$ used by ourselves and EPA (1985); and the use by ENVIRON (1986) of the Hartley-Sielken procedure for adjusting for less than lifetime exposure and observation.

SESSION V: THE USE OF PHARMACOKINETIC MODELING IN RISK ASSESSMENT: CASE STUDIES

CANCER RISK ASSESSMENT OF 1,3-BUTADIENE

Steven Bayard

The U.S. Environmental Protection Agency's (EPA) risk assessment of 1,3-butadiene was published in September, 1985 (U.S. EPA, 1985) and represents knowledge available on the mutagenicity and carcinogenicity up to that time. The information on the carcinogenicity of 1,3-butadiene includes positive cancer response studies in both rats and mice (Hazleton, 1981; National Toxicology Program, 1984). In the Hazleton study, groups of male and female Charles River (Sprague-Dawley) rats were exposed for 2 years, 6 hours/day, 5 days/week to 1,000 and 8,000 ppm 1,3-butadiene via inhalation. There was significantly decreased survival in both the male and female high dose groups. Increased tumors were observed in males in leydig cell, pancreatic exocrine, and zymbal glands, with increases being statistically significant only at the highest concentration and only for the two former sites. Female rats showed mainly increased mammary and uterine tumors, with to a lesser degree increased Zymbal gland and thyroid follicular cell tumors. In comparison to the response in rats, cancer response in both male and female $B6C3F_1$ mice, at comparatively lower concentrations of 625 and 1,250 ppm, was both massive and rapid. Survival in both sexes at both doses was affected such that the studies had to be terminated after 60 and 61 weeks. Early tumors, especially malignant lymphomas and hemangiosarcomas (mainly heart) were responsible for most of these deaths. Other statistically increased tumor sites included lung and forestomach (both sexes) and liver, mammary and ovarian glands (females).

Human evidence for the carcinogenicity of 1,3-butadiene at the time of EPA's publication was considered inadequate for classification, although excess cancers of the lymphatic and hematopoietic tissues were seen in some studies (Meinhardt et al., 1982; Matanoski et al., 1982; and McMichael et al., 1976). More recent information presented at a 1988 conference on 1,3-butadiene (NIEHS, 1988; see also Downs et al., 1987) provides additional confirmatory evidence that 1,3-butadiene exposure is associated with these cancers in humans, but accurate contemporary exposure estimates are lacking.

Using the above evidence of positive cancer response in two animal species and inadequate epidemiologic data, the EPA in 1985 classified 1,3-butadiene as a "probable" human carcinogen, Group B2 according to EPA's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1984; see also U.S. EPA, 1986).

An estimate of the carcinogenic potency of 1,3-butadiene to humans was based on the most sensitive animal sex-species. In this case, since the cancer responses in the male and female mice were very similar, both sets of results were used by taking the geometric mean of the 95% upper-limit estimates derived by the use of the linearized multistage model, using the computer program Global 82 (Howe and Crump, 1982).

To derive estimates of target tissue dose, the data from an unpublished study (NTP, 1985) on male mouse total body burden were used in EPA's risk assessment (U.S. EPA, 1985). However, following EPA's publication additional experiments and further analysis by NTP led to a publication of final results (Bond et al., 1986) which differ from those in the unpublished report. These final results are used for the body burden and absorption estimates in this risk assessment. Estimates of total body burden were used instead of estimates of target organ dose, because of the high number of affected sites and the different dose and time response characteristics of the different tumor sites. In estimating internal dose from external exposure, estimates of the low exposure retention of 1,3-butadiene and/or metabolites following 6 hour exposures were 20% over a two order-of-magnitude range of concentrations (up to 7 ppm). At the higher exposures of 70 and 930 ppm, retention decreased to 8% and 4% respectively. Since metabolic clearance of 1,3-butadiene in mice (and rats) follows linear pharmacokinetics below exposure concentrations of about 1,000 ppm (Kreiling et al., 1986), the decreases at high concentrations in micromoles body burden/ppm concentration after 6 hours exposure are assumed to result from decreased lung absorption rates. When body burden doses are adjusted for this decreased absorption at higher atmospheric concentrations, potency estimates in the female rat and female mouse are within a factor of 3 of each other, but estimates based on the male mouse are still between one and two orders of magnitude greater than those based on the male rat.

When the linearized multistage model is used to extrapolate from the cancer response in the National Toxicology Program (NTP) mouse bioassay, a correction factor for early termination, and the estimates of body burden based on the data of (Bond et al., 1986), the upper-limit estimate of carcinogenic potency for humans (assuming a 70-year continuous exposure) is 0.25 per ppm. This finding is very close to the potency estimate based on the analysis by Hattis, also presented at this workshop (Hattis, 1988). Hattis' estimate of 0.20 per ppm reflects a decrease in risk due to his higher body burden estimates than those of EPA's. This is partially offset, however, by his final selection of the male mouse response and by his adding of risks for each tumor site separately compared with EPA's risk, which is based on total number of animals with at least one of the increased tumor types. Other factors used in the two risk assessment are similar.

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SESSION V: THE USE OF PHARMACOKINETIC MODELING IN RISK ASSESSMENT: CASE STUDIES

METHYLENE CHLORIDE MODEL - INHALATION DATA

Richard Reitz

Methylene chloride (dichloromethane, CH_2Cl_2) is a low-molecular-weight volatile solvent with slight solubility in water. It is widely used in industrial processes, consumer products, and food processing. As a consequence, the toxicity of CH_2Cl_2 has been widely studied. In general, this chemical has a low order of subchronic toxicity, but recent reports have shown that chronic exposure to vapors of CH_2Cl_2 is associated with increased incidences of lung and liver tumors in mice (NTP, 1985). The obvious question raised by these new findings is whether humans exposed to CH_2Cl_2 are likely to develop the same kinds of tumors seen in mice. While there are many biological factors to consider in answering this question, one of the primary considerations involves differences in the delivery of the ultimate toxicant (a metabolite of CH_2Cl_2) to the target tissue of different species under different exposure paradigms. This subject has been the focus of a recent workshop conducted by the National Academy of Sciences (NAS, 1987).

This paper reviews the development and validation of a physiologically based pharmacokinetic (PBPK) model capable of providing a quantitative description of metabolite production in mice, rats, hamsters, and humans. A detailed description of the model development and validation may be found in the work of Andersen et al. (1987). Refinement of the model by inclusion of in vitro enzyme studies with human and animal tissues has been described by Reitz et al. (1988a), and application of the model to quantitative cancer risk assessments (which form the major basis of this case example) have been described by Reitz et al. (1988b).

Incorporation of "internal dose" data from the PBPK model reduced quantitative estimations of human risk by about two orders of magnitude. A portion of this reduction in the estimated risk arises from the shift in metabolic pathways (from oxidation to conjugation) as doses of CH_2Cl_2 are increased, and the remainder arises from the different levels of activating enzymes present in the different species.

Use of PBPK models in the future would be strengthened by collection of pharmacokinetic data in chronically exposed animals, studies in animals of various ages, and development of in vitro systems for estimating metabolic capacity in humans. Development of systems for estimating metabolic activity in

humans may be the most important of these three areas, since it is likely that this information will be lacking in most cases. Many uncertainties remain in the risk assessment process, but analyses that properly consider the role of physiology and pharmacokinetics should be considerably more reliable than those that do not.

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SESSION V: THE USE OF PHARMACOKINETIC MODELING IN RISK ASSESSMENT: CASE STUDIES

METHYLENE CHLORIDE MODEL - INGESTION DATA

Michael J. Angelo

The presentation will be introduced with an historical perspective reviewing the 2-year bioassays performed by the National Toxicology Program (NTP), in which rats and mice received daily oral gavage administrations of methylene chloride in a corn oil carrier, and the 2-year bioassay sponsored by the National Coffee Association (NCA), in which rats and mice were exposed to methylene chloride ad libitum in drinking water.

NCA believed the drinking-water administration to be a more relevant exposure scenario, but questions arose as to the manner in which administered dose levels were to be compared between a bolus "mg/kg" dose and a "mg/kg/day" drinking-water dose, which was consumed gradually over a 24-hour period.

Pharmacokinetic information on methylene chloride was believed to be very important in understanding the different internal disposition patterns that resulted from these two dosing protocols. Limited pharmacokinetic data did exist early in these studies, but not enough were available to quantify the "delivered" doses to suspected sites or metabolic mechanisms of toxicity.

General Foods decided to contribute to the methylene chloride safety assessment effort by contracting with the Huntingdon Research Centre to collect pharmacokinetic data that would be used to understand tissue distribution and metabolism of methylene chloride as a function of several dosing factors such as dose level, route of exposure, dosing vehicle, and frequency of administration. With this information, a physiologically based pharmacokinetic model was to be constructed that would aid in interpreting the different disposition phenomena, quantify "delivered" doses vs. administered doses, and provide a mechanism for simulating the pharmacokinetics resulting from different exposure patterns in studies which would otherwise be too complicated or costly to perform.

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SESSION V: THE USE OF PHARMACOKINETIC MODELING IN RISK ASSESSMENT: CASE STUDIES

THE IMPACT OF PHARMACOKINETICS ON THE RISK ASSESSMENT OF DICHLOROMETHANE

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Although it was not the U.S. Environmental Protection Agency's (EPA) first use of pharmacokinetic data and physiologically based pharmacokinetic models (PBPK) for risk assessment purposes, dichloromethane is certainly the most 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, it 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. Rather, we 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 that 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 nonlinearities 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 nonlinearities 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 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 that must be extrapolated from in vitro to in vivo conditions and those that 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 that 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 be accurate only 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 that 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 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 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 are often lacking. This leads to either expensive and time-consuming laboratory experiments or to models that 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 endpoint of any exposure assessment, but in reality it 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 that 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 modeling 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. The way 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 (U.S. EPA, 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 glutathione-S-transferase (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 promoter 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 nonlinearities in the dose-response relationship do not arise solely from pharmacokinetic nonlinearities.

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-a-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 2,000 times the volume, and presumably 2,000 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, we have examined 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 when the pharmacokinetics turn 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 it 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 when 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 nonpulmonary 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-Anderson pharmacokinetic model show that at similar air concentrations, humans and mice do 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 because 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 dose to low dose component and not to species differences in metabolism, although for other compounds this could be different.

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APPENDIX C

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WORKSHOP ON BIOLOGICAL DATA FOR PHARMACOKINETIC MODELING AND RISK ASSESSMENT

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