

Applications of computational toxicology methods at the Agency for Toxic Substances and Disease Registry

Hisham A. El-Masri, Moiz M. Mumtaz, Gangadhar Choudhary, William Cibulas, Christopher T. De Rosa

Computational Toxicology Laboratory, Division of Toxicology, Agency for Toxic Substances and Disease Registry, Atlanta, GA

Received September 13, 2001 · Accepted November 7, 2001

Abstract

In its efforts to provide consultations to state and local health departments, other federal agencies, health professionals, and the public on the health effects of environmental pollutants, the Agency for Toxic Substances and Disease Registry relies on the latest advances in computational toxicology. The computational toxicology laboratory at the agency is continually engaged in developing and applying models for decision-support tools such as physiologically based pharmacokinetic (PBPK) models, benchmark dose (BMD) models, and quantitative structure-activity relationship (QSAR) models. PBPK models are suitable for connecting exposure scenarios to biological indicators such as tissue dose or end point response. The models are used by the agency to identify the significance of exposure routes in producing tissue levels of possible contaminants for people living near hazardous waste sites. Additionally, PBPK models provide a credible scientific methodology for route-to-route extrapolations of health guidance values, which are usually determined from a very specific set of experiments. Also, scientists at the computational toxicology laboratory are using PBPK models for advancing toxicology research in such areas as joint toxicity assessment and child-based toxicity assessments. With BMD modeling, all the information embedded in an experimentally determined dose-response relationship is used to estimate, with minimum extrapolations, human health guidance values for environmental substances. Scientists in the laboratory also rely on QSAR models in the many cases where consultations from the agency are reported for chemicals that lack adequate experimental documentation.

Key words: Computational – models – PBPK – QSAR – benchmark – toxicology

Introduction

In the environment, we are exposed to hundreds of chemicals and an exponential number of their combinations as mixtures. Thus, ideally, these chemicals and their combinations need be experimentally tested. However economically and otherwise it is impossible to test them all. Since the start of

toxicology testing at the National Toxicology Program, a very small fraction of the chemicals and a much smaller number of their combinations have been tested (Yang, 1996). Among the substances tested are a few pharmaceuticals, drugs, nutrients and medically important chemicals. Of the top 250 priority environmental chemicals, it has been suggested that only a small fraction have been char-

Corresponding author: Hisham A. El-Masri, ATSDR, Division of Toxicology, 1600 Clifton Road, NE, MS E-29, Atlanta, GA 30333, USA. Phone: ++404 498 0697, Fax: ++404 498 0092, E-mail: hbe4@cdc.gov

acterized sufficiently, but toxicity data for the remainder are scarce. Hence, for the past decade, the Agency for Toxic Substances and Disease Registry (ATSDR) has explored the use of alternative methods for laboratory testing and computational tools to augment our current knowledge in the areas of hazard identification and toxicity evaluation. In 1994, ATSDR hosted an international symposium of experts in the application of computational models to decision-support methodologies for human risk assessment of toxic substances. The recommendations of these experts led to the establishment of a state-of-the-art computational toxicology laboratory in 1998. Since that time, ATSDR has supported *in vitro* and limited *in vivo* toxicity testing that could be guided through computational toxicology modeling to further advance our understanding of chemical toxicity and health effects of chemicals and our knowledge of advances in computational methods development.

Computational toxicology, an applied science, utilizes the latest advances in mathematics, biology, chemistry, and computer technologies. Integrating all of these sciences into a biologically based computational model enables the researcher to numerically investigate, either pharmacokinetically and/or pharmacodynamically, the impact of exposure to environmental chemicals on people. Decision-support models in computational toxicology routinely used by ATSDR include physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models, benchmark dose (BMD) analysis, and quantitative structure-activity relationships (QSAR). PBPK/PD models are used to describe the relevant biochemical and physiologic processes that relate exposure to toxicity of a chemical or multiple chemicals. They also can be used to identify a biologically active dose for which extrapolations among species and routes of exposure can be performed. In this manner, PBPK/PD is a step farther in the direction of employing current knowledge about toxic mechanisms to estimate risks to humans. BMD methods use the complete dose-response relationship to determine doses that cause a pre-determined response (e.g., 10% of the population with documented toxicologic response). QSAR methods rely on structural similarities between chemicals to predict toxicity based on statistical analysis of experimental databases. Therefore, QSAR is used to screen chemicals for toxic end points when experimental information about them is not available.

In this paper, we provide an overview of ATSDR's use of decision-support computational toxicology tools. We explain how the tools have been used to

address substance-specific priority data needs, and to support chemical-specific health consultations and public health assessments.

Computational toxicology models used at ATSDR

The computational toxicology laboratory at ATSDR is equipped with several state-of-the-art personal computers and one workstation. The laboratory is made up of 4 stations; each is equipped with a computer and audio/visual instructional devices for use in training and invited seminars by experts in the field. The laboratory library includes many software programs widely used in computational toxicology modeling. PBPK model development and utilization is carried out with two widely used modeling software programs: Matlab (The Math Works Inc.) and ACSL (The AEGIS Technologies Group). QSAR is applied using TOPKAT (Pharmacoepia, Inc.) and CaseTox (Multicase, Inc.).

In general, applications of computational toxicology models serve the agency in its efforts to provide scientifically credible health risk consultations of human exposures to environmental chemicals, and to further advance research in the areas of chemical mixtures toxicity and risk assessment. These efforts are illustrated in the following examples of activities conducted in the computational toxicology laboratory.

PBPK modeling

Pharmacokinetics (PK) involves the study of the rates of absorption, distribution, excretion, and biotransformation of chemicals and their metabolites. PK models can be used to reconstruct extensive data sets based on small numbers of kinetic parameters (Andersen, 1995). These models can be used to predict the results of new experiments and integrate studies of kinetics, disposition and metabolism in various animal species (Wagner, 1981). In physiologically based pharmacokinetic (PBPK) models, compartments correspond more closely to actual anatomical structures, defined with respect to their volumes, blood flows, chemical binding (partitioning) characteristics, and the ability to metabolize or excrete the compounds of interest (Figure 1). Because the kinetic parameters of these models reflect tissue blood flows, partitioning, and biochemical constants, these models are more readily scaled from

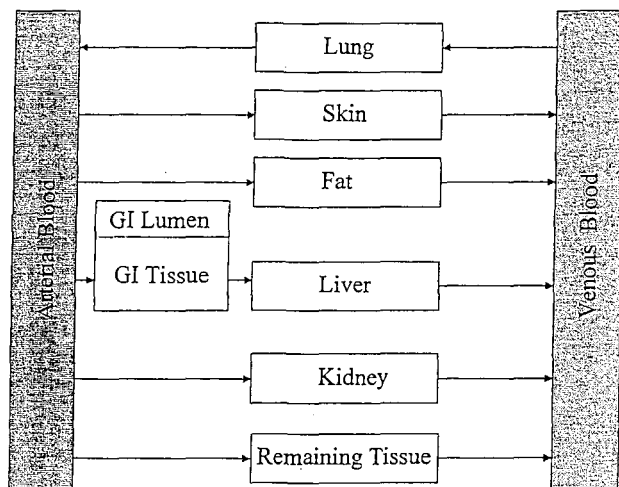


Fig. 1. A schematic of a physiologically based pharmacokinetic (PBPK) model. Each compartment represents a tissue of interest. The tissues are mathematically described using mass balance equations accounting for the storage, clearance and/or metabolism of the chemical under investigations. Each tissue is characterized by a set of parameters reflecting its volume, blood perfusion rates and the chemical's partitioning in it. The resulting set of differential mass balance equations are solved simultaneously using available software packages. The solutions yield a temporal profile of the chemical of interest in blood or any modeled tissue.

one animal species to another (Dedrick, 1973). Quantitative applications of PBPK models in risk assessment date to the development of a number of PBPK models for methylene chloride in the mid-1980s (Andersen et al., 1987). Today the use of PBPK models in toxicology research and chemical risk assessment is primarily related to their ability to make more accurate predictions of target tissue dose for different exposure situations in different animal species, including humans.

Highlighted below is an example of the application of PBPK modeling in agency programs and activities relating to the public health assessment of hazardous substances found at national priority list (NPL) sites.

Example: Application of PBPK models to identify exposure routes of PCBs near a waste site

The major route of human exposure to polychlorinated biphenyls (PCBs) is through ingestion of contaminated food. Nationally, the average range in serum of PCBs is 4–7 $\mu\text{g/l}$ (Agency for Toxic Substances and Disease Registry, 2000a). However, a much higher range of total PCBs levels (76.3 to 187.5 $\mu\text{g/l}$) was found in the serum of some residents living in a highly contaminated residential area. Total PCB soil levels in this area ranged from 17.4 to 840 mg/kg, levels much higher than the maximum

soil level of 1.5 mg/kg reported in a national survey. We chose this example to estimate the contribution of exposure by soil ingestion to the levels of serum PCBs, both nationwide and in this residential area. This was achieved by using PBPK models, which are useful in relating environmental exposure to biological markers such as blood levels.

Preliminary efforts to address the contribution of contaminated soil ingestion to blood levels in the studied area centered around the use of oral human PBPK models of the 25 most common PCB congeners. The basic PBPK model structure was reconstructed according to an earlier published structure for several PCB congeners (Lutz et al., 1977). Parameters such as partition coefficients and metabolic constants used in the PBPK models were determined using quantitative structure-activity relationships (QSAR) based on published procedures (Parham et al., 1997, 1998). All simulations were run using a soil ingestion default rate of 50 mg/day for a lifetime exposure scenario. Using average soil levels nationwide, the model estimate for the level of total PCBs in blood is 0.06 $\mu\text{g/l}$. The 95% percentile nationwide blood levels of total PCBs in the United States is estimated at 10 $\mu\text{g/l}$ (Agency for Toxic Substances and Disease Registry, 2000a). Hence, the model average estimate is only 0.6% of the reported nationwide percentile, this indicates that soil ingestion is not a major route of PCB exposure nationwide. Specifically, in the highly contaminated residential area, a probabilistic distribution model for PCB blood levels was derived based on actual PCB soil measurements to partially address exposure variability within the community. This distribution was then applied to the 25 PBPK models to derive a distribution of predicted total PCBs in blood for lifetime exposure scenarios. The derived distribution of blood levels was superimposed on the actual distribution of measured serum levels estimated in the same community (Figure 2). The distribution of actual blood levels for 9 out of 10 persons falls within the modeled exposure-range. For this sample of 9 individuals, the mean of the actual blood levels distribution falls within the 2 percentile lower end of the simulated curve. The superimposition at the lower end of the simulation curve indicates that contributions of soil levels to actual blood levels can be assumed when model simulations are done based on lower rate of soil intake.

The initial results of this example are not conclusive but can be used to warrant a more in-depth analysis of the contribution of soil contamination to the high observed blood levels in the community. Because of lack of actual exposure scenarios for the people in the area, the PBPK models assumed

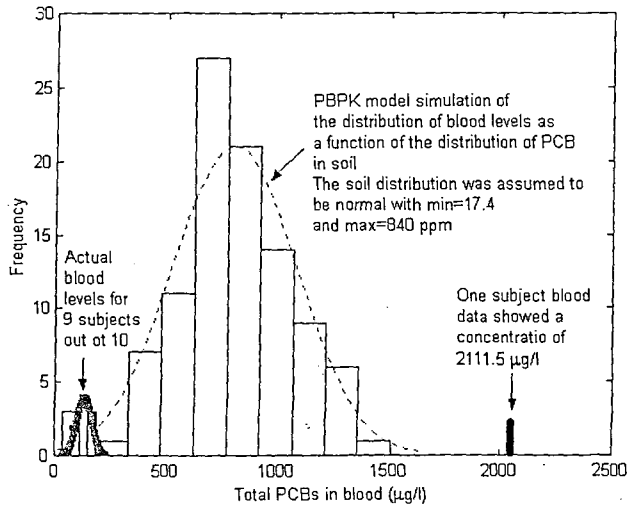


Fig. 2. Data on the levels of total PCBs in blood from a sample of 9 out of 10 persons near a waste site are converted into a mathematical distribution. The distribution is given by the solid line fit over the calculated histograms. This distribution is superimposed on another one which is predicted by PBPK models using various values of soil concentrations in the site. The PBPK simulated distribution is given in the dashed line over the simulated histograms. The wider spread of the simulated model is indicative of the variance in soil levels at the site. Given this wide variance, the actual data distribution coincided with the lower tail of the predicted distribution. This result indicates that soil, when ingested at lower levels, can be a contributing factor to the blood levels seen in the sample of people living next to the site.

continuous daily exposure of soil levels at a default uptake rate of 50 mg/day. Coupled with the use of a wide range of soil levels in the vicinity of the waste site, a wider range of model-predicted blood levels than the actual ones is obtained as seen in Figure 2. For a better understanding of soil exposure contribution, the PBPK models have to be fine tuned to address exposure more explicitly. This can only be done by looking at factors such as age, time of residency in the area, and more detailed geographic distribution of PCBs soil levels near the residences of the affected populations. Scientists at the computational laboratory are continuing to improve on these models in addition to analyzing other exposure scenarios to PCBs such as dietary intake and air levels.

Benchmark dose (BMD) modeling

A BMD is a statistical lower confidence limit for a dose that produces a predetermined change in response rate of an adverse effect (called the benchmark response or BMR) compared to background. Unlike the no-observed-adverse-effect-level (NOAEL), the

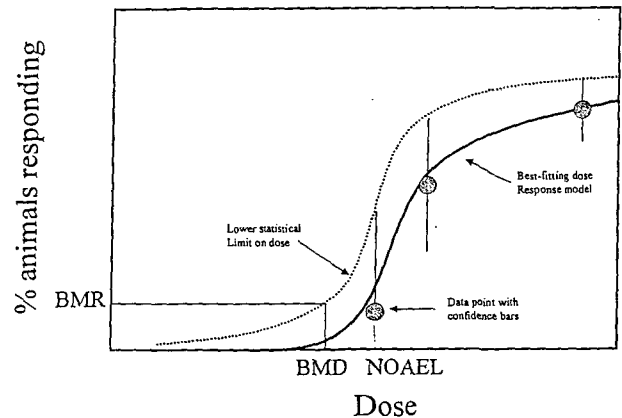


Fig. 3. An example of a BMD calculation. BMR is the target response level used to define the BMD. In the default method, the single value of NOAEL is experimentally determined as the dose at which a significant response was detected above background. This value is then adjusted by using different extrapolation factors to define a guidance value more closely related to humans. The resulting guidance value usually falls outside the data curve. Alternatively, the BMD method uses the complete dose-response relationship by fitting the data mathematically to a curve. The curve is also bound by confidence levels resulting from the data and the fitting process. This fitting process allows the assessor to use the complete experimental data set to define a BMD that will fall inside the experimental dose-response.

BMD takes into account dose-response information by fitting a mathematical model to dose-response data. The BMR is generally set near the lower limit of responses that can be measured directly in animal experiments of typical size. Thus, unlike the default risk assessment methods, the BMD method does not extrapolate to doses far below the experimental range. A graphical explanation of the benchmark calculation is shown in Figure 3. ATSDR uses the benchmark dose-modeling approach to evaluate minimal risk levels (MRLs) for specific chemicals. An MRL is defined as an estimate of the daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects that are non carcinogenic. Manganese is an example of a chemical for which a BMD model was used to support the derivation of an inhalation chronic MRL.

The most sensitive and significant effects caused by inhalation of manganese dusts are neurological deficits with progressive increased injury with prolonged exposures (Agency for Toxic Substances and Disease Registry, 2000b). The BMD approach was used to provide a surrogate NOAEL to derive a chronic inhalation MRL based on published dose response experiments. The BMD method defines an adverse effect as a risk level of more than an established percentage (usually 10%) above background. This risk level is determined by estimating a

lower confidence limit (e.g., 95%) on a dose corresponding to a predetermined increase (e.g., 10%) in the incidence of a particular adverse effect. Sufficient data on individual participants' exposure levels and neurological test performance results are available in literature (Agency for Toxic Substances and Disease Registry, 2000b). When BMD models were applied to these data, a chronic inhalation NOAEL was estimated to be 0.074 mg/m³ at the 95% confidence limit for a 10% increase in risk. Based on this BMD value, a chronic inhalation MRL of 0.00004 mg/m³ was derived using appropriate uncertainty and conversion factors.

Quantitative structural activity relationship (QSAR) models

In instances where bioassay data are limited or absent, QSAR studies have been supported at ATSDR. QSAR approaches have been applied formally or informally for decades in several disciplines including pharmacology, pesticide chemistry, and the food-drug industry. However, since the 1980s and particularly during the 1990s these approaches have been formalized into computer software models to include that can be used to predict a variety of toxicity end points (Enslin et al., 1990; Gombar et al., 1995).

At ATSDR, scientists in the computational toxicology laboratory use two commercially available QSAR software models: TOPKAT (Pharmacopeia, Inc.) and CaseTox (Multicase, Inc.). Both models allow multiple toxicity end-point evaluations that are important to public health by enabling profiling of the inherent toxicity of the candidate chemicals of interest. Both software applications use a linear chemical structure entry system called SMILES (Simplified Molecular Input Line Entry System) for entering the structure of the chemical. The chemical structure is visually verified before the toxicity of the chemical is evaluated. After the structure is confirmed, the investigator selects one of the several models available in the software package and submits the chemical for evaluation. Within a minute, the model searches all the chemicals in the specific database and generates the results. Using sound scientific judgement, an experienced toxicologist analyzes and interprets these results before accepting and further using the output of the model.

TOPKAT and CaseTox models perform chemical-structure based-toxicity assessments and correlate the toxicity with a set of structural descriptors that are present in a particular model's accompanied

database. Thus, the resulting predictions capture the collective knowledge of all the chemicals that have been experimentally tested. For several end points the model-predicted toxicity values are transformed into probability values. These probability estimates help the researcher determine if the chemical in question is active or not towards the modeled toxicity end point (e.g., developmental or cancer). To add more confidence in the models' predictions, researchers in the computational toxicology laboratory use a special feature of the models, the similarity search, extensively. This search yields a similarity distance on a scale of 0.0–1.0; the smaller this distance, the greater is the similarity in structure between the studied chemical and those that exist in the database. For example, if a chemical such as benzene is present in the database, and, if it is entered as a query chemical, the similarity distance will be 0.0. However, if toluene is entered in the same model, the model could give a similarity distance of 0.23. Based on experience in analyzing such data and on precedence, scientists in the laboratory developed and used the following "decision analytic" to express our confidence in the estimates (Figure 4). The decision analytic is based on a cut-off similarity distance value of 0.25 and, at a minimum, the comparison of experimental and predicted results of the four nearest neighbors of the queried chemical.

Recently, we used the TOPKAT software models at ATSDR to evaluate a series of unusual chemicals identified by the New Jersey Department of Health and Senior Services. The chemicals were tetrachlorophthalic acid, tetrachlorophthalic anhydride, chlorendic anhydride, chlorendic acid, o-chlorostyrene, m-chlorostyrene, p-chlorostyrene, alpha beta dichlorostyrene, bis (4-chlorophenyl) sulfone, triallyl isocyanurate, 1,2-diphenylhydrazine diphenylamine, N-ethyl-p-toluenesulfonamide, N-methyl-p-toluenesulfonamide, and styrene-acrylonitrile dimer. The end points evaluated were mutagenicity, carcinogenicity, developmental toxicity, rat oral LD₅₀, and octanol water partition coefficient. Literature searches were conducted for all the chemicals so that the QSAR results could be interpreted in view of the known and published toxicity data for each of the chemicals. QSAR data analysis shows that 9 of the 15 chemicals have a potential for carcinogenicity, 6 have a potential for developmental toxicity, and 6 can cause mutagenicity. The confidence in these conclusions varies from low to high according to the scheme developed in Figure 4. These toxicologic predictions are based on QSAR analysis and are not assessments of the health effects that may be expected to occur in human populations living in the vicinity of this site. Most of the predicted

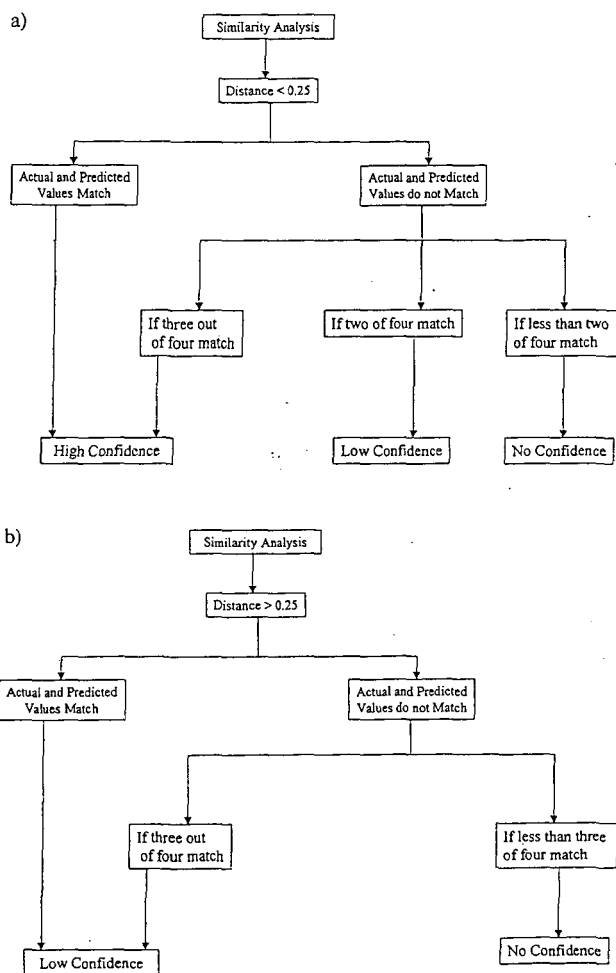


Fig. 4. A flow chart depicting a decision tree used by scientists at the computational toxicology laboratory to estimate confidence levels in QSAR models predictions when TOPKAT software is used. The chart depends on analysis of the similarity distance between the studied chemical and the database in TOPKAT. If similarity distance is less than 0.25 with an existing chemical in the database, flow chart (a) is used. However, flow chart (b) is used when similarity distance is more than 0.25. In either case actual results from the database of the similar chemical are compared with the predicted results for the same chemical. A higher degree of confidence is given to the models predictions of the studied chemical whenever the predictions and actual results (from the existing database) agree for other similar chemicals. This process is done for at least four chemicals similar to the one for which predictions are made.

effects are based on animal rather than human studies. The potential for a chemical to cause adverse effects can be addressed only after information on site-specific conditions and exposures has been collected and evaluated. A chemical cannot cause a toxic effect if there is no exposure. If chemical exposures are sufficient in terms of level, frequency, and duration, then a biologically plausible basis may exist for associating health effects with the exposure.

Discussion

The computational toxicology laboratory at ATSDR is constantly developing and applying mathematical models to address issues of concern to the agency's various constituencies, particularly residents of communities near superfund hazardous waste sites. The applications of these models are intended to increase our understanding of the underlying mechanisms by which toxic substances may cause injury to people. This understanding is then transformed into more scientifically credible estimates for risk to people by performing extrapolations that are better linked to biological and chemical processes than risks estimated by the usual default risk analysis methods.

Health risk assessment of human exposure to environmental chemicals is usually based on experimental findings in test animals for single chemicals. Current assessment methods include two issues that are constantly debated in the scientific community. First, using these findings to estimate health risks to humans is routinely done by applying default uncertainty factors, the purpose of which is to extrapolate between experimental settings and real life situations among different doses, animal species, and exposure scenarios. The acceptance of these uncertainty factors by the scientific community reflects the lack of understanding of physiological and biochemical mechanisms behind the toxicity of environmental chemicals. Second, in most cases, risks are only estimated for exposures to single chemicals in the environment. This scenario also is an oversimplification of actual exposure situations where people are exposed to multiple chemicals concurrently. Biologically based computational models can address both issues by linking tissue levels, pharmacokinetically, or mechanisms of actions, pharmacodynamically, with exposure levels. This linkage provides a numerical procedure that can be used to investigate the role of physiological (e.g., body weight, cardiac output) or biochemical factors (such as metabolism or protein binding) in determining risk estimates among different species and different exposure scenarios (high and low). Whenever possible, mechanisms of interactions, such as metabolic inhibitions or competition on protein binding sites, can also be introduced into the models to address the toxicologic effects of exposure to multiple chemicals on human health.

References

- Agency for Toxic Substances and Disease Registry: Toxicological profile for polychlorinated biphenyls (Update): U.S. Department of Health and Human Services, Atlanta, GA 2000a.
- Agency for Toxic Substances and Disease Registry: Toxicological profile for Manganese (Update): U.S. Department of Health and Human Services, Atlanta, GA 2000b.
- Andersen, M. E., Clewell, H. J., III, Gargas, M. L., Smith, F. A., Reitz, R. H.: Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol.* 87, 185-205 (1987).
- Andersen, M. E.: Development of physiologically based pharmacokinetic and physiologically based pharmacodynamic models for applications in toxicology and risk assessment. *Toxicol. Lett.* 79, 35-44 (1995).
- Dedrick, R. L.: Animal scale-up. *J. Pharmacokinet. Biopharm.* 1, 435-461 (1973).
- Enslein, K., Blake, B. W., Borgstedt, H. H.: Prediction of probability of carcinogenicity for a set of ongoing NTP bioassays. *Mutagenesis* 5, 305-306 (1990).
- Gombar, V. K., Enslein, K., Blake, B. W.: Assessment of developmental toxicity potential of chemicals by quantitative structure-toxicity relationship models. *Chemosphere* 31, 2499-2510 (1995).
- Lutz, R. J., Dedrick, R. J., Matthews, H. B., Eling, T. E., Anderson, M. W.: A preliminary pharmacokinetic model for several chlorinated biphenyls in the rat. *Drug Metab. Dispos.* 5, 386-396 (1977).
- Parham, F. M., Kohn, M. C., Matthews, H. B., DeRosa, C., Portier, C. J.: Using structural information to create physiologically based pharmacokinetic models for all polychlorinated biphenyls. *Toxicol. Appl. Pharmacol.* 144, 340-347 (1997).
- Parham, F. M., Portier, C. J.: Using structural information to create physiologically based pharmacokinetic models for all polychlorinated biphenyls. II. Rates of metabolism. *Toxicol. Appl. Pharmacol.* 151, 110-116 (1998).
- Wagner, J. G.: History of pharmacokinetics. *Pharmacol. Ther.* 12, 537-562 (1981).
- Yang, R. S.: Some current approaches for studying combination toxicology in chemical mixtures. *Food Chem. Toxicol.* 34, 1037-1044 (1996).