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BIOLOGICALLY BASED PHARMACODYNAMIC MODELS: Tools for Toxicological Research and Risk Assessment

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INTRODUCTION

In broad terms, pharmacodynamics is the study of the time course and intensity of biological responses arising from exposure to or treatment with particular chemicals. These compounds may have beneficial, therapeutic effects (drugs) or noxious, deleterious effects (toxic chemicals). The emphasis in this article is on the pharmacodynamics of toxic chemicals. Pharmacodynamic models attempt to quantitatively describe the relationship between exposure of the organism and the time course of the response. The models may be (*a*) correlational, (*b*) based on empirical equations whose parameter values are fit to data or, (*c*) based on knowledge of the biology of the test species, the interactions of chemical with tissues in the test species, and the response of the tissues to the interaction with chemical. These latter descriptions are termed biologically based pharmacodynamic (BB-PD) models. There are relatively few formal BB-PD models validated for tissue responses to particular drugs/toxic chemicals and there is no intention in this review to catalog the various models attempted to date. Instead, this review traces the development of strategies for creating BB-PD models, examines the

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interrelationships of these models with physiologically based pharmacokinetic models for chemical disposition, and discusses the perceived future development of this field, especially as it relates to toxicology and quantitative risk assessment for chemical carcinogens.

EMPIRICAL MODELS

Many empirical pharmacodynamic (PD) models have been developed to describe the often complex relationship between blood concentration of a drug and its therapeutic effect (for reviews see 1–3). In these PD models polyexponential equations are first used to create a multicompartment pharmacokinetic (PK) submodel. A "link" equation then describes the amount of drug in an "effect" compartment as a function of drug in the central compartment of the PK submodel. An "effect model" in turn defines the therapeutic effect as a function of drug in the effect compartment. These PD models are empirical in the sense that their mathematical structure is selected to be consistent with the PK and effect data being modeled. The purpose of an empirical PD model is to provide a concise, mathematical description of the relationship between dose of drug and therapeutic effect. For example, Sheiner et al (4) developed an empirical PD model for paralysis by d-tubocurarine, a competitive binding antagonist of acetylcholine. In this model, the rate of change of drug in the effect compartment was defined by the following link equation:

$$dA_e/dt = k_{1e}A_1 - k_{e0}A_e \qquad 1.$$

A_e is the amount of drug in the effect compartment; A_1 is the amount of drug in the central (blood) compartment of the pharmacokinetic model; k_{1e} and k_{e0} are first-order compartmental transfer constants. The effect model, relating paralysis to drug in the effect compartment, was given by:

$$E = \frac{(A_e)^g}{(A_e)^g + A_e(50)^g} \qquad 2.$$

E is the intensity of paralysis as a fraction of the maximal effect, $A_e(50)$ is the drug concentration in the effect compartment at 50% effect, and g is a parameter allowing sigmoidicity in the relationship between A_e and the pharmacological effect. The parameter values of Equations 1 and 2 were estimated by fitting the model to time course data on both blood concentration and biological (therapeutic) response (Figure 1).

As noted above, empirical PD models are developed to provide convenient mathematical descriptions of dose-response relationships previously mea-

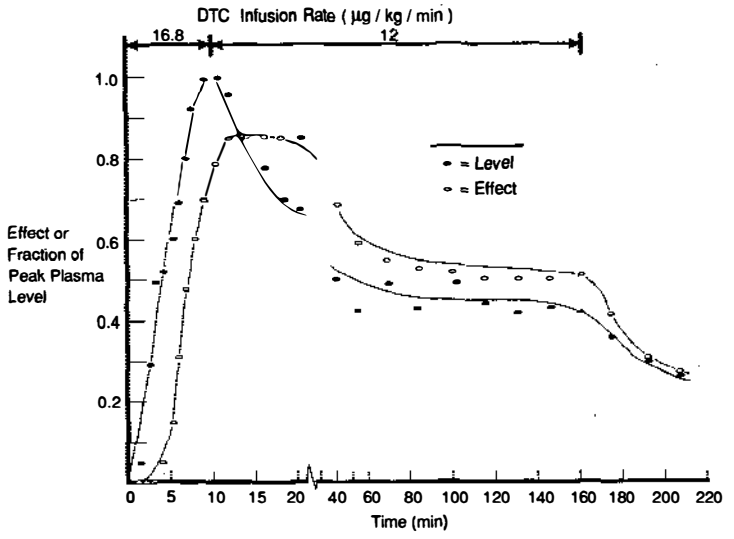

Figure 1 Plasma concentration (level) of d-tubocurarine (DTC) and effect (paralysis) relationship vs time for a normal parient. The data depicted by ● and ○ are actual data points. The plasma level is normalized to the peak concentration actually observed. The solid line represents the best fit of the model to the data. The patient received an infusion of 16.8 µg/kg/min for 10 min followed by 1.2 µg/kg/min for 150 min. Note break in graph at 20 to 30 min due to change of scale on time axis. (Reproduced from (4) with permission by C. V. Mosby Co.)

sured in the laboratory. Although the sites where d-tubocurarine interacts with the acetylcholine receptor could be described in biologically realistic terms, in the empirical approach the effect compartment is hypothetical. It primarily allows for a delay, or hysteresis, between the response and blood time course of drug. Similarly, the main criterion in selecting the mathematical structure of the effect model is consistency with the PK and PD data. The goals in construction of biologically based models, described below, are quite different.

BIOLOGICALLY BASED MODELS

In developing biologically based models, known steps involved in the delivery of chemical to target tissues, in its interactions with tissues, and in the response of the tissue are described to the extent *necessary*. There is a rapidly growing literature in the currently most active area of biologically based model development–physiologically based pharmacokinetic models (PB-PK; 5–9). Instead of having empirical compartments inferred from fitting a model to data, in PB-PK models compartments represent the physical structure of the organism and are defined realistically with respect to tissues, blood flows,

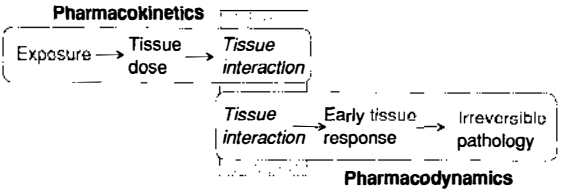

Figure 2 Overlap between pharmacokinetics and pharmacodynamics when using a biologically based description. The first step in a pharmacodynamic sequence, e.g. binding to a receptor or enzyme, is also a determinant of pharmacokinetic behavior.

reaction pathways, tissue solubilities of toxicant, and specific binding proteins.

Models With Receptor Binding

METHOTREXATE To accurately describe pharmacokinetic behavior, PB-PK models often define interactions that are crucial to the ultimate toxic/therapeutic response and that might easily be considered part of pharmacodynamics rather than pharmacokinetics. The now classic PB-PK model for methotrexate (MTX), an antineoplastic agent which acts by inhibiting the enzyme dihydrofolate reductase (DHFR), described binding of MTX to DHFR in target tissues for MTX toxicity (10). In contrast to empirical models then, this model for MTX described actual target tissue sites for the binding of MTX to DHFR. This was needed to obtain an accurate description of MTX concentration in the target tissues because total MTX concentration in a tissue reflects both its free and bound forms. MTX binding to DHFR is also an important factor in MTX-mediated cytotoxicity and, as such, is part of the PD sequence linking the tissue dose of MTX to its ultimate therapeutic effect. This illustrates the difficulty in clearly distinguishing between kinetics and dynamics that ensues from developing a biologically based description. With empirical PD models, the difference between the PK and PD components of the model is clear and conceptually useful. In the case of the PB-PK model for MTX, binding to an enzyme, part of its pharmacodynamics, is also an important determinant of its pharmacokinetic behavior and no absolute demarcation between the two is possible (Figure 2).

With MTX, sites of action (effect compartments) were realistically defined, but the biological details of the model linking DHFR binding to cytotoxicity were not. More extensive descriptions of a biologically based model for MTX toxicity have been reported in which polyglutamination of MTX plays an important role in maintaining intracellular MTX concentrations, thus enhancing its therapeutic, cytotoxic action (11).

ORGANOPHOSPHATES A biologically based approach has also recently been pursued in developing PK models for acetylcholinesterase (AChE) inhibition by soman (12) and by diisopropylfluorophosphate (DFP; 13). With DFP the PB-PK model accounts for tissue solubility of the organophosphate (OP), its hydrolysis by nonspecific esterases, and its reaction rate constants with AChE in various tissues. The basal rates of AChE synthesis and degradation are also described to account for normal AChE activity and for the time course of recovery of activity after OP-mediated AChE inhibition.

Using the BB model for DFP, Gearhart et al (13) simulated pharmacokinetics and AChE inhibition in mice and rats after single or multiple doses and by different routes of administration (Figure 3). The model accurately simulated plasma DFP concentrations in male Dublin ICR mice shortly after single tail vein injections (Figure 3a) and brain AChE activity in male Wistar rats during repeated subcutaneous injections (Figure 3c). However, actual plasma DFP concentrations in mice at 15 and 30 minutes after injection (Figure 3b) were higher than predicted and rat brain AChE activity returned to normal levels after cessation of dosing more slowly than predicted (Figure 3d). In the empirical approach models are fit to data with very few constraints. The fitting process is not necessarily intended to improve the mechanistic understanding of the animal-toxicant system. With biologically based models, on the other hand, discrepancies between real data and simulations are cause to revaluate our understanding of the biological structure represented by the mathematical description in the model. It is also possible that the discrepancy arises not from an error in model structure but, rather, from the data being analyzed. For the mouse, the discrepancy between data on concentrations of DFP in blood and simulated concentrations is probably due to the assumption that all extractable tritium radiolabel is DFP. Radioactivity measured at longer times is presumably not associated with DFP because DFP is rapidly degraded in vivo. With rat brain cholinesterase, there is no good explanation at present for the less rapid return to basal AChE activity—the model is incomplete and brain AChE activities are affected by factors other than those included in the version of the model described here. This example illustrates how working with a biologically based model helps to identify specific problems in need of further research.

In addition to the role that biologically based models play in testing our understanding of the animal-chemical system, they are also useful for predictive extrapolation of PK and PD behaviors to situations that have not been, and often cannot be, tested experimentally. Common extrapolations in toxicology are from high doses to low and from experimental species to people. In animals and people, PK and PD behaviors at both high and low doses are determined by the biochemistry of the drug or toxicant and the biological structure of the organism. If the *relevant* biological structure and

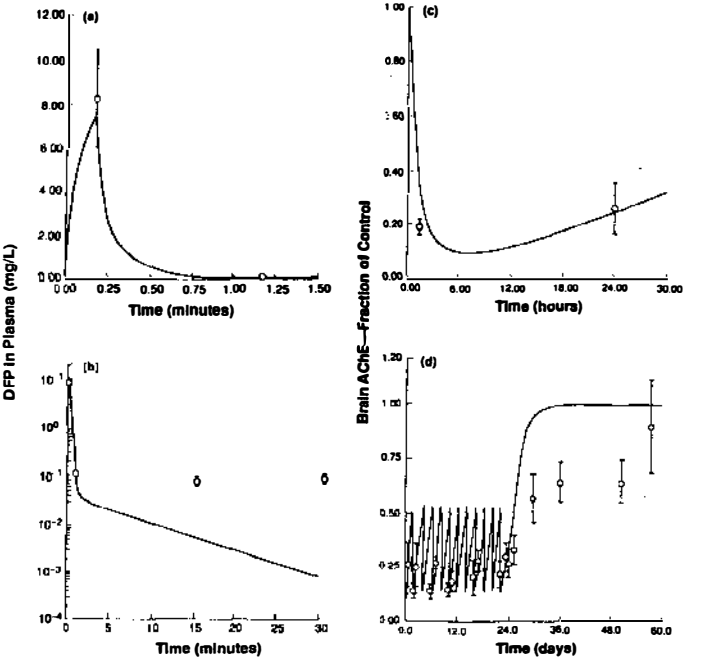

Figure 3 Figures 3a and 3b show the free DFP concentration (mg/L) in plasma of male, Dublin ICR mice after tail vein injection of 1 mg DFP/kg. Figures 3c and 3d show brain AChE activity in male Wistar rats injected subcutaneously with DFP (first dose of 1.1 mg DFP/kg, then 0.7 mg/kg every other day for 22 days). AChE activity data are percent of control. Solid line depicts computer simulation. Each data point represents mean of 5 or 6 animals. (Reproduced from (13) with permission by Academic Press, Inc.)

chemical-specific parameters are correctly described, then a biologically based model will accurately simulate PK and PD behavior over a range of doses. Similarly, species differences in PK or PD behaviors for a given chemical are due to quantitative and/or qualitative species differences in biological structure. To the extent that these differences are adequately described in a biologically based model, the model will faithfully simulate actual interspecies differences in PK and PD. In other words, biologically based models can be powerful tools for extrapolation, but they are only as good as the investigator's understanding of the biological processes shaping PK and PD behaviors in the species of interest and ability to measure pertinent parameter values.

Scaling biologically based models from animals to people requires estimating values of human parameters. Critical parameters of both the MTX and OP models described above could be measured in human tissues in vitro. For MTX this would include the MTX binding constant to DHFR and, for

OP-mediated AChE inhibition, the rates of OP hydrolysis by nontarget esterases and the interaction of the OP with AChE. When parameter values are measured in vitro the relationship between in vitro and in vivo values must be considered. A current approach is to measure in vitro/in vivo ratios of parameter values in animal tissues, use human tissue in vitro, and then apply the in vitro/in vivo ratio from the animal to estimate the human in vivo value. This procedure was used by Reitz et al (14) in a PB-PK model for methylene chloride to scale the description of metabolism from rodents to humans. While this type of empirical in vitro/in vivo ratio may be useful, in the long run it will be desirable to develop a more mechanistic understanding of factors controlling in vitro-in vivo differences.

While the models for soman and DFP do not yet simulate toxic effects of these OPs, they describe a biological effect, AChE inhibition in target tissue, that is believed to be causally associated with toxicity (15). These first generation biologically based response models will, with further development, be extended to predict acute toxic effects and associated chronic OP toxicity in animals and humans for realistic exposure scenarios. More complete models will need to incorporate a biologically based description of the actual processes linking AChE inhibition to clinical OP toxicity. Correlational or empirical linkages could be used. Biologically based descriptions will be more difficult to construct but they will ultimately be more useful in improving our understanding of the mechanisms of OP toxicity and human health risks associated with OP exposure.

Models With Induction of Protein Synthesis

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (DIOXIN) Although the mechanisms of dioxin toxicity and carcinogenicity are not understood, they are thought to involve effects on gene regulation, induction of the synthesis of various proteins, and alterations of cell growth and differentiation. In recent years a PB-PK model has been developed for dioxin. The steps taken in developing this model illuminate, once again, the value of a quantitative description of a biologically realistic structure in improving our understanding of toxicant effects in experimental animals (and people) and the close intertwining of kinetics and dynamics that becomes evident in this approach.

Dioxin is highly lipophilic, yet it is usually found at higher concentrations in liver than in fat of exposed animals (16). Initial kinetic models of similar lipophilic, hydrophobic chemicals attributed this behavior simply to their intrinsic solubilities in these tissues (17). In developing a PB-PK model for dioxin, Leung et al (18) attributed elevated hepatic concentrations relative to fat to the presence of specific hepatic binding sites for dioxin. Selected parameters of the PB-PK model were optimized to estimate the binding affinity and capacity of these sites, predictions that were then substantiated by

direct experimentation with an analogy of dioxin, 2-iodo-3,7,8-trichlorodibenzo-p-dioxin (19, 20). This experimental work also showed that the hepatic binding capacity for dioxin was induced by dioxin pretreatment.

The PB-PK model for dioxin was then refined to more accurately account for the tissue time course of dioxin concentration by describing induction of the hepatic dioxin binding protein (probably cytochrome P-450d; 20, 21). This description of enzyme induction is extremely parsimonious. Dioxin binds to a cytosolic protein, the Ah receptor, and the dioxin-Ah complex is assumed to regulate gene transcription. The binding affinity of dioxin for the Ah receptor was estimated by computer optimization to be about 15 picomolar, which is consistent with studies by Bradfield et al (22). An instantaneous, linear relationship was described in the model between fractional occupancy of the Ah receptor by dioxin and the level of binding protein induction.

Dioxin is very persistent in rodents, which is not surprising given its lipophilicity, very tight binding to the Ah receptor, and induction of the specific hepatic binding protein. The assumption in the model of an instantaneous linkage of binding protein induction and receptor occupancy, while not biologically realistic, probably works because the system is observed on a time scale of many days to weeks, not the hours to a few days needed for enzyme induction. Future development of the model will involve explicit description of gene regulation and RNA transcription and translation that, in the current version, are subsumed in the empirical linkage between Ah receptor occupancy and the level of protein induction. This increased biological realism will provide a basis for investigating the scaling of this PB-PK model across species.

PREDNISOLONE With dioxin the PB-PK model is fairly extensive but the biological details of protein induction are not specified. Another approach has been used by Jusko and colleagues (23) to examine induction of the enzyme tyrosine aminotransferase (TAT) by the synthetic glucocorticoid prednisolone. Blood concentrations of prednisolone were represented by a fit of an empirical PK model to time course data and free prednisolone in liver was equated to total plasma prednisolone. In the liver, prednisolone binding to the cytosolic glucocorticoid receptor was specifically described with association and dissociation rate constants and the translocation of the prednisolone-receptor complex to the nucleus was also specified. Time course studies included direct measurements of receptor concentration in the cytosol. An empirical linkage was used to connect the nuclear accumulation of prednisolone-receptor complex (i.e. its disappearance from cytosol) with induction of TAT. This linkage contains a series of time delays that allow for transcription of RNA and its translation into protein, though these biological steps are not actually described.

Unlike the dioxin model, this model for prednisolone does not include a biologically based PK model. It does contain more realistic descriptions than the dioxin model of, for example, ligand-receptor translocation to the nucleus and of the linkage of induction to the nuclear concentration of the ligand-receptor complex. Taken together, the dioxin and prednisolone models illustrate the process of developing quantitative, biologically realistic descriptions of many of the events connecting exposure of the organism and distribution of a drug or toxic chemical to target tissue with regulation of gene expression leading to induction of a protein in the target tissue. These efforts lay the groundwork for future models in which realistic descriptions of pharmacokinetics, target tissue dosimetry, and gene regulation will be joined with biologically based descriptions of subsequent events leading to frankly toxic (or therapeutic) effects.

Halogenated Hydrocarbon Cytotoxicity

1,1-DICHLOROETHYLENE(1,1-DCE) PB-PK models have been successfully developed for a wide range of volatile halogenated hydrocarbons. With certain of these materials, metabolism of the parent chemical produces intermediates that are much more toxic than the parent chemical itself. 1,1-DCE is metabolized via oxirane intermediates to chloracetylchloride, which is either detoxified by reaction with glutathione (GSH) or reacts with cellular constituents leading to hepatic cytolethality, increased plasma liver enzyme levels, and death of the animal if the liver damage is sufficiently extensive (24–26). D'Souza & Andersen (27) developed a model for 1,1-DCE toxicity in which lethality following a specific exposure regimen was correlated with the amount of metabolized 1,1-DCE that escaped conjugation with GSH. This effort required development of a quantitative model for the control of hepatic GSH levels in these rats and linking the kinetic models for 1,1-DCE metabolism and GSH together into a unified model of 1,1-DCE toxicity. While this approach uncovered a consistent correlation between the amount of non-GSH reacting metabolite and lethality, it did not specify how the explicit cellular processes of damage and repair interact in the eventual development of frank hepatotoxicity. It would only be possible to generalize the correlation between metabolism and lethality, i.e. to extrapolate it across doses and species, if the biological linkage between metabolite production and eventual frank toxicity was explicitly described in the model and if the interspecies scaling of its individual components were understood. These areas are natural subjects for future investigations. Again, we see that the power of the biologically based model is simply a reflection of how well the investigator understands the system of interest at a mechanistic level. The model facilitates better understanding by serving to integrate knowledge of the system in a formal, quantitative manner and by serving to sharply define areas of continuing ignorance.

CHLOROFORM With 1,1-DCE the biologically based model was developed to test a pharmacodynamic hypothesis: Does toxicity correlate with the specific amount metabolized via specific pathways in a particular exposure situation? A more comprehensive approach to cytotoxicity has been attempted with chloroform. In addition to its cytotoxicity, chloroform is carcinogenic in male and female B6C3F1 mouse liver and male Osborne-Mendel rat kidney after chronic dosing by corn oil gavage (28, 29). However, exposure of these same animals to chloroform in drinking water, at concentrations giving doses equivalent to the oil gavage studies, is not carcinogenic (30). Although it is not appreciably genotoxic (31), tumorigenic doses of chloroform do tend to be hepatotoxic (28). This observation is consistent with a growing body of evidence indicating that chemicals that stimulate cell proliferation may be carcinogenic without direct DNA reactivity (32–34).

A biologically based model for chloroform cytotoxicity was developed to improve our understanding of how physiological differences between mice and rats, and kinetic differences associated with oral gavage and drinking water dosing, contributed to the different outcomes of the various bioassays. The working hypothesis used was that the hepatocarcinogenicity of chloroform is a direct consequence of its cytolethality. The model described both the pharmacokinetics of chloroform and an empirical linkage between the hepatic metabolism of chloroform and cell killing (hepatotoxicity). Stimulation of cell replication was assumed to reflect a restorative process after cytolethality.

Corley et al (35) constructed a PB-PK model for chloroform to simulate pharmacokinetic data in mice and rats. Two kinds of tissue response data were also obtained in male B6C3F1 mice (36). Chloroform-stimulated cell replication was measured after inhalation exposure and hepatocyte death was quantitated histologically after corn oil gavage. Development of the cytotoxicity model involved specification of an appropriate measure of chloroform dose to the liver and identification of an empirical relationship between this measure of tissue dose and cell killing.

Chloroform-mediated cell killing did not correlate with the total amount of its hepatic metabolism per unit liver weight. Similar amounts of chloroform were metabolized by mice after drinking water exposure and corn oil gavage, despite the different tumor outcomes. The amount of metabolism after gavage was also similar in mice, a sensitive species, and rats, a nonsensitive species. Instead, cell killing correlated more closely with the instantaneous rate of chloroform metabolism per unit liver weight (36). At the bioassay doses, corn oil gavage results in peak rates of chloroform metabolism several times greater than those achieved by drinking water exposure. Similarly, the peak rate of metabolism after corn oil gavage in the B6C3F1 mouse is significantly greater than in the Osborne-Mendel rat (Figure 4). This latter observation

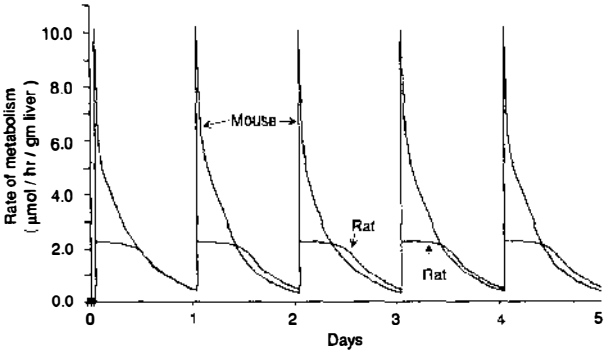

Figure 4 Simulation of rate of metabolism when male B6C3F1 mice and male Osborne-Mendel rats are gavaged with 300 and 180 mg/kg in corn oil, respectively, once a day for 5 days. (From a modification by RBC of models described in (35) and (36)).

reflects the larger V_{max} per unit tissue weight for chloroform in the mouse (35). Correlation of toxicity with rate of metabolism is also consistent with the chemistry of phosgene and hydrochloric acid, the presumed major oxidative metabolites of chloroform (37). Phosgene is expected to produce mostly labile macromolecular carbamyl adducts. The cellular adduct burden should correlate more closely with the instantaneous rate of chloroform metabolism than with the total amount metabolized over a period of hours or days. Similarly, the time course of any disturbance of cellular pH by hydrochloric acid should be more closely linked to the instantaneous rate of chloroform metabolism.

This work with chloroform illustrates the process of development of a biologically based model to test a proposed mechanism of tissue response. This use of a model is reminiscent of the classical approach used in the discipline of chemical kinetics in which time course studies are examined in an attempt to resolve the detailed molecular mechanism(s) of chemical reactions. As with chemical kinetics, it should be stressed that kinetic studies never by themselves prove that a particular mechanism is correct. They show that a mechanism is *consistent* with observation. Other chemical or biological evidence then must be gathered to bolster the case for a particular mechanism. With chloroform, defining the rate of metabolism as the appropriate dose surrogate to link with cytolethality is consistent with both the cytotoxicity and the bioassay data, whereas other measures of dose, such as total amount metabolized, are not. Use of the rate of metabolism surrogate is in turn consistent with the expected chemistry of phosgene and hydrochloric acid in the cell. As with 1,1-DCE, no account has yet been made for explicit inclusion of repair processes in the response model for chloroform.

The chloroform model, developed to the point of identifying the relation-

ship between rate of chloroform metabolism and cytotoxicity, is a description of the biological processes that the investigator thinks determine the hepatotoxicity of chloroform and that is consistent with the available data. As such, the model actually represents a quantitative hypothesis about the mechanism of chloroform cytotoxicity. In addition, the model serves as a conceptual framework that helps to identify additional experiments needed to confirm or reject the hypothesis. For example, future experiments might attempt to identify transient macromolecular adducts and changes in intracellular pH associated with chloroform metabolism. Simulations generated with the model could be used to guide the design of some aspects of such experiments. The data obtained from these experiments would either serve to support the hypothesis (model) or identify the need for its revision.

The mouse-rat difference in susceptibility to chloroform hepatocarcinogenicity (29) parallels the susceptibility to chloroform hepatotoxicity (28). While hepatotoxicity may be a critical determinant of this carcinogenicity, a sequence of subsequent molecular and cellular events links the hepatotoxicity with development of tumors. Cytotoxicity and this sequence of cellular events together determine the shape of the dose response curve for chloroform hepatocarcinogenesis. An understanding of species differences in susceptibility to chloroform hepatotoxicity is, therefore, only a partial explanation of species differences in dose response curves for chloroform hepatocarcinogenicity. A fuller understanding requires a quantitative, biologically based description linking cytolethal events with cell cycle dynamics and tumor formation and, finally, knowledge of how this description scales from animals to people.

Towards Biologically Realistic Models for Chemical Carcinogenesis

The recognition that carcinogens such as 2,3,7,8-tetrachlorodibenzo-p-dioxin, saccharin, and chloroform do not exert their primary effects on DNA (32–34) underscores the need for incorporation of mechanistic information into carcinogen risk assessments. A biologically based tissue response model for chemical carcinogenesis, relating carcinogen exposure to tumor formation, would be useful for examination of how pharmacokinetic behavior and different biochemical mechanisms of carcinogen action (i.e. DNA reactive, mitogenic, cytotoxic) influence the shape of the dose-tumor response curve. Such knowledge could, in turn, be used in carcinogen risk assessment to improve the estimation of safe exposure levels.

METHYLENE CHLORIDE, ETHYLENE DICHLORIDE The carcinogenicity of some halogenated hydrocarbons has been associated with their metabolism to reactive metabolites. With two of them, methylene chloride and ethylene

dichloride, biologically based models for pharmacokinetics and target tissue metabolism were developed and used to estimate the amounts of parent compound metabolized via specific pathways (7, 38). In each case, metabolism is either by oxidation or by conjugation with GSH with metabolites in the GSH pathway believed to be involved in the processes leading to tumor development. For these cancer models, this target-tissue dose surrogate (i.e. the daily amount of parent chemical metabolized via the GSH pathway to the putative ultimately toxic metabolite at the site of tumor formation) was linked to a linearized, multistage (LMS) cancer model defining the shape of the dose-tumor response curve. These combined dosimetry-effect models were used to predict expected tumor outcome for low-dose rodent and human exposure situations. The LMS response model is theoretical and not well supported by the current understanding of chemical carcinogenesis for either DNA reactive or other classes of carcinogens. Its use for interspecies and high dose-low dose extrapolation is based more on federal regulatory policy than on the accuracy of its representation of critical events in chemical carcinogenesis. The challenge, in future refinement of models like these, is to develop mechanistic descriptions of the effects of toxic chemicals on the parameters related to stochastic behaviors of individual cells, i.e. the manner in which cell birth and death rates change with chemical treatment and the manner in which chemical exposure changes the probability of procarcinogenic mutation per cell division (mutation frequency). It would then be possible to combine biologically based models for the PK and PD behavior of chemicals like methylene chloride and ethylene dichloride with realistic, biologically based descriptions of the processes by which normal cells become malignant.

A PROPOSED COMPREHENSIVE, BIOLOGICALLY BASED MODEL FOR CHEMICAL CARCINOGENESIS Conolly et al (39–41) described a comprehensive, biologically based model for chemical carcinogenesis comprised of three individual elements: (*a*) pharmacokinetics, (*b*) mechanism of action, and (*c*) carcinogenesis. A PB-PK submodel defines the target-tissue dose of the carcinogen. A biologically based submodel defines the mechanism of action that links the dose of carcinogen at its site of action with its particular effect on the processes by which normal cells become malignant. Generic linking mechanisms were described for DNA reactive, cytotoxic, and mitogenic carcinogens. These generic links can be modified as necessary to be consistent with actual mechanisms of action of specific carcinogens. The cancer submodel, called the MVK model due to its initial description by Moolgavkar, Venson, and Knudson (42–44), specifies that two critical mutations are required for malignancy and defines the roles of target cell birth and death processes in the accumulation of these mutations (Figure 5).

At present, detailed, quantitative knowledge of biochemical mechanisms of

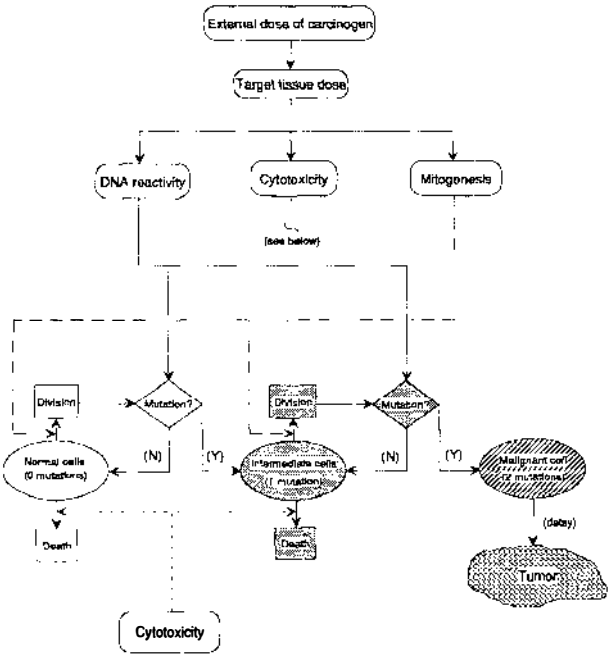

Figure 5 Comprehensive, quantitative model for chemical carcinogenesis. A physiologically based pharmacokinetic submodel translates external dose of carcinogen into dose in a target tissue. Biologically based submodels (DNA reactive, cytotoxic, mitogenic) describe sequences of events linking target tissue dose of carcinogen with the parameters of the cancer submodel. The cancer submodel defines the roles of cell division, death, and mutation in the development of malignant cells and tumors. Note that the different linking submodels each affect different parameters of the cancer submodel. (Modification by RBC of chemical carcinogenesis models described in (39–41))

action at the cellular and molecular levels is the weakest link in the PK-PD-tumor development sequence that comprises this biologically based model of chemical carcinogenesis. In contrast to the lack of biologically detailed information available for mechanisms of action, both PB-PK models and the MVK model are biologically well-defined, though they will also, like descriptions of mechanisms of action, continue to evolve as knowledge increases. A better understanding of mechanisms of action is clearly a pressing research need as this determines how carcinogenic chemicals affect the birth, death, and mutation frequency parameters of the carcinogenesis submodel (Figure 5).

The MVK model readily accommodates nongenotoxic mechanisms of carcinogen action by specifying how cytotoxicants and promoters can accelerate the process of mutation accumulation and malignant tumor formation

without direct interaction with DNA (Figure 5). This is accomplished as follows: Mitogens increase rates of normal and intermediate cell division, though the two cell types are not necessarily affected equally. Cytotoxicants also increase division rates but do so indirectly. Cytotoxicant-induced cytolethality leads to an increase in the division rate of surviving cells to replace lost tissue mass. As with mitogens, normal and intermediate cells do not necessarily have to be equally sensitive to cytotoxicity. For both mitogens and cytotoxicants, an increase in the rate of mutation accumulation, and eventually of tumor formation, will result as long as the probability of procarcinogenic mutation per cell division is greater than zero. The roles of DNA repair in decreasing the rate of procarcinogenic mutation per cell division and of immune surveillance in removing genetically altered cells are not explicitly described in the model, nor is the loss of newly created malignant cells simply because they happen to die before their first division. Instead, the rates of mutation accumulation and tumor formation in the MVK model should be thought of as the *effective* rates in the presence of these protective processes.

Reitz et al (36) used the MVK model implicitly in conducting a biologically based risk assessment for chloroform hepatocarcinogenicity. They used a safety factor approach to estimate human carcinogenic risk. This approach was justified by two assumptions. First, that chronic regenerative cellular proliferation secondary to cytotoxicity was the driving force for tumor development in the chloroform bioassays. This is consistent with the cytotoxic potency of chloroform, the role of cytotoxicity in the MVK model for cancer, and presence of a dose threshold for chloroform cytotoxicity (which is equivalent to saying that more than one molecule of chloroform is needed to kill an hepatocyte). The second assumption was that other mechanisms of carcinogen action such as DNA reactivity, or mitogenesis unrelated to cytotoxicity, are not operative with chloroform. If chloroform did have such effects in addition to its cytotoxicity, the assumption of a dose threshold for its carcinogenicity would be difficult to justify, given the current understanding of the dose-response behavior of these other mechanisms.

CARCINOGENS WITH MULTIPLE MECHANISMS OF ACTION Unlike the assumption used in the risk assessment for chloroform described above, many chemicals do act by multiple mechanisms of action. Diethylnitrosamine is both mutagenic and cytotoxic (45) and formaldehyde may also act by both of these mechanisms (46, 47). Description of how simultaneous action by two or more mechanisms would affect the parameters of the MVK model, and ultimately tumor incidence, is theoretically straightforward and can readily be described in biologically based simulation models (39–41), though there are to date no published examples of this approach for specific carcinogens.

Further developments in this area will be of great interest. It may, eventually, be possible to identify the individual contributions to total cancer risk for a single chemical of two or more different mechanisms of action. Of particular interest would be knowledge of the dose-response behavior for each mechanism. It is possible, for example, that a chemical that is both cytotoxic and DNA reactive might exhibit a dose threshold for the component of its carcinogenic risk mediated by cytotoxicity but no threshold for the component mediated by DNA reactivity. Biologically based models providing quantitative insights into the individual contributions of multiple mechanisms over a broad range of doses would do much to rationalize the process of carcinogen risk assessment.

THE HAZARD FUNCTION The hazard function, the rate at which malignant tumors arise in previously tumor-free animals, is an important index of carcinogenic effect. (Tumor prevalence in a population, which is simply the probability of tumor multiplied by the population size, is often erroneously called "incidence" or "hazard" but is, in fact, a quite different quantity [see, for example, 44]). In a biologically based description of cancer using the MVK model, the hazard function describes the quantitative linkage between the population of intermediate cells having one critical mutation and the effective rate of formation of malignant cells with two critical mutations that will expand clonally into clinically detectable tumors. In the context of the rodent carcinogen bioassay, the hazard function can be thought of as the rate at which malignant tumors arise in previously tumor-free rodents.

Moolgavkar et al (44) have described both "approximate" and "exact" forms of the hazard function. Explanation of this distinction is beyond the scope of this review, but it can be said here that the exact solution is more specific than the approximate in its use of the cell birth, death, and mutation rate parameters. Use of the exact hazard function is therefore preferable since the goal in developing any biologically based model is understanding the precise roles of component processes, in this case cell birth, death, and mutation rates, in the overall response. The exact hazard function is also more accurate than the approximate when the probability of tumor is high, which is often the case in rodent carcinogenicity bioassays. The exact hazard function, probability of tumor, and the growth of foci of intermediate cells can all be calculated analytically (44, 48). Moolgavkar et al (49) recently analyzed lung tumor data obtained from radon-exposed rats and estimated both the approximate and exact hazard functions. They found that, while the two hazard functions fit the data equally well, the overall shapes of time-to-tumor curves calculated with the approximate and exact solutions were quite different. Given that the analytical form of the exact solution of the hazard function for the MVK model is available and is the more accurate representation of the

behavior of the model (44), there is no reason to use the approximate solution for analysis of tumor data.

BIOLOGICALLY BASED DESCRIPTIONS OF NON-TUMOR ENDPOINTS Tumor development is just one of the endpoints in the overall process of chemical carcinogenesis for which development of biologically based models can be expected to lead to a better quantitative understanding and improved risk assessment. When a procarcinogenic mutation occurs during division of a normal cell, proliferation of the resulting intermediate cell (Figure 5) may give rise to a focus or clone of cells that are phenotypically different from the surrounding normal cells. These clones are identified by histochemical and enzyme staining of liver sections (50). Moolgavkar et al (51), working with a data set obtained from Lewis rats treated with N-nitrosomorpholine, have shown that the number and size of hepatic foci of intermediate cells can be analyzed within the framework of the MVK model to assess the "initiation" and "promotion" potencies of a carcinogen. Initiation potency refers to an effect on the rate of mutation per cell division per unit of carcinogen dose. This is a determinant of the rate at which intermediate cells arise from the normal cell population. Promotion potency refers to effects on the rates of intermediate cell division and death or terminal differentiation per unit of dose. It determines the relationship between the total number of intermediate cells at any point in time and the number created from mutation of normal cells. Promotion potency is also a determinant of the size distribution of clones of intermediate cells. In addition to providing estimates of initiation and promotion potency, analysis of the growth kinetics of cell populations involved in the carcinogenic process can be used to identify parameter values for biologically based cancer models. Future biologically based risk assessments for chemical carcinogens will very likely utilize this kind of data on foci development in target tissues from treated animals.

TIME-DEPENDENT PARAMETERS In the biologically based analysis of bioassay data, the intermittent nature of carcinogen dosing means that chemical-dependent effects on the parameters of the cancer model (Figure 5) are also time-dependent. The exact nature of the time-dependence reflects both the pharmacokinetics of the carcinogen and the mechanism of action linking the tissue dose with the cancer model parameters. At their present level of development the analytical forms of the MVK model cannot accommodate time-dependent variation of the cell division, cell death, and mutation rate parameters for both the normal and initiated cell populations (44, 52). Quinn (52) has recently described a carcinogenesis model based on the MVK model that contains an analytical solution for the exact hazard function and where the growth and mutation rate parameters for the intermediate cell population can

be time-dependent. Moolgavkar et al (49) have also recently described a method for incorporating time-dependent variation of the parameters of intermediate cell birth, death, and mutation rates but no method is yet available that allows for time-dependent effects on normal as well as intermediate cells.

Simulation models of carcinogenesis, such as that described by Conolly et al (39–41), readily accommodate time-dependent variations in parameter values. Analytical and simulation models are complementary approaches in the study of biological problems including, but not limited to, cancer. For the simulation

sistent with the mathematical theory of a system as described by its analytical solution. On the other hand, there are often cases where the system being studied is so complex that no analytical solution is possible and simulation must be used. PB-PK models are good examples of this latter case.

SUMMARY

Models, Reality, and Experimental Design

The cancer model contains extensive biological detail, but is not fully validated. It is extremely useful for organizing what is known about malignant transformation, for linking measures of tissue dose with biological processes, and for proposing new experiments. It became very clear, as the cancer model was developed, that we lack an adequate understanding of the details of biochemical mechanisms of carcinogen action linking the target-tissue dose of carcinogen with effects on cell birth, death, and mutation rates as specified by the MVK model. This problem extends to noncarcinogenic toxicants such as methotrexate and the organophosphates. In these cases the biologically based descriptions of toxicity stop at the first interaction of the chemical with tissue. The linkage from the primary interaction to ultimate toxic effect is a "black box" that, at present, can only be described empirically rather than in biologically based terms. The models for dioxin and prednisolone do illustrate how biological events several steps removed from the initial tissue interaction of the toxicant can be described in quantitative, biologically based terms. As such they point the way for future development of more realistic descriptions in which the use of purely empirical linkages is reduced.

Rigorous organization of current knowledge and specification of a logical sequence of new experiments encompass the major reasons for developing biologically based (structural) descriptions of any process. It bears emphasis that biologically based tissue response models, for cancer, for chloroform cytotoxicity, for functional impairment following organophosphate exposure, etc, are in reality quantitative formulations of an hypothesis or proposed mechanism incorporating the pharmacokinetics of the chemical and the link-

age between target-tissue dose and toxic effect(s). The quantitative model allows us to see if the hypothesis is consistent with pertinent data and aids in designing experiments to define, refute, or bolster the general nature of the proposed linkages between tissue dose, tissue response and the whole organism's response to the chemical exposure. In this sense these biologically based models are quantitative descriptions of the current level of understanding of toxic effects in animals.

We began this review article by looking at the literature on empirical tissue-response models to educate ourselves on the history of PD modeling. We rapidly found that the clear distinction between PK and PD models described in the literature blurs when one moves to biologically based modeling of tissue disposition and tissue response. Many articles on physiologically based pharmacokinetics turn out on close scrutiny to be as closely related to tissue response as they are to kinetics (Figure 2). The process of exposure, tissue dose, early tissue response, and irreversible tissue injury is a continuum. This continuum has to be looked at in its entirety to understand the factors that determine eventual organism response from a particular exposure. It is no wonder that kinetic and dynamic responses become intertwined and difficult to disentangle from one another.

Literature Cited

1. Holford, N. H. G., Sheiner, L. B. 1981. Pharmacokinetic and pharmacodynamic modeling in vivo. *CRC Crit. Rev. Bioeng.* 5:273–322
2. Dingmanse, J., Danhof, M., Breimer, D. D. 1988. Pharmacokinetic-pharmacodynamic modeling of CNS drug effects: An overview. *Pharmacol. Ther.* 38:1–52
3. Tucker, G. T. 1990. Pharmacokinetic and pharmacodynamic models. In *Advances in Pain Research and Therapy, Vol 14*, ed. C. Benedetti, C. R. Chapman, G. Giron. 14:181–201. New York: Raven Press
4. Sheiner, L. B., Stansky, D. R., Vozeh, S., Miller, R. D., Ham, J. 1979. Simultaneous modeling of pharmacokinetics and pharmacodynamics: Application to d-tubocurarine. *Clin. Pharmacol. Ther.* 25:358–71
5. Matthews, H. B., Dedrick, R. L. 1984. Pharmacokinetics of PCBs. *Annu. Rev. Pharmacol. Toxicol.* 24:85–103
6. Ramsey, J. C., Andersen, M. E. 1984. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol.* 73:159–75
7. Andersen, M. E., Clewell, H. J. III, Gargas, M. J., Smith, F. A., Reitz, R. H. 1987. Physiologically-based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol.* 87:185–205
8. Paustenbach, D. J., Clewell, H. J. III, Gargas, M. L., Andersen, M. E. 1988. A physiologically based model for carbon tetrachloride. *Toxicol. Appl. Pharmacol.* 96:191–211
9. Freeman, R. A., Rozman, K. K., Wilson, A. G. E. 1989. Physiological model of hexachlorobenzene in the rat. *Health Phys.* 57:139–47 (Suppl. 1)
10. Bischoff, K. B., Dedrick, R. L., Zaharko, D. S., Longstreth, J. A. 1971. Methotrexate pharmacokinetics. *J. Pharm. Sci.* 60:1128–33
11. Morrison, P. F., Dedrick, R. L., Lutz, R. J. 1987. Methotrexate: Pharmacokinetics and assessment of toxicity. In *Pharmacokinetics in Risk Assessment*, 8:410–27. Washington, DC: Natl. Acad. Press
12. Maxwell, D. M., Vlahacos, C. P., Lenz, D. E. 1988. A pharmacodynamic model for soman in the rat. *Toxicol. Lett.* 43:175–88
13. Gearhart, J. M., Jepson, G. W., Clewell, H. J. III, Andersen, M. E.,

Conolly, R. B. 1990. A physiologically-based model for in vivo inhibition of acetylcholinesterase by diisopropyl-fluorophosphate. *Toxicol. Appl. Pharmacol.* 106:295–310

14. Reitz, R. H., Mendrala, A. L., Park, C. N., Andersen, M. E., Guengerich, F. P. 1988. Incorporation of in vitro enzyme data into the physiologically-based pharmacokinetic (PB-PK) model for methylene chloride: implications for risk assessment. *Toxicol. Lett.*, 43:97–116
15. Taylor, P. 1985. Anticholinesterase Agents. In *The Pharmacological Basis of Therapeutics*, ed. A. G. Gilman, L. S. Goodman, T. W. Rall, F. Murad, pp. 110–29. New York: MacMillan
16. Abraham, K., Krowke, R., Neubert, D. 1988. Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Arch. Toxicol.* 622:359–68
17. King, F. G., Dedrick, R. L., Collins, J. M., Matthews, H. B., Birnbaum, L. S. 1983. A physiological model for the pharmacokinetics of 2,3,7,8-tetrachlorodibenzofuran in several species. *Toxicol. Appl. Pharmacol.* 67:390–400
18. Leung, H-W., Ku, R. H., Paustenbach, D. J., Andersen, M. E. 1988. A physiologically based pharmacokinetic model for 2,3,7,8,-tetrachlorodibenzo-p-dioxin in C57BL/6J and DBA/2J mice. *Toxicol. Lett.* 42:15–28
19. Poland, A., Teitelbaum, P., Glover, E., Kende, A. 1989. Stimulation of in vivo uptake and in vitro hepatic binding of [^{125}I]2-lodo-3,7,8-trichlorodibenzo-p-dioxin by the administration of agonists for the Ah receptor. *Mol. Pharmacol.* 36:121–27
20. Poland, A., Teitelbaum, P., Glover, E. 1989. [^{125}I]2-lodo-3,7,8-trichlorodibenzo-p-dioxin-binding species in mouse liver induced by agonists for the Ah receptor: Characterization and identification. *Mol. Pharmacol.* 36:113–20
21. Leung, H-W., Pausenbach, D. J., Murray, F. J., Andersen, M. E. 1990. A physiological pharmacokinetic description of the tissue distribution and enzyme-inducing properties of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. *Toxicol. Appl. Pharmacol.* 103:399–410
22. Bradfield, C. A., Kende, A. S., Poland, A. 1988. Kinetic and equilibrium studies of Ah receptor-ligand binding: Use of [^{125}I]2 - lodo - 7,8 - dibromodibenzo -p-dioxin. *Mol. Pharmacol.* 34:229–37

22a. McConnell, E. E., Lucier, G. W., Rumbaugh, R. C., Albro, P. W., Harvan, D. J., et al. 1984. Dioxin in soil: Bioavailability after ingestion by rats and guinea pigs. *Science* 223:1077–79

23. Nichols, A. I., Boudinot, F. D., Jusko, W. J. 1989. Second generation model for prednisolone pharmacodynamics in the rat. *J. Pharmacokinet. Biopharm.* 17:209–27
24. Henschler, D. 1977. Metabolism and mutagenicity of halogenated olefins. *Environ. Health Perspect.* 21:61–64
25. Jaeger, R. J., Conolly, R. B., Murphy, S. D. 1973. Diurnal variation of hepatic glutathione concentration and its correlation with 1,1-dichloroethylene toxicity in rats. *Commun. Chem. Pathol. Pharmacol.* 6:465–71
26. Jenkins, L. J. Jr., Trabulus, M. J., Murphy, S. D. 1972. Biochemical effects of 1,1-dichloroethylene in rats: Comparison with carbon tetrachloride and 1,2-dichloroethylene. *Toxicol. Appl. Pharmacol.* 23:501–10
27. D'Souza, R. W., Andersen, M. E. 1988. Physiologically-based pharmacokinetic model for vinylidene chloride. *Toxicol. Appl. Pharmacol.* 95:230–40
28. Eschenbrenner, A. B., Miller, E. 1945. Induction of hepatomas in mice by repeated oral administration of chloroform with observations on sex differences. *J. Natl. Cancer Inst.*, 2:251–55
29. National Cancer Institute, 1976. Carcinogenesis bioassay of chloroform. *NCI, Bethesda, MD, Natl. Tech. Info. Serv.* No. PB264018/AS
30. Jorgensen, T. A., Meierhenry, E. F., Rushbrook, C. J., Bull, R. J., Robinson, M. 1985. Carcinogenicity of chloroform in drinking water in male Osborne-Mendel rats and female B6C3F1 mice. *Fundam. Appl. Toxicol.* 5:760–69
31. deSerres, F. J., Ashby, J., eds. 1981. Evaluation of short-term tests for carcinogens. In *Progress in Mutation Research, Vol. 1*. Amsterdam: Elsevier 827 pp.
32. Lutz, W. K., Maier, P. 1988. Genotoxic and epigenetic chemical carcinogenesis: one process, different mechanisms. *Trends Pharmacol. Sci.* 9:322–26
33. Butterworth, B. E. 1990. Considerations of both genotoxic and nongenotoxic mechanisms in predicting carcinogenic potential. *Mutat. Res.* 239:117–32
34. Goldsworthy, T. L., Morgan, K. T., Popp, J. A., Butterworth, B. E. 1991. Guidelines for measuring chemically-induced cell proliferation in specific rodent target organs. In *Chemically-Induced Cell Proliferation: Implications for Risk Assessment*, ed. B. E. Butter-

worth, T. J. Slaga. New York: Wiley-Liss. In press
35. Corley, R. A., Mendrala, A. L., Smith, F. A., Staats, D. A., Gargas, M. L., Conolly, R. B., Andersen, M. E., Reitz, R. H. 1990. Development of a physiologically-based pharmacokinetic model for chloroform. *Toxicol. Appl. Pharmacol.* 103:512–27
36. Reitz, R. H., Mendrala, A. L., Corley, R. A., Quast, J. F., Gargas, M. L., et al. 1990. Estimating the risk of liver cancer associated with human exposures to chloroform. *Toxicol. Appl. Pharmacol.* 105:443–59
37. Pohl, L. 1979. Biochemical toxicology of chloroform. In *Reviews in Biochemical Toxicology*, ed. E. Hodgson, J. R. Bend, R. M. Philpot, 1:79–107. New York: Elsevier
38. D'Souza, R. W., Francis, W. R., Buce, R. D., Andersen, M. E. 1987. Physiologically based pharmacokinetic model for ethylene dichloride and its application in risk assessment. See Ref. 11, pp. 286–301
39. Conolly, R. B., Reitz, R. H., Clewell, H. J. III, Andersen, M. E. 1988. Pharmacokinetics, biochemical mechanism and mutation accumulation: A comprehensive model of chemical carcinogenesis. *Toxicol. Lett.* 43:189–200
40. Conolly, R. B., Reitz, R. H., Clewell, H. J. III, Andersen, M. E. 1988. Biologically-structured models and computer simulation: Application to chemical carcinogenesis. *Comments Toxicol.* 2:305–19
41. Conolly, R. B., Reitz, R. H., Clewell, H. J. III, Andersen, M. E. 1989. Computer simulation of chemical carcinogenesis. In *Biologically-based Methods for Cancer Risk Assessment*, ed. C. Travis, pp. 301–17. New York: Plenum
42. Moolgavkar, S. H., Venzon, D. J. 1979. Two-event models for carcinogenesis: Incidence curves for childhood and adult tumors. *Math. Biosci.* 47:55–77
43. Moolgavkar, S. H., Knudson, A. G. 1981. Mutation and cancer: A model for human carcinogenesis. *J. Nat. Cancer Inst.* 66:1037–52
44. Moolgavkar, S. H., Dewanji, A., Venzon, D. J. 1988. A stochastic two-stage model for cancer risk assessment. I. The hazard function and the probability of tumor. *Risk Anal.* 8:383–92
45. Magee, P. N., Montesano, R., Preussmann, R. 1976. N-Nitroso compounds and related carcinogens. *ACS Monogr.* 173:491–625
46. Craft, T. R., Bermudez, E., Skopek, T. R. 1987. Formaldehyde mutagenesis and formation of DNA-protein cross-links in human lymphoblasts in vitro. *Mutat. Res.* 176:147–55
47. Morgan, K. T., Patterson, D. L., Gross, E. A. 1986. Responses of the nasal mucocilliary apparatus of F-344 rats to formaldehyde gas. *Toxicol. Appl. Pharmacol.* 82:1–13
48. Dewanji, A., Venzon, D. J., Moolgavkar, S. H. 1989. A stochastic two stage model for cancer risk assessment. II. The number and size of premalignant clones. *Risk Anal.* 9:179–87
49. Moolgavkar, S. H., Luebeck, G. 1990. Two-event model for carcinogenesis: Biological, mathematical, and statistical considerations. *Risk Anal.* 10:323–41
50. Harada, T., Maronpot, R. R., Morris, R. W., Stitzel, K. A., Boorman, G. A. 1989. Morphological and stereological characterization of hepatic foci of cellular alteration in control Fisher 344 rats. *Toxicol. Pathol.* 17:579–93
51. Moolgavkar, S. H., Luebeck, E. G., de Gunst, M., Port, R. E., Schwarz, M. 1990. Quantitative analysis of enzyme-altered foci in rat hepatocarcinogenesis experiments I: Single agent regimen. *Carcinogenesis* 11:1271–78
52. Quinn, D. W. 1989. Calculating the hazard function and probability of tumor for cancer risk assessment when the parameters are time-dependent. *Risk Anal.* 9:407–13