# Application of Physiologically Based Pharmacokinetic/Pharmacodynamic Modeling in Cumulative Risk Assessment for *N*-Methyl Carbamate Insecticides

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## NEED FOR CUMULATIVE RISK ASSESSMENTS FOR *N*-METHYL CARBAMATE INSECTICIDES

Human exposure to xenobiotics may occur through multiple pathways and routes of entry punctuated by exposure intervals throughout a work or leisure day. Exposure to a single environmental chemical along multiple pathways and routes (aggregate exposure) may have an influence on an organism if the exposure dose is absorbed and distributed to target tissues. Exposure to multiple chemicals belonging to a class of similar compounds having a common mechanism of action may have a cumulative effect on target tissues (cumulative toxicity). Under this situation, the evaluation of the toxic effect from one chemical is obviously not enough. Instead, the net effect from all chemicals should be considered. Whenever such scenarios are

encountered, cumulative risk assessment (CRA) is needed in order to evaluate the net cumulative toxicity caused by the aggregate exposure from all routes of entry for a single chemical or a group of chemicals that have a common mechanism of toxicity (U.S. EPA, 2002a).

The application of pesticides for the purpose of insect pest control creates such possible scenarios, not only in occupational settings but also in the general population. Organophosphorus compounds, *N*-methyl carbamates (NMCs), and pyrethroids are three popular classes of insecticides widely used in the United States and worldwide. For the general population, these pesticide residues may enter exposure pathways in food, drinking water, breathable air, and on residential surfaces where exposure may occur by ingestion, inhalation, and dermal contact. Recognizing the risk imposed by exposure to multiple chemicals, the Food Quality Protection Act (FQPA) of 1996 in the United States and Regulation (EC) No. 396/2005 in the European Union both mandate CRAs on human health resulting from exposure to multiple chemicals that exert their toxicity by a common mechanism of action. The FQPA requires the U.S. Environmental Protection Agency (U.S. EPA) to consider the cumulative effects to human health that can result from pesticides and other substances that have a common mechanism of toxicity. To know the background and history of regulations regarding CRA, readers are directed to U.S. EPA (2002b) for the available papers.

As insecticides, *N*-methyl carbamates (NMCs) share a common chemical structure with the general formula $ROC(=O)NHCH_3$ for *N*-methylcarbamates and $ROC(=O)N(CH_3)_2$ for dimethylcarbamates. The detailed chemical structures of each member in this class can be found in Table 1. NMCs have a common mechanism of action toward insect pests and unintended toxicity to non-target organisms including humans; i.e. acetylcholinesterase (AChE) inhibition

by carbamylating the serine hydroxyl group in the active site of the enzyme in the nervous system, leading to the persistent action of the neurotransmitter, acetylcholine, on cholinergic postsynaptic receptors (O'Brien, 1967; Kuhr and Dorough, 1976; Matsumura, 1985; Baron, 1991; Ecobichon, 1991; Knaak *et al.*, 2008). Therefore, these pesticides are recognized as a common mechanism group (CMG) (U.S. EPA, 2007). Unlike the organophosphorus insecticides, inhibition of cholinesterase by NMCs is reversible and the onset and recovery of inhibition is rapid, with the maximum inhibition occurring between 15 and 45 minutes and recovery starting from minutes to hours (U.S. EPA, 2007).

## METHODOLOGIES FOR PERFORMING CUMULATIVE RISK ASSESSMENTS FOR *N*-METHYL CARBAMATES

A CRA begins with the identification of a CMG of chemicals, which exert toxic effects by a common mechanism of action (U.S. EPA, 1999). After the identification of a CMG, individual chemicals are selected based on perceived risk and exposure potential. This subgroup of chemicals within the CMG is sorted into cumulative assessment groups. Many approaches have been investigated and used in CRA (Boobis *et al.*, 2008). Basically, there are four methodologies that include: 1) a toxicological index method, 2) a margin of exposure method, 3) a relative potency factor (RPF) method, and 4) physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling. Algorithms used in the first three methods are summarized in Table 2.

The index method accounts for cumulative risk by summing all risk indexes calculated as the ratio of exposure level to the reference value for each individual chemical. This method uses the basic principle for risk assessment of calculating the Hazard Index, which is the inverse of the margin of exposure. Readers are referred to the detailed description for various index methods presented in Boobis *et al.* (2008). It should be noted that, even though the algorithms are different, these methods are interchangeable when the evaluation of all chemicals is based on the same toxicological endpoint and study design, thus the outcome of the assessment is the same regardless of the method used (Boobis *et al.*, 2008).

The RPF and PBPK/PD modeling methods will be described further in the following sections with particular emphasis on the PBPK/PD modeling approach. The use of PBPK modeling for the CRA of pesticides has been discussed or performed by Lowit *et al.* (2004), Conolly *et al.* (2005); and Zhang *et al.* (2008). The U.S. EPA has issued a report providing guidance for using the PBPK method in risk assessment (U.S. EPA, 2006a).

**Relative Potency Factor Approach Using an Index Chemical**

The U.S. EPA developed the RPF approach which uses an index chemical to carry out the CRA for organophosphorus insecticides (U.S. EPA, 2006b) and NMCs (U.S. EPA, 2007), and has released guidance for performing CRAs for aggregate exposures involving multiple routes for a single chemical (aggregate risk) (U.S. EPA, 2001) and for a CMG which incurs cumulative risk (U.S. EPA, 2002a).

Briefly, an index chemical is selected based on the completeness and representativeness of available data, and is used as the point of reference for comparing the absorbed dose for the rest of the chemicals in the cumulative assessment group. The doses for the other CMG chemicals are converted into the equivalent dose of the index chemical so that the aggregate exposure can then be lumped together as the equivalent dose of the index chemical.

The cumulative risk is then evaluated by comparing the level of exposure against the point of departure (PoD) on the dose response curve of the index chemical. The PoD is defined as a dose-response point that marks the beginning of a low dose extrapolation from laboratory animals to humans. Under most situations, the PoD is based on an external exposure or administered dose that leads to the observed responses. The selected PoD is used to depart from the observed range of empirical response data for extrapolating risk in laboratory animals to the exposure anticipated in the human population (U.S. EPA, 2002a). The PoD that is usually used is either a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or benchmark dose (BMD) of the index chemical. The U.S. EPA prefers the use of BMD rather than NOAEL or LOAEL (U.S. EPA, 2007).

For the NMCs, the endpoint of relative potency is brain AChE inhibition measured at peak inhibition following oral gavage exposures in Long Evans rats (Padilla *et al.*, 2006) and studies submitted by the registrants. The central estimate of 10% brain AChE inhibition ($BMD_{10}$) is used as the response level to develop RPFs. In the family of NMCs, oxamyl is used as the index chemical because of the availability of high-quality dose response data for various routes. Brain AChE activity was selected as the endpoint to calculate RPFs from the PoDs (U.S. EPA, 2007).

Based on the available brain AChE activity data in the rat, an empirical dose-time-response exponential model was developed for each NMC and the central estimate of the $BMD_{10}$ is used to determine the relative potency. The lower confidence limit of the $BMD_{10}$ (i.e., $BMDL_{10}$) for the index chemical, oxamyl, is used as the PoD. The mathematical equations describing the dose-time-response exponential model can be found in U.S. EPA (2007). An RPF is defined as the ratio of the $BMD_{10}$ of oxamyl divided by the $BMD_{10}$ of each NMC. With this algorithm, the RPFs for all ten NMCs range from 0.02 (pirimicarb) to 4 (aldicarb) with the RPF of the index chemical as 1. The exposure doses are then converted to an equivalent dose of oxamyl by multiplying the estimated dose of each NMC with its RPF to calculate the Index Equivalent Residue (used in Equation 1). The aggregate exposure is then summed together to obtain the total exposure of oxamyl as indicated in the denominator in Equation 1. After further uncertainty factors are applied, the PoD is adjusted to extrapolate the exposure dose for humans. The targeted acceptable margin of exposure (as calculated in Equation 1) for the NMC CRA is larger than 10.

Equation (1)
$$MOE = \frac{PoD_{index}}{\sum_{i=1}^{n} \text{Residue}_i \times PF_i \times RPF_i}$$

where *MOE* is the margin of exposure and *PF* refers to the chemical-specific processing factor.

The RPF method is based on several assumptions. First, the dose of each chemical is assumed to be additive; i.e. there is no synergism or competitive interaction from all AChE inhibitors in the process of AChE inhibition. This assumption is based on a multi-NMC mixture study (Padilla *et*

*al.*, 2007) in which interactions among NMCs were not observed. Second, the RPF method largely depends on the availability and quality of the toxicological database on the index chemical. Consequently, any uncertainty or incompleteness in this database would be propagated in the downstream algorithm. Meanwhile, the assumption of the ratio of toxic potencies between the index chemical and the specific NMC as being constant across all dose ranges need further experimental testing. Moreover, the aggregated exposure (summed dose) is assumed to occur as a single time event rather than a series of events, and not as discrete or punctuated events which can actually happen in reality. Lastly, subject differences (age, gender, and metabolic polymorphism) are not considered. Therefore, the RPF method cannot reflect the variation in the targeted human population. With the advances in PBPK modeling described in the next section, however, these assumptions can be tested.

**Advances in the PBPK/PD Modeling Approach**

Theoretically, PBPK models can be regarded as the "electronic copy" of the laboratory animal or human test system. "Exposure" can be simulated *in silico* and tissue dosimetry can be estimated or predicted prior to further (focused) animal testing. Mathematically, a PBPK model is a group of mass balance differential equations describing the rate of change of xenobiotics and metabolites in a simulated organism that includes the basic or more detailed physiological structures (Reddy *et al.*, 2005). Toxic effects can be studied with a linked pharmacodynamic module to examine dose-response relationships in a computer-generated environment.

*Parameters needed to build PBPK/PD models* - The procedure for developing a PBPK model is a dynamic process where model structure can include a variety of physiological, biophysical, biochemical, and pharmacodynamic parameters as illustrated in Figure 1. Physiological parameters refer to blood flow, compartment volume, cardiac output, and so on. Values and plausible ranges for many parameters can be found in Davies and Morris (1993), Brown *et al.* (1997), and U.S. EPA (2006a) and the references cited therein.

Biophysical parameters mainly include partition coefficients for parent compounds and metabolites. For the convenience of building PBPK models for pesticides, the partition coefficients in various tissues or organs in the rat and human have been predicted with computational models (Poulin and Krishnan, 1995a, 1995b, 1996a, 1996b, 1998, and 1999; Poulin *et al.*, 1999; Knaak *et al.*, 2004, 2008, and 2009). For NMCs, their partition coefficients were predicted without consideration of protein binding and reported in Knaak *et al.*, 2008. The main biochemical parameters refer to metabolic and excretionary parameters, such as $V_{max}$ and $K_m$, when the metabolism and excretion are modeled as saturable Michaelis-Menten kinetics (Belfore, 2005; Krishnan and Andersen, 2008). Usually these metabolic parameter values come from the *in vitro* studies and more recently the *in vitro* data predicted with Quantitative Structure-Activity Relationship (QSAR) models provide modelers at least initial values for their model construction in the expectation of saving much intensive lab work.

For risk assessment purposes, AChE inhibition is the toxicological endpoint for organophosphorus insecticides and NMCs. The investigation of AChE activity in the brain or red blood cells consists of the pharmacodynamic module in an intact PBPK/PD model. This

toxicological event is modeled as a bimolecular enzyme inhibition process by NMCs (Knaak *et al.*, 2008; Zhang *et al.*, 2007). The most important parameter is the bimolecular enzyme inhibition rate constant ($k_i$), which describes the inhibition capability of an inhibitor toward the enzyme. Experimentally, AChE inhibition (the $k_i$ values for 55 insecticides) has been measured in electric eel and bovine erythrocytes (Herzsprung *et al.*, 1992). These reported $k_i$ values can be used as the initial values to start a draft model. A complete review of the $k_i$ values for organophosphorus insecticides can be found in Knaak *et al.* (2004) and for the NMCs in Knaak *et al.* (2008).

*Application of the Exposure-Related Dose Estimating Model* - The U.S. EPA's National Exposure Research Laboratory has developed the Exposure-Related Dose Estimating Model (ERDEM) for PBPK/PD modeling of exposure and dose resulting from environmental chemicals (U.S. EPA, 2006c; Blancato *et al.*, 2006). The differential mass balance equations describing the disposition and toxicity of chemicals in the body can be found in Blancato *et al.* (2006). The intact package is downloadable for free from the EPA website (U.S. EPA, 2006c). The most recent downloadable version of ERDEM is Version 5.1. ERDEM exports particular model specifications, which are input into a graphical user interface (GUI), into an advanced continuous simulation language (ACSL) command file to conduct a PBPK/PD model simulation. The ERDEM modeling framework has the tools to handle a wide variety of model parameters and perform studies using both simple and complex model structures. Typically, the model has been used as a flow-limited construct. ERDEM is a robust modeling platform that allows specific simulation of mass transport and internal doses within the human body. One example of a

detailed PBPK model structure with full gastrointestinal (GI) compartments is shown in Figure 2, which has been used in a rat model for carbofuran (Zhang *et al.*, 2007).

*Model calibration/validation* - One important concept that is usually used by modelers is dose metrics. This refers to the target tissue dose that is closely related to ensuing adverse responses in an organism (U.S. EPA, 2006b). In the PBPK/PD modeling process, it is the point of interest that the modelers are trying to simulate or predict, e.g., the toxic moiety concentration in the blood or brain AChE activity in the toxicity study of NMCs. With the model structure determined and metabolic reactions/metabolites selected, the next step is to find out the values for various model parameters. It is always a challenge to find the experimental evidence or, in a better situation, there are some experimentally measured values available, but they cannot be used directly in the model. A complete literature search is necessary to reveal the variation of parameter values. Moreover, the diverse experimental data sets can be identified for possible use in model calibration and validation. With the initial values and experimental pharmacokinetic or pharmacodynamic data available, the draft model will run against the dose metrics reported in the literature. It is worth mentioning that sometimes raw data values are not directly reported in the references while only plots and curves are available. Special digitizing software, such as DigitizeIt (ShareIt!, Cologne, Germany), is needed to digitize the plots into numerical time-course values. In other situations, the mathematical units need to be converted from the *in vitro* data into *in vivo* data that can be further used in the model construction. *In vitro* metabolic studies are one of the typical examples that the unit conversion needs to be considered. Based on the results of the initial fitting, the preliminary simulation may not be satisfactory; consequently, some model parameter values may be deemed necessary for consideration of adjustment within a

reasonable range. This adjustment of parameter values is referred to as the process of parameter value optimization. The optimization process is usually iterative and the process is repeated until the best available fit to the experimental measurements is seen. The above procedures to derive a finalized PBPK model are summarized in Figure 1.

Under most situations, the model-predicted dose metrics cannot fit to all of the data sets from different studies closely and concurrently, or the dose metrics may be good for just part of the time history data. Therefore, not surprisingly, the more experimental dose metrics that are included in the simulation, the more likely it will be that discrepancies will be seen since uncertainties exist both in the model itself as well as in the experimental data sets from different studies. However, a model constructed with as many diversified data sets as possible will be more dependable, and will have less uncertainty when extrapolations are performed than a model constructed with fewer experimental data sets. A PBPK/PD model for carbofuran in the Sprague-Dawley (SD) rat was built with diverse data sets including different exposure routes and doses (Zhang *et al.*, 2007). For the purpose of evaluating goodness-of-fit, traditional statistical procedures aimed to analyze whether the underlying distributions of the two data sets are similar or not, such as t-, Mann-Whitney, two-sample $\chi^2$, and two sample Kolmogrov tests, are not applicable (U.S. EPA, 2006a). Recognizing the difficulty of regular statistical approaches, so far the most convenient and still acceptable way to judge the goodness-of-fit is by visual inspection to evaluate how close the simulated curves are to the data points (U.S. EPA, 2006a). Readers are reminded that visual judgment is affected by how the simulated curves are presented. For example, a logarithmic scale of the dose metrics will visually reduce the discrepancy between the simulated curve and the experimental data points.

Traditional one- and two-compartment kinetic models are useful in describing the kinetics of a chemical for any available data set, but these models cannot be used for extrapolating beyond the data used to develop the model (U.S. EPA, 2006a). A good and useful PBPK model is one that, theoretically, can simulate an independent data set from a different group of researchers or any independent exposure scenarios that have never been used in the model parameter optimization process. The models that are useful in risk assessment should have the capabilities that concurrently integrate diverse pharmacokinetic data under various exposure routes and are able to make prediction on tissue dosimetry or toxicity beyond the data sets used for model optimization. Since these data sets are not used for model parameter optimization, simulated results running against these data will provide strong evidence of the predictability of the model. Such models are valuable for risk assessment since they are capable of predicting the *in vivo* pharmacokinetic profiles at very low exposure levels which are applicable to human environmental exposures (U.S. EPA, 2006a). Whether a constructed model is useful or not is also decided by general model behavior. A dependable model should take into account the important pharmacokinetic characteristics of a chemical, such as the overall half-life, bioavailability, percentage of dose excreted through kidneys and bile, and dose eliminated in feces. Such an effort had been implemented in the carbofuran model construction for the SD rat (Zhang *et al.*, 2007)

*Post-Model Construction Analysis* - It should be noted that the calibrated and validated PBPK model represents the average individual in laboratory animal or human populations. The model parameter values stands for the population mean and do not reflect population variability. Thus,

compared with the probabilistic approach, the constructed model is deterministic. Sometimes, it is called a baseline model by some modelers (Zhang *et al.*, 2007). After a model has been completed with a fit to all the available experimental data as satisfactorily as possible, modelers may further perform extra analyses on the finished model. These analyses are generalized here as post-model construction analysis, which includes sensitivity analysis for input model parameters, variability analysis for model output predictions, and uncertainty analysis for input model parameters. The concepts and methodologies of these analyses will be briefly discussed here. Readers can find more discussion on these topics in Chiu *et al.* (2007). Sensitivity analysis is meant to find out how model input parameter values influence the estimates of the dose metrics or model predictions. When only one parameter varies at a time, it is named as local (or functional) sensitivity analysis, while global sensitivity analysis refers to all parameters being varied simultaneously. Parameters that have the greatest impact on the model outputs of interest can then be studied in future efforts to reduce the uncertainty of these key players. For detailed methodology to perform sensitivity analysis, readers are referred to U.S. EPA (2006a) for principles and Zhang *et al.* (2007) for an example of sensitivity analysis in which only one parameter value was perturbed at one time while all the others were held constant (local sensitivity analysis).

The next step is variability analysis which is meant to evaluate the impact of the variability of model input parameters on the variability of the model output (dose metrics). The model calibrated with experimental data represents only the average individual of an animal or human population. By considering the fluctuation of input model parameter values in a population, the population range or variability of the dose metrics needs to be known. For this purpose, Monte

Carlo sampling techniques based on the distributions of input parameters have been used. Readers can go to U.S. EPA (2006a) for further details and can find the examples in the cited references therein. In performing the Monte Carlo simulations, it should be noted that dependency among some model parameters should be considered whenever a parameter value is perturbed in sensitivity and variability analysis. The rest of the dependent parameters will also need to change their values in order to keep the mass balance. For example, the fractional blood flows and compartment volume should be summed to 100%. No matter what value will be selected to perturb for any parameter, the rest of the parameter values need to be updated in order to keep the total as 100%. An example with such a consideration can be found in Zhang *et al.* (2007).

Uncertainty analysis for PBPK models will evaluate the impact of lack of information about either the numerical values of model parameters or model structure on model predicted dose metrics (U.S. EPA, 2006a). Uncertainty analysis is particularly useful when a PBPK model does not simulate the experimental data well enough. Quantitative uncertainty analyses can be performed by using a traditional Monte Carlo approach, a Bayesian Markov chain Monte Carlo analysis, a stochastic response surface method, and a fuzzy simulation approach (U.S. EPA, 2006a and the references therein). In general, sensitivity, variability, and uncertainty analyses can improve the credibility of PBPK models and can also help prioritize research needs to reduce uncertainties in the developed model used in risk assessment. So far such analyses may not be required for all PBPK models for the purpose of risk assessment (U.S. EPA, 2006a).

## APPLICATION OF THE CONSTRUCTED PBPK/PD MODEL

**Toxicity Study of Carbofuran**

The PBPK/PD model for carbofuran has been constructed for the SD rat (Zhang *et al.*, 2007) and the corresponding human model was derived by replacing the rat physiological structure with that of the human and compensating for the lower oxidation of carbofuran. The physiological structure included arterial blood, brain, skin, fat, GI-tract, kidney, liver, rapidly perfused tissue, slowly perfused tissue, static lung, portal blood, and venous blood. Non-perfused tissue was not modeled as a separate compartment. A full GI compartment model including stomach, duodenum, lower small intestine, and colon was utilized to better describe carbofuran GI absorption, biliary circulation, and fecal elimination with considerable elaboration (Figure 2). Both the parent and its oxidized metabolite, 3-hydroxy carbofuran, are AChE inhibitors (Herzsprung *et al.*, 1992 and Knaak *et al.*, 2008). With this consideration, a complete metabolic pathway for carbofuran was incorporated into the model for SD rats (Zhang *et al.*, 2007). The AChE inhibition process was modeled as bimolecular inhibition process (Hetnarski and O'Brien, 1975) using a bimolecular enzyme inhibition rate constant ($k_i$) as indicated in Figure 3A.

Once going through all the necessary procedures mentioned above, the constructed PBPK/PD model can be further used for various applications. With the cabofuran model in the SD rat available (Zhang *et al.*, 2007), the dose-response relationship by oral exposure in the rat was studied by simulating a series of exposure doses using ERDEM. These results are not published anywhere else and they are presented here only for the purpose of demonstrating the methodology. The simulations were based on the scenario reported by Ferguson *et al.* (1984)

which was used as the major data set for model construction. The NOAEL was then selected from the dose-response curve by targeting a 10% AChE inhibition in the blood ($BMD_{10}$). A dose range from 1 to 5000 μg/kg was simulated at the needed increment from 5 to 1000 μg/kg by oral exposure to the SD rat. Time, carbofuran dosage, and AChE activity (% of control) was plotted in a 3-D curve (Figure 4). Without considering the time to reach the minimum activity (or maximum inhibition), the minimum AChE activity under each dosage was plotted against the exposed dosages. This minimum AChE curve is actually the bottom (the blade) of the 3-D valley-shaped sheet. For convenience of presentation, this bottom curve was plotted in a logarithmic time-course change shown as an S-shaped curve in Figure 5. The toxicological endpoint, such as NOAEL or BMD, could be determined from this curve. As a result, a NOAEL or $BMD_{10}$ value (central estimation) of 10 μg/kg was determined by targeting 90% of control AChE activity (10% inhibition) in red blood cells (RBC) as the endpoint. This $BMD_{10}$ value was actually the same as what was used for the critical toxicity endpoint in the risk characterization for carbofuran in the California Environmental Protection Agency (Cal/ EPA) report (Cal/EPA, 2006) in which an acute regulatory $LED_{05}$ value (the lower bound on the effective dose at the 95% confidence limit) of 0.01 mg/kg was assigned. The advantage that can be seen here is that the application of the constructed PBPK/PD model enables the dose-response study and the toxicological endpoints to be estimated *in silico,* something which cannot be easily achieved by regular bench work. But readers are reminded again that only when a well-calibrated and validated PBPK/PD model is constructed would these computerized study findings be useful.

**Construction and Application of a Cumulative PBPK/PD Model of Three NMCs**

When multiple chemicals of a CMG are studied for cumulative toxicity, a model will be developed that includes all parent chemicals plus corresponding metabolites and associated metabolic pathways. Furthermore, when multiple exposure pathways are to be examined, the cumulative model should also include multiple routes of entry. Therefore, a cumulative PBPK/PD model is one that simulates concurrent multiple chemical exposures and includes all related routes of entry. Such a PBPK/PD model for a mixture has no formal nomenclature. The term, cumulative model, is used here for convenience. A cumulative model is built by combining the individual PBPK/PD models for each chemical together into one system. These individual models must have gone through the model construction procedures including model parameter optimization, model calibration/validation, and post-model construction analysis in addition to quality assurance so that confidence on using these models can be established.

A PBPK/PD model for the binary mixture of chlorpyrifos and diazinon in the rat has been built (Timchalk and Poet, 2008). Similarly, a cumulative model for three NMC insecticides (carbaryl, carbofuran, and aldicarb) has been constructed (Zhang *et al.*, 2008, 2009a, and 2009b). For simplification, the completed individual model for each exposed chemical may not necessarily be entirely included into the cumulative model. For example, metabolites which are not used as biomarkers can be removed from the individual mode. Only those aspects which are related to the dose metrics of interest or toxicities are needed while the same physiological structures are kept. The simplified individual models have a reduced number of parameters which lessens the burden of computer simulation for the assembled cumulative model. In the cumulative model of three NMCs, the interactions from the mixture of AChE inhibitors, e.g., the competitive interaction from all enzyme inhibitors, are not considered in the model. This individual action

toward AChE activity was shown in Figure 3B. However, if there is strong evidence to show a competitive interaction, such as competition for the metabolic enzymes (e.g., cytochrome P450) among metabolic substrates, then the competition for the enzymes by various substrates should be considered. If the exposure level is very low such that the tissue concentration of one substrate is far less than its corresponding $K_i$ value ($I<<K_i$), then the competition for the metabolic enzyme by this substrate would be ignored. This rule can be verified in the equation of competitive action toward the metabolic enzyme shown in Equation (2), when the mechanism is described by the competitive Michaelis-Menten kinetics.

Equation (2) $$V = V_{max} \frac{[S]}{K_m(1+\frac{[I]}{K_i})+[S]}$$

where V is the reaction rate; $V_{max}$ is the maximum reaction rate for one substrate; $K_m$ is its Michaelis constant; [S] is its concentration; [I] is the concentration of the competitor; and $K_i$ is the Michaelis constant of the competitor.

*Exposure Assessment as Input for the Cumulative Model* - To use the cumulative model in risk assessment forwardly (compared to using it for exposure reconstruction), the model required exposure input that describes the potential chemical exposure at the time of contact with human body. The methodology is briefly summarized in Figure 6. For pesticide exposure, the major sources include food and drinking water ingestion, contact with residues in breathable air, and dermal contact with surfaces (U.S. EPA, 2002a).

The cumulative PBPK/PD model simulated aggregate exposure to three NMC insecticides: 1) carbaryl from food intake, 2) aldicarb in drinking water via drinking water ingestion, and 3) carbofuran in food via dietary ingestion. Exposure inputs for the three NMCs via the oral pathway were taken from outputs from the Stochastic Human Exposure Dose Simulation (SHEDS) model (Zartarian *et al.,* 2007). SHEDS as developed by the U.S. EPA National Exposure Research Laboratory is a probabilistic model that predicts longitudinal 1-year exposure profiles for pesticide exposure assessment (U.S. EPA, 2007; Zartarian *et al.*, 2000). The PBPK/PD model for aldicarb was developed parallel to carbaryl and carbofuran models (Zhang *et al.*, 2008, 2009a, and 2009b).

*Application of the Cumulative NMC Model for Cumulative Risk Assessment* - In this section, the methodology of using the cumulative model to predict the toxicological endpoints will be demonstrated. Readers should be aware that this pilot work has not as yet been published in peer-reviewed scientific journals and is only cited to demonstrate its potential feasibility. It is recommended that readers focus on the process rather than emphasizing the results or making any conclusions. The resulting exposure event timeline scenarios were run to predict the outcome using the cumulative NMC PBPK/PD model (Zhang *et al.*, 2008). The simulation results were then projected to the U.S. population (Table 3). Distributions of AChE activities in RBCs and brain, and urine biomarker concentrations were evaluated. The model prediction of the minimum AChE activities in the blood and brain were above 99.99% of control level for all age and gender groups at the 95th percentile (Table 3). These findings were expected to be consistent with levels of the urinary biomarkers for carbaryl and carbofuran in the same general population (Zhang *et al.*, 2008). Conceptually, model predictions of elimination are expected to

agree with biomonitoring findings for the same or similar human populations. Substantial or significant differences between model predictions and biomonitoring results might indicate model deficiencies or unaccounted pathways and routes of exposure when biomonitoring results are greater than modeling outcomes.

To evaluate how close these predictions were to reality, the modeling outcomes were compared with the National Health and Nutrition Examination Survey (NHANES) biomonitoring results reported by the U.S. Centers for Disease Control and Prevention (CDC)'s National Center for Health Statistics (CDC, 2005). NHANES assesses the exposure of the U.S. population to environmental chemicals using biomonitoring in which chemicals or their metabolites were measured in blood and urine samples. In one example (carbaryl), the modeled cumulative elimination of 1-napthol was significantly lower than the measured concentrations at the $90^{th}$ and $95^{th}$ percentiles for groups in the national population sorted by age and gender (Table 4). This disparity might be attributed to the commonality of 1-napthol as a biomarker for other chemicals or the incompleteness of the exposure that might involve additional unrepresented pathways and routes in the model simulation for carbaryl. This is one of the weaknesses that the readers should be aware of in using the PBPK method for CRA. The reliability of using the PBPK model approach will be limited if the exposure inputs to the model are only partial.

Related to carbofuran, carbofuran phenol was measured as the biomarker of carbofuran exposure. The model simulated biomarker levels were in trace level and close to what had been detected (Zhang *et al.*, 2008). Even though this comparison did not use any statistical procedure due to the lack of information on both sampled populations, it shows that the application of the

cumulative model demonstrates a plausible method for *in silico* human population risk assessment. However, results from NHANES will be the final gold standard for comparison.

## ADVANTAGES AND WEAKNESSES OF THE CUMULATIVE PBPK/PD STRATEGY

The PBPK/PD model approach showed progress in simulating the exposure scenarios in a more pharmacologically representative fashion, thus providing another choice in the analysis of the cumulative risk from random exposures. Moreover, toxicological endpoints such as AChE activity and urinary biomarker levels could be studied directly at any time without uncertainty factors. Therefore, a PBPK/PD model approach permits the CRA to be performed with more realistic assumptions, which provides a more realistic risk assessment by closely simulating the exposure scenarios and making fewer assumptions. But in the SHEDS simulations, the cumulative model for the three NMCs considered only body weight as the individual difference. Population variations such as compartment volumes, blood flows, and metabolic polymorphisms were not included (Zhang *et al.*, 2008) even though they were technically plausible. This study demonstrated that the use of a composite PBPK/PD model linked to an exposure model, such as SHEDS, for pesticide residues in humans may provide a promising way to do *in silico* human population risk assessment.

The method of using PBPK/PD modeling in CRA has been regarded as one of the approaches, but only when a highly refined assessment is needed (Boobis *et al.*, 2008). It should be noted that not all PBPK models are useful for risk assessment applications. If the ultimate goal of developing a model is for this purpose, the requirements for the quality of the model should be

higher. Therefore, this data intensive task will require experimental studies to be developed specifically for modeling purposes. Experimental pharmacokinetic data targeted for laboratory animal PBPK model construction are needed. Meanwhile, a complete set of standardized pharmacokinetic data should be performed, preferably by the same group of researchers. But for human models, it is always a challenge to have any data related to the dose metrics concerned in risk assessment. Alternatively, a human model must be built by extrapolation from an animal model. Therefore, the uncertainty during the model extrapolation may be carried down to the human model. As mentioned previously, PBPK modeling provides the foundation for the study of dose metrics in a targeted human population by the stochastic technique. To do so, the model parameters need to reflect the population variability and distribution in order for the Monte Carlo sampling technique to be implemented. This information is still a challenge to all modelers. To demonstrate model reliability, which is especially important for regulatory decision making, formal statistical analyses are necessary; e.g., uncertainty analysis for the model and Bayesian analysis for parameter optimization. Intensive computer-based data analyses are not always possible for every risk assessor. Even though the range and distribution have been determined for those physiological parameters (U.S. EPA, 2006a), the parameters such as partition coefficients, metabolic parameters, and even pharmacodynamic parameters still need more investigation. Therefore a true meaningful population model simulation will require more research

## FUTURE NEEDS FOR THE APPLICATION OF PBPK MODELING IN RISK ASSESSMENT

First of all, the use of PBPK modeling in risk assessment has been increasingly regarded as an important component of chemical risk assessment. Obviously, uncertainties still exist in the developed PBPK models in the aspects of model structure, parameter values, or even experimental data used for parameter optimization. But should the existence of these uncertainties be an obstacle for the use of the PBPK models in risk assessment? Frontier application of computational technique will serve as light guiding us walking through the "dark room" of risk assessment. "Better judgments are made as result of the light, even a dim one" (Blancato, 2009). More investigations aiming to reduce the uncertainties are needed. Second, the model structures and the parameters used to describe the absorption, distribution, metabolism, excretion, and toxicity (ADMET) need international harmonization so that models from different researchers can be convertible or even "cloned" into another system. Different modelers may use different model parameters to describe the same biological process. For example, to describe GI absorption, the absorption rate constant ($K_a$, $h^{-1}$) has been used for chlorpyrifos (Timchalk *et al.*, 2002) and carbofuran (Zhang *et al.*, 2007), while the oral absorption fraction ($f_{abs\text{-}oral}$) was used in a malathion model (Bouchard *et al.*, 2003). A standardized or recommended set of model parameters needs to be available for use by modelers. In addition, there needs to be an effort to perform experiments and standardize the measurement of parameter values. Thirdly, Good Modeling Practice (GMP) for PBPK model development, characterization, documentation, and evaluation has been proposed (Loizou *et al.*, 2008). The development and implementation of GMP for PBPK modeling will increase the transparency of model development and model documentation. These efforts will make the work of quality assurance and quality control more efficient and can increase the credibility of a constructed model so that the developed PBPK model can be used for risk assessment with more confidence.

## DISCLAIMER

Although this work was reviewed by the U.S. EPA and approved for publication, it does not necessarily reflect official Agency policy or the official views of General Dynamics Information Technology (GDIT). Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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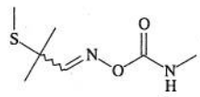
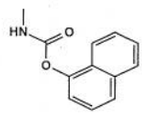
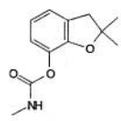
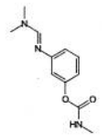
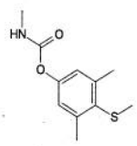
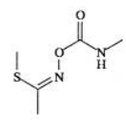
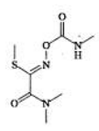
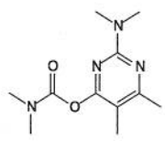
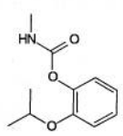
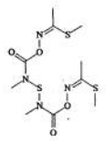
**Table 1**
General Information on the Carbamate Insecticides[a]

| Common Name | *N*-methyl Carbamate Class | Molecular Weight | CAS No. | Chemical Structure |
|---|---|---|---|---|
| Aldicarb | oxime | 190.26 | 116-06-3 |  |
| Carbaryl | aryl | 201.20 | 63-25-2 |  |
| Carbofuran | aryl | 221.25 | 1563-66-2 |  |
| Formentanate | aryl | 221.26 | 22259-30-9 |  |
| Methiocarb | aryl | 225.31 | 2032-65-7 |  |
| Methomyl | oxime | 162.21 | 30558-22-0 |  |
| Oxamyl | oxime | 219.26 | 23235-22-0 |  |
| Pirimicarb | aryl | 238.29 | 23103-98-2 |  |

| | | | | |
|---|---|---|---|---|
| Propoxur | aryl | 209.24 | 114-26-1 |  |
| Thiodicarb | oxime | 354.47 | 3919618-4 |  |

[a]Revised from Knaak *et al.* (2008)

**Table 2**
Non-PBPK-Modeling Methods for the CRA for the Compounds in the Same Cumulative Assessment Group

| Methodology | Criteria used for CRA | Algorithm | Acceptable level |
|---|---|---|---|
| Index method | Hazard Index (HI) | $HI = \frac{Exp1}{RV1} + \frac{Exp2}{RV2} + \frac{Exp3}{RV3} + \cdots$ | HI < 1 |
| Index method | Cumulative risk index (CRI) | $CRI = \frac{1}{Exp1/_{RV1} + Exp2/_{RV2} + Exp3/_{RV3} + \cdots}$ | CRI > 1 |
| Index method | Reference point index (RPI) | $RPI = \frac{Exp1}{RP1} + \frac{Exp2}{RP2} + \frac{Exp3}{RP3} + \cdots$ | RPI < 1 |
| Margin of exposure | Combined margin of exposure ($MOE_T$) | $MOEt = \frac{1}{(1/_{MOE1}) + (1/_{MOE2}) + (1/_{MOE3}) + \cdots}$ | $MOE_T$ > 100 |
| Potency factor | Relative potency factor (RPF)[a] | $MOE = \frac{PoD_{index}}{\sum_{i=1}^{n} \text{Residue}_i \times PF_i \times RPF_i}$ | MOE > 10 |

Summarized from Boobis *et al.* (2008)
[a]RPF method converts other chemicals to the equivalent doses of the selected index compound to derive the total equivalent exposure.
MOE = margin of exposure; RV = reference value; PF = processing factor; PoD = point of departure.

**Table 3**
Cumulative model simulation for the minimum AChE activity (% of control) in the blood and brain using the SHEDS exposure data for one day and a seven-day longitudinal study

| Simulation duration | Age groups | n | Compartment | Mean | Selected Percentiles | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 25th | 50th | 75th | 90th | 95th |
| One day [a] | 6-59 | 19,724 | Brain | 100 | 100 | 99.9998 | 99.9993 | 99.9966 | 99.9931 |
| | | | Venous blood | 100 | 100 | 99.9999 | 99.9996 | 99.9981 | 99.9964 |
| Seven days [b] | 6-14 | 2,602 | Brain | 99.9966 | 100 | 99.9999 | 99.9986 | 99.9953 | 99.9911 |
| | | | Venous blood | 99.9982 | 100 | 99.9999 | 99.9992 | 99.9973 | 99.9954 |
| | 15-59 | 7,589 | Brain | 99.9974 | 100 | 99.9998 | 99.9992 | 99.9971 | 99.9936 |
| | | | Venous blood | 99.9983 | 100 | 99.9999 | 99.9996 | 99.9983 | 99.9966 |

[a] SHEDS database provided two non-consecutive days of oral exposure data. They were treated as two days in terms of person-days. The distribution was for SHEDS sample population instead of projected US population.
[b] The distribution is based on the projected 1995 US population.

**Table 4**
Cumulative model simulation for the urinary biomarker (1-naphthol) concentration for carbaryl using the SHEDS exposure data for one day and seven-day longitudinal studies and comparison with NHANES

| Study | Age groups | n | Unit of urinary concentration [a] | Mean | Selected Percentiles | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 25th | 50th | 75th | 90th | 95th |
| NHANES (2001-2002) | | 2,748 | µg/L | — [b] | 0 | 1.72 | 4.76 | 12.5 | 22.3 |
| Meeker *et al.* (2007) | | 370 | µg/L | — | 0 | 2.86 | 4.49 | 7.61 | 13.3 |
| One day [c] | 6-59 | 19,724 | µg/L | 0.213 | 0 | 0 | 0 | 0.171 | 0.620 |
| Seven days [d] | 6-14 | 2,602 | µg/L | 0.322 | 0 | 0 | 0 | 0.251 | 0.884 |
| | | | µg/g Cn | 0.364 | 0 | 0 | 0 | 0.266 | 0.948 |
| | 15-59 | 7,589 | µg/L | 0.202 | 0 | 0 | 0 | 0.170 | 0.582 |
| | | | µg/g Cn | 0.186 | 0 | 0 | 0 | 0.151 | 0.534 |

[a] Urinary concentration was calculated based on the cumulative excretion normalized either by the volume or the creatinine (Cn).
[b] — not reported.
[c] SHEDS database provided two non-consecutive days oral exposure data. They were treated as two days in terms of person-days.
[d] The distribution is based on the projected 1995 US population.

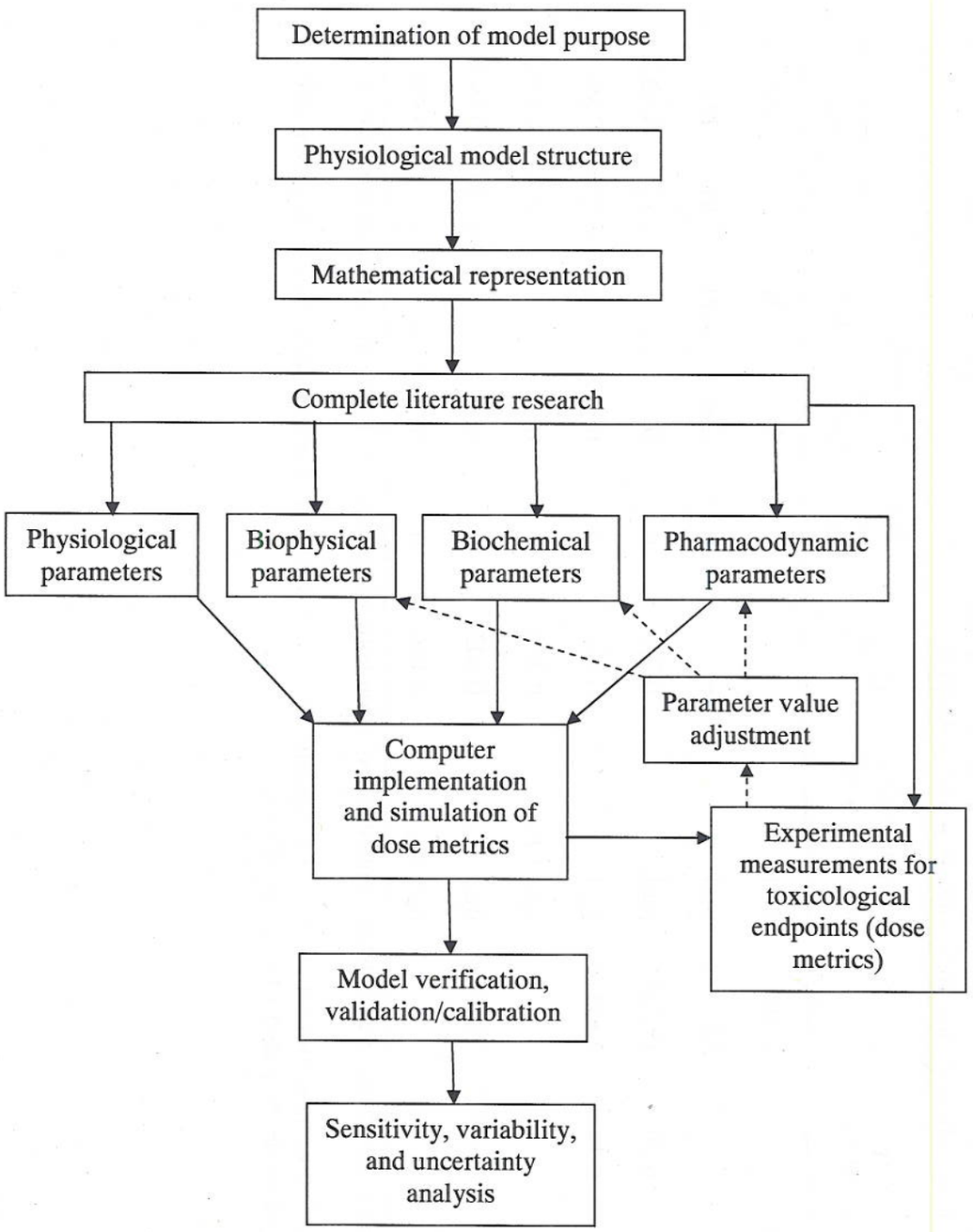



**Figure 1** Procedures for the construction of PBPK/PD models. Dashed arrows indicate the need for parameter value adjustment within a reasonable range.

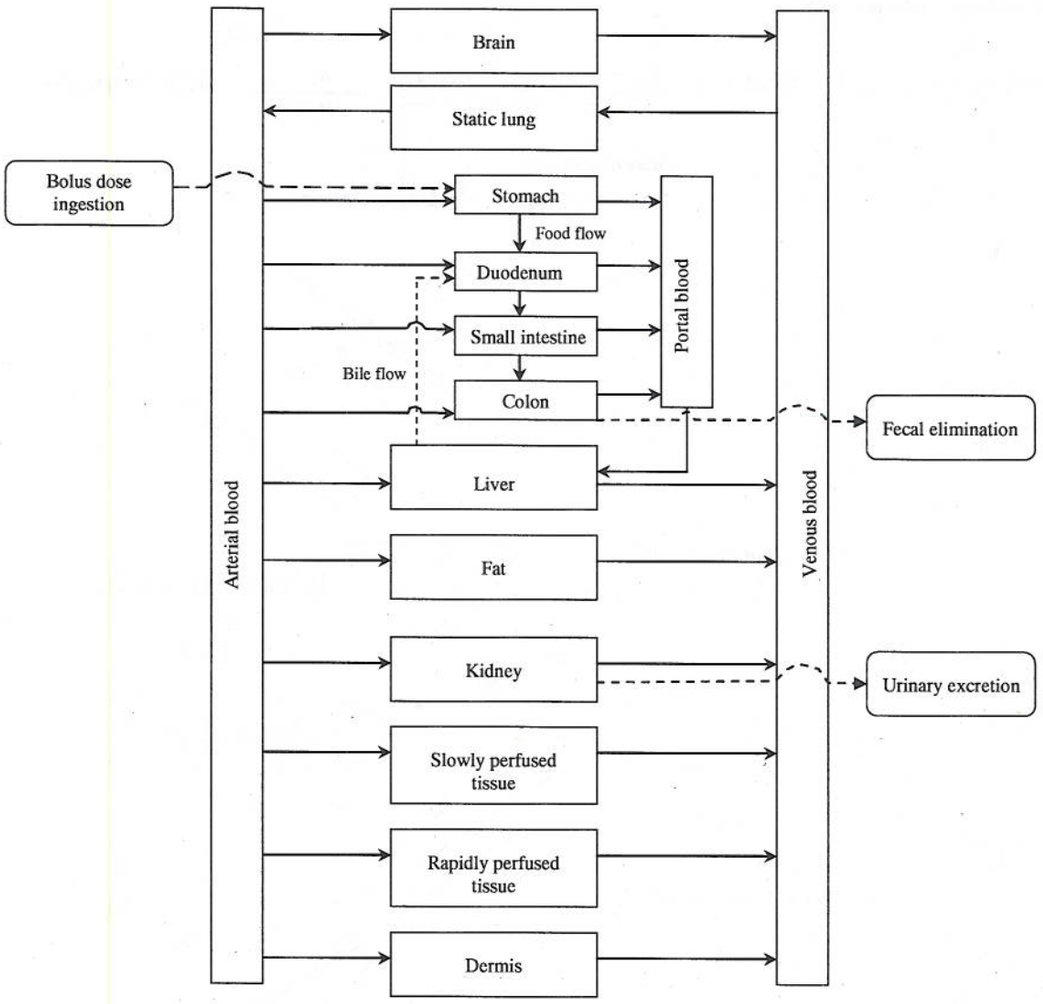



**Figure 2** ERDEM PBPK/PD model structure in the rat for the exposure scenario of bolus oral ingestion of carbofuran. A full gastrointestinal compartment including enterohepatic circulation of glucuronic acid conjugates was implemented (Zhang *et al.*, 2007) (cited with permission).

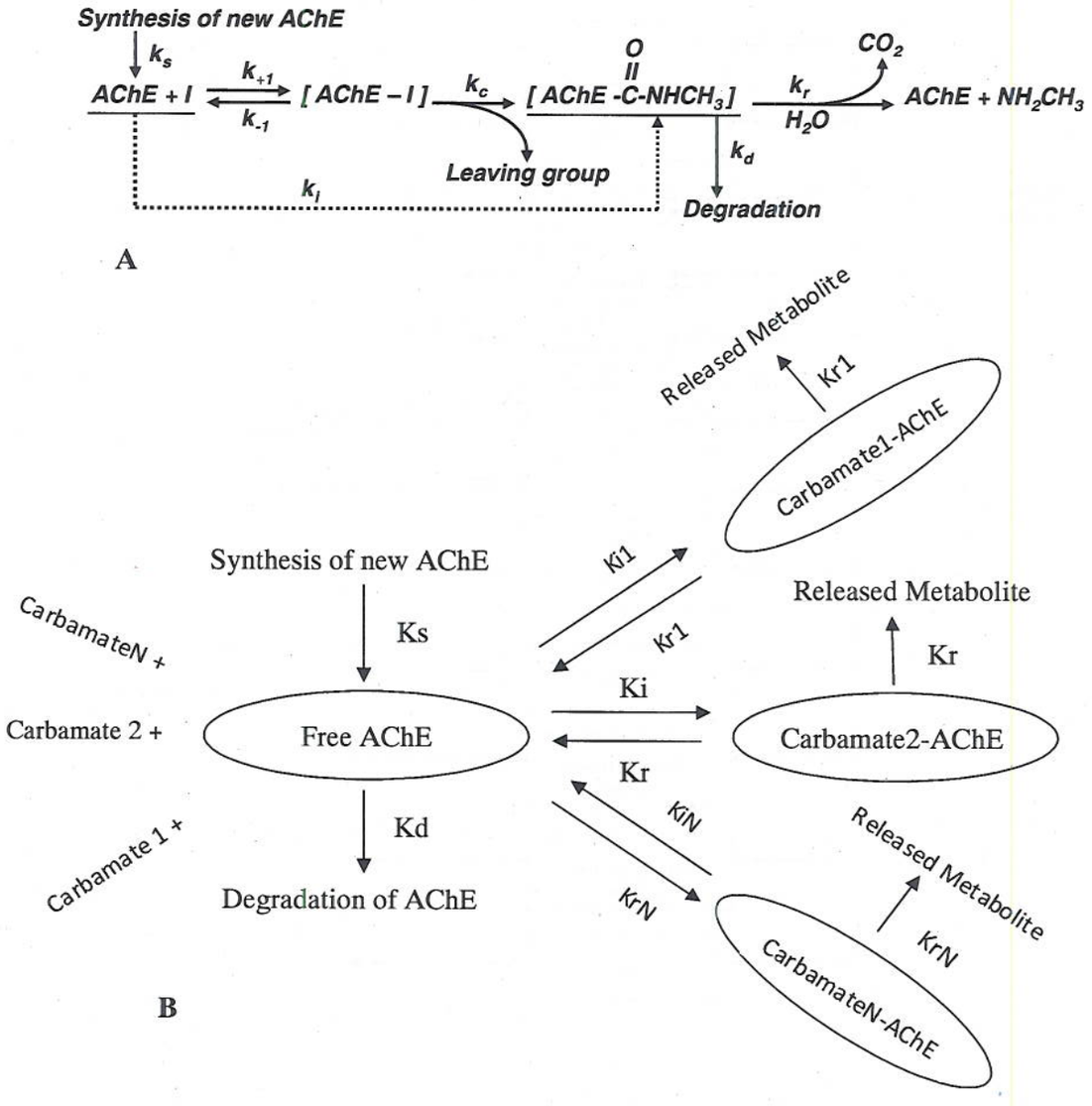
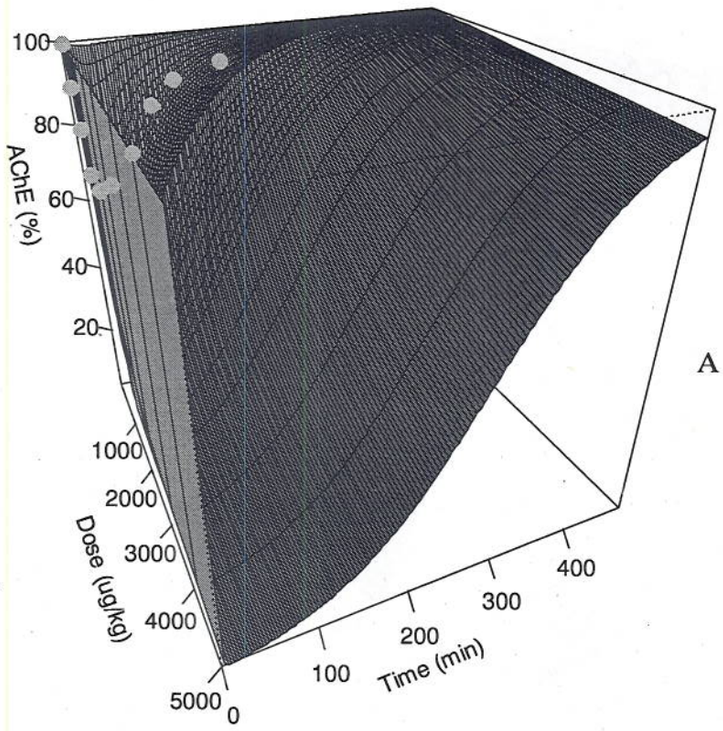



**Figure 3** Pharmacodynamic model for AChE inhibition by carbofuran and its metabolite, 3-hydroxycarbofuran (A) (Zhang *et al.*, 2007) (cited with permission), and the model in the cumulative PBPK/PD model for NMCs (B). There is no interaction among the members of NMCs and neither is there competition from the NMCs with AChE. I = Inhibitor; $k_s$ = resynthesis rate constant of enzyme; $k_d$ = degradation rate constant of the inhibited enzyme; $k_r$ = regeneration rate constant of the inhibited enzyme; $k_i$ = enzyme inhibition rate constant; $k_{+1}$, $k_{-1}$, and $k_c$ were not modeled.


100
80
60
40
20
AChE (%)
1000
2000
3000
4000
5000
Dose (ug/kg)
0
100
200
300
400
Time (min)
A

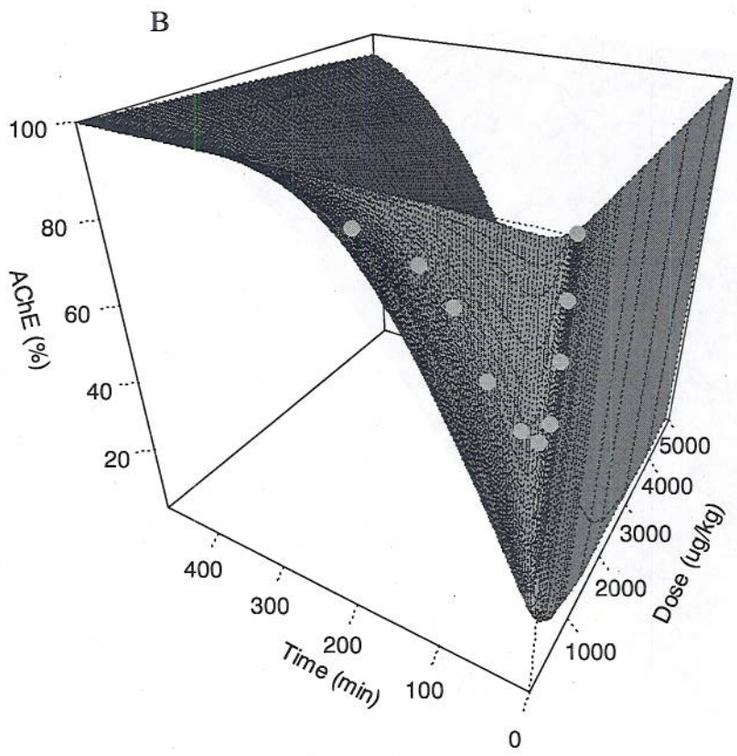



**Figure 4** Three-dimensional plotting of the time course of AChE activity in the blood simulated by the model at different dosages administered orally to the rat. A= front view. B = back view. The dots represent the experimental data reported by Ferguson *et al.* (1984).

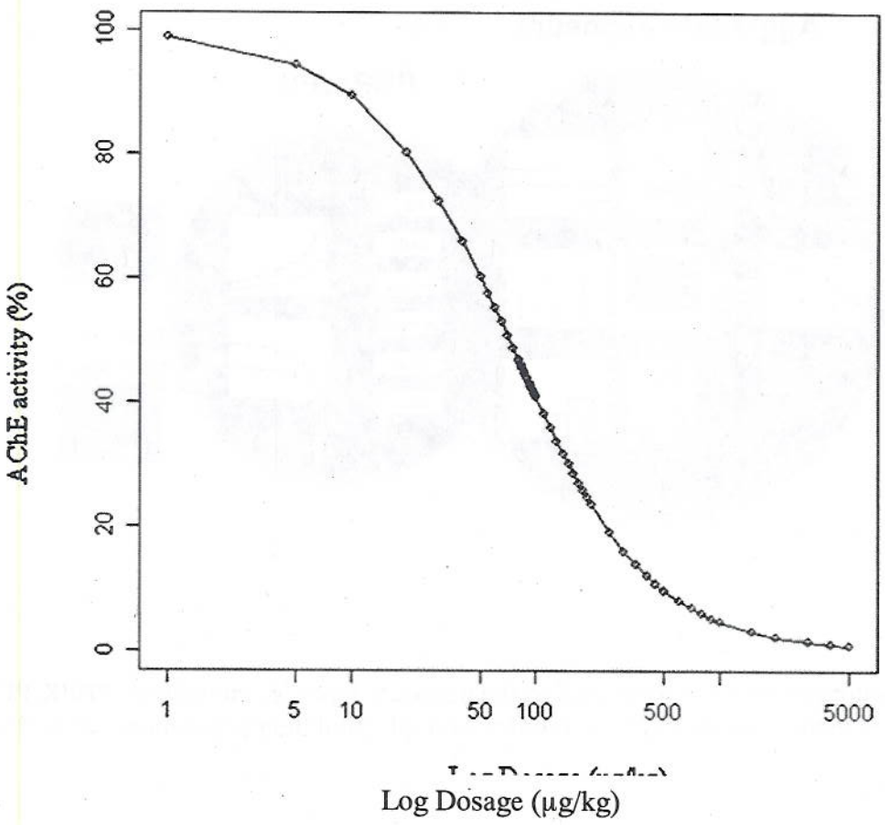



**Figure 5** Minimum AChE activity (% of control) in the blood plotted with logarithmic change of different dosages after carbofuran oral exposure to the rat.

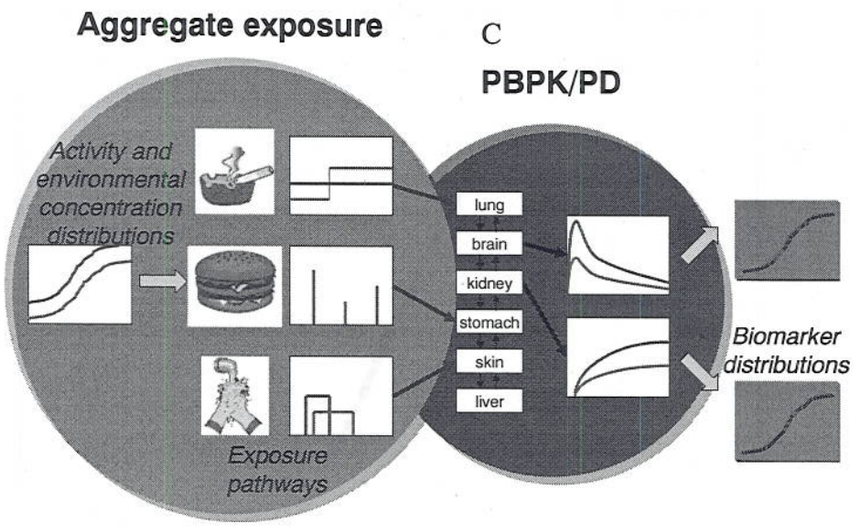



**Figure 6** Aggregate exposures are used as the exposure input for cumulative PBPK/PD models so that the dose metrics (biomarker level in this example) and their population variability can be predicted.