Charge the SAP for the October 24-27 2017

Meeting on Physiologically Based Pharmacokinetic Modeling to Address Pharmacokinetic Differences Between and Within Species

The 2009 National Research Council report "Science and Decisions"[1] recommends that the agency uses the best, most current science, to support or revise the default assumptions in the agency's risk assessments. In addition, the 2013 Institute of Medicine report on "Environmental Decisions in the Face of Uncertainty"[2] further recommends replacing default uncertainty factors with data-derived extrapolation factors (DDEFs) that delineate the differences between and within species, which would decrease uncertainty in risk assessment. DDEFs for inter- and intra-species extrapolation can be estimated using physiologically based pharmacokinetic (PBPK) modeling to organize data that describe the absorption, distribution, metabolism, and excretion (ADME) of chemicals that enter the body.

PBPK models incorporate the relevant biology that determines the ADME processes, and thus, they can be used to predict internal dosimetry related to a certain chemical within and outside the testing conditions of specific studies (e.g., species, dose ranges). PBPK models have been used to assist high-to-low dose, route-to-route, and inter-species extrapolations necessary for estimating human health risks on the basis of animal toxicity studies. The physiological structure of PBPK models also allows for examining the effects of changing physiology, such as aging or early life-stage. The agency has recognized that PBPK model analysis is a scientifically sound approach to estimate the internal dose of a chemical at a target site, and as a means to evaluate and describe the uncertainty in risk assessment (EPA, 2006)[3]. Several registrants, including the Council for the Advancement of Pyrethroid Human Health Risk Assessment (CAPHRA), Tessenderlo Kerley Inc. (TKI), FMC, and Syngenta, recently approached the agency with their intention to develop PBPK models for dimethoate, malathion, carbaryl, deltamethrin, permethrin, and acibenzolar, for supporting chemical-specific risk assessment.

The 2006 EPA document on "Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment" and the 2010 World Health Organization (WHO) document on "Characterization and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment"[4] recommend that evaluation of PBPK models intended for risk assessment applications should include considerations for model purpose and scope, model structure and biological characterization, mathematical representation of absorption, distribution, metabolism, excretion (ADME), parameter estimation and analysis, computer implementation, and model predictive capacity, along with sensitivity, variability, and uncertainty analyses.

---

[1] National Research Council. 2009. Science and Decisions: Advancing Risk Assessment. Washington, DC: The National Academies Press. https://doi.org/10.17226/12209.

[2] Institute of Medicine. 2013. Environmental Decisions in the Face of Uncertainty. Washington, DC: The National Academies Press. https://doi.org/10.17226/12568.

[3] U.S. Environmental Protection Agency. 2006. Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment (Final Report). Washington, D.C., EPA/600/R-05/043F.

[4] World Health Organization. 2010. Characterization and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment. International Programme on Chemical Safety Harmonization Project Document No. 9. Geneva, Switzerland.

Consistent with the recommendations set forth in these guidance documents, the agency will solicit comments from the members of the October 2017 Scientific Advisory Panel (SAP) on the evaluation of the models for carbaryl, deltamethrin, and *cis*-permethrin for their capabilities to predict internal dose metrics in humans from birth to adulthood, as well as to estimate DDEFs and derive scenario-specific human points of departure (PoDs) for use in human health risk assessment. The members of the SAP will also be asked to comment on the potential utility of a population-based *in vitro* to *in vivo* extrapolation (IVIVE)-PBPK modeling approach carried out by Syngenta; who utilized acibenzolar as a case example, to facilitate the use of *in vitro* and *in silico* tools for a more efficient and predictive human health risk assessment. Finally, the agency will seek advice from the SAP members to refine the rat PBPK/PD models for malathion and dimethoate, so that these two models may be used in the near future to inform the development of human models for predicting the human pharmacokinetic (PK) and pharmacodynamic (PD) properties from exposure to these two chemicals, and to ultimately derive DDEFs and/or scenario-specific human PoDs.

1. Carbaryl is an *N*-methyl carbamate (NMC) pesticide. NMCs share the ability to inhibit acetylcholinesterase (AChE) inhibition via carbamylation of the active site. NMCs are characterized by rapid onset of AChE inhibition (15-60 minutes) and rapid reactivation of the enzyme, leading to ½ lives of recovery within 1-4 hours. The human life-stage PBPK/PD model for carbaryl predicts the disposition of carbaryl and its inhibition of AChE in red blood cells (RBC) and in brain over time.

a) Please comment on whether the model structure is appropriate for describing the PK and PD properties of carbaryl, such as the assumptions of diffusion-limited compartments, the inclusion of major metabolic pathways, descriptions of oral, dermal and inhalation routes, and incorporations of cholinesterase binding in blood and other tissues.
b) Please comment on the correctness of mathematical equations used to describe the model structure, and their implementation in the programming language R. Additionally, comments are needed on the appropriateness of the integration of algorithm and integration internals selected for this model. Lastly, comments are needed on the mass balance of the model.
c) Please comment on the selection and/or estimation of values and distributions for all model parameters, including age- and gender-dependent physiological parameters and enzyme ontogeny, tissue/blood partition coefficients, metabolic constants, and pharmacodynamic parameters that determine the binding of carbaryl to cholinesterase's active site, carbamylation of the active site, and subsequent reactivation of enzyme activity.
    i. Please comment on whether the model properly accounts for human variability in PK and PD by considering age- and gender-differences.
    ii. Please comment on the correctness of allometric scaling of parameters. Please comment on the appropriateness of methods used to extrapolate *in vitro*-derived data (e.g., metabolism constants) to an *in vivo* system. Please comment on the IVIVE and enzyme ontogeny approach for estimating intrinsic clearance for humans at different ages.
    iii. Please comment on the justification and appropriateness of using empirical ratios to adjust an *in vitro* measured bimolecular rate to fit *in vivo* AChE inhibition data in brain and in plasma.

d) Please comment on the evaluation of model performance to simulate the general trend of published human time-concentration data, as well as time profiles of AChE inhibition in RBC.
e) Please comment on the results from sensitivity and uncertainty analyses, and their implications on the estimation of DDEFs and derivation of scenario-specific human PoDs.
f) Please comment on the approach used to determine the most sensitive age and gender combination that results in the highest peak AChE inhibition in RBC and in brain. Please comment on the subsequent use of the most sensitive age and gender combination for a specific exposure scenario to estimate the PoD for that scenario.
g) Please comment on the appropriateness of the Monte Carlo simulations in estimating distributions of the peak AChE inhibition in RBC and in brain and for use in DDEF derivation.
h) In the context of panel responses to 1a-1g, please comment on the appropriateness of the carbaryl PBPK/PD model for use in human health risk assessment to
    - replace the default inter-species and intra-species with the DDEFs estimated using the human life-stage PBPK/PD model
    - derive scenario-specific PoDs.
i) In the context of panel responses to 1a-1g, please comment on the potential to apply a read-across approach that uses one generic model structure in conjunction with chemical-specific parameter values, primarily *in vitro* metabolism and AChE binding measurements, to construct PBPK/PD models for other NMCs.

2. Permethrin and deltamethrin are synthetic pyrethroids. Synthetic pyrethroids share the ability to interact with voltage-gated sodium channels (VGSCs) in the central and peripheral nervous systems, leading to changes in neuron firing and, ultimately, neurotoxicity. The toxicity profiles for pyrethroids are characterized by rapid absorption, metabolism, and time-to-peak effect. The single dose and repeat dosing studies show that repeat exposures do not result in lower PoDs (i.e. there is no evidence of increasing toxicity with an increased duration of exposure). Therefore, for the purpose of exposure assessments, only single day risk assessments need to be conducted for permethrin and deltamethrin. The human life-stage PBPK models for deltamethrin and *cis*-permethrin predict the disposition of deltamethrin and cis-permethrin, respectively, over time.

a) Please comment on whether the model structure, which is the same for both deltamethrin and *cis*-permethrin and the same for rats and humans, is appropriate for describing the PK properties of the two pyrethroids, such as the assumptions of flow-limited and diffusion-limited compartments, the inclusion of major metabolic pathways in different tissues, and descriptions of different exposure routes.
b) Please comment on the correctness of mathematical equations used to describe the model structure, and their implementation in the programming language R. Additionally, comments are needed on the appropriateness of the integration of algorithm and integration internals selected for this model. Lastly, comments are needed on the mass balance of the model.
c) Please comment on the selection and/or estimation of values and distributions for all model parameters, including age- and gender-dependent physiological parameters and enzyme ontogeny, tissue/blood partition coefficients, and metabolic constants.

i. Please comment on whether the model properly accounts for human variability in PK by considering age- and gender-differences

ii. Please comment on the correctness of allometric scaling of parameters. Please comment on the appropriateness of methods used to extrapolate *in vitro*-derived data (e.g., metabolism constants) to an *in vivo* system. Please comment on the IVIVE and enzyme ontogeny approach for estimating intrinsic clearance for humans at different ages.

iii. Please comment on the justification and appropriateness of using an empirical factor to adjust the apparent Km from an *in vitro* system to an *in vivo* free-concentration based K_m.

d) Please comment on the evaluation of model performance simulating the general trend of published time-concentration data measured in post-natal day 90 (PND90) and PND15 rats. Please comment on the appropriateness of using the parallelogram approach to support the development of a human life-stage PBPK model in the absence of human *in vivo* data.

e) Please comment on the results from sensitivity and uncertainty analyses, and their implications on the estimation of DDEFs and derivation of scenario-specific human PoDs.

f) Please comment on the approach used to determine the most sensitive age and gender combination that results in the highest peak deltamethrin and *cis*-permethrin concentrations in plasma and in brain. Please comment on the subsequent use of the most sensitive age and gender combination for a specific exposure scenario to estimate the PoD for that scenario.

g) Please comment on the appropriateness of the Monte Carlo simulations in estimating distributions of the peak deltamethrin and *cis*-permethrin concentrations in plasma and in brain, and for use in DDEF derivations.

h) In the context of panel responses to 2a-2g, please comment on the appropriateness of the deltamethrin/*cis*-permethrin PBPK model for use in human health risk assessment to

- replace the default inter-species and intra-species safety factors with the DDEFs estimated using the human life-stage PBPK model
- derive scenario-specific PoDs.

i) In the context of panel responses to 2a-2g, please comment on the potential to apply a read-across approach that uses one generic model structure in conjunction with chemical-specific model parameters, primarily *in vitro* metabolism measurements, to construct PBPK models for other pyrethroids.

3. Acibenzolar is a fungicide and plant growth regulator. Acibenzolar is metabolized via rapid hydrolysis by carboxylesterase to acibenzolar-acid. This metabolism process is so rapid that time-concentration profiles of acibenzolar-acid following intravenous infusion of either acibenzolar or acibenzolar-acid are found to be superimposable. The human life-stage PBPK model for acibenzolar predicts the disposition of the major metabolite, acibenzolar-acid, over time.

a) Please comment on whether the model structure, such as the three-compartment structure, is appropriate for describing the PK properties of acibenzolar and acibenzolar-acid.

b) Please comment on the correctness of mathematical equations used to describe the model structure.

c) Please comment on the approaches used to select and/or estimate values and distributions for chemical-specific parameters, such as optimization of volume of distribution using *in vivo* time-concentration data obtained from rats, *in vitro* measurements of metabolic constants, IVIVE approach.
    a. Please comment on appropriateness of the selected oral absorption rate, which was obtained by fitting model predictions to *in vivo* time-concentrations in rats, for use in the human model.
    b. Please comment on the correctness of allometric scaling of parameters. Please comment on the appropriateness of methods used to extrapolate *in vitro*-derived data (e.g., metabolism constants) to an *in vivo* system.

d) Please comment on the evaluation of model performance simulating the general trend of time-concentration data measured in rats after exposed to acibenzolar via intravenous infusion and oral dosing. Please comment on the simulated variability in acibenzolar-acid concentrations in blood with observed variability in rats. Please comment on the appropriateness of scaling volume of distribution estimates for rats to those in humans.

e) Please comment on the results from sensitivity analysis, and their implications on the estimation of DDEFs.

f) In the context of panel responses to 3a-3e, please comment on the appropriateness of the approach that uses animal PoD and model-predicted human PoD in estimating inter-species DDEF for PK.

g) In the context of panel responses to 3a-3f, please comment on the appropriateness of using Monte Carlo simulations to predict distribution of clearance for adults and children between 1-2 years old for intra-species PK DDEF derivations.

h) The PBPK model for acibenzolar was developed using a proprietary PBPK modeling software, Simcyp, which is a simulation platform routinely used in drug development but not commonly used in risk assessment of environmental chemicals. Syngenta has submitted, as supporting material, 1) a detailed report summarizing the model's development using Simcyp; 2) references that describe Simcyp platform history and construction, key algorithms, and features; and 3) input/output files for the acibenzolar PBPK model. However, the agency has not been able to review the model code. Please comment on the degree to which the acibenzolar PBPK model has (or has not) is sufficiently transparent and, if appropriate, provide suggestions to increase the transparency when using such a software platform.

4. Malathion is an organophosphate (OP) pesticide. Like other OPs, the initiating event leading to cholinergic toxicity for malathion involves inhibition of AChE, which leads to an accumulation of acetylcholine and ultimately neurotoxicity in the central and/or peripheral nervous system. Malathion requires metabolic activation to the oxon metabolite (malaoxon) to inhibit AChE, with subsequent metabolism that leads to detoxification. The human life-stage PBPK/PD model for malathion predicts the disposition of malathion and its metabolite, malaoxon, and inhibition of AChE by malaoxon in red blood cells (RBC) and in brain over time.

a) Please comment on the appropriateness of the general metabolic scheme included in the model structure and offer an alternative interpretation(s) of the references, as appropriate?

Please comment on the completeness of the studies currently included to develop the metabolic scheme.

b) Please comment on the completeness of selected studies on rat and human *in vivo* time-concentration and AChE inhibition data. Are the summarized references sufficient to inform the model structure and calibrate model parameters? Are there other references that are missing and should be used to inform the model structure?

c) Please comment on the appropriateness of using the chlorpyrifos PBPK/PD model structure, but with malathion and malaoxon-specific parameters, to describe the PK and PD properties of malathion.

d) Please comment on the correctness of mathematical equations used to describe the model structure, and implementation of these equations in the programming language acslX. Please comment on the use of the competitive inhibition equation to describe malathion inhibition of carboxylesterase, which can also metabolize malaoxon. Please comment on the appropriateness of the integration of algorithm and integration internals selected for this model. Please comment on the mass balance of the model.

e) Please comment on the selection and/or estimation of values and distributions for all model parameters, including age- and gender-dependent physiological parameters, tissue/blood partition coefficients, metabolic constants, and pharmacodynamic parameters that determine the binding of malaoxon to cholinesterases active site. Please comment on the correctness of allometric scaling of parameters.

f) Please comment on the *in vitro* studies that were conducted by Mississippi State University to inform malathion and malaoxon metabolism, as well as malaoxon inhibition of AChE. Please comment on the appropriateness of methods used to extrapolate *in vitro*-derived data (e.g., metabolism constants) to an *in vivo* system.

g) Please comment on the strategy used to calibrate the PBPK/PD model, by first fitting the model simulations to time-concentration data for urinary metabolites and to time profiles of AChE inhibition in RBC obtained from rats exposed to malaoxon.

h) Keeping in mind the need to conduct a refined human health risk assessment in the near future, please provide advice on an appropriate model fitting strategy or on additional studies critical for improving the model predictions of time profiles of AChE inhibition in RBC obtained from rats exposed to malathion.

5. Dimethoate is an organophosphate (OP) pesticide. Like other OPs, the initiating event leading to cholinergic toxicity for dimethoate involves inhibition of AChE, which leads to an accumulation of acetylcholine and ultimately neurotoxicity in the central and/or peripheral nervous system. Dimethoate requires metabolic activation to the oxon metabolite (omethoate) to inhibit AChE, with subsequent metabolism that leads to detoxification. The human life-stage PBPK/PD model for dimethoate predicts the disposition of dimethoate and its metabolite, omethoate, and inhibition of AChE by omethoate in RBC and in brain over time.

a) Please comment on the appropriateness of the general metabolic scheme included in the model structure and offer an alternative interpretation(s) of the references, as appropriate? Please comment on the completeness of the studies currently included to develop the metabolic scheme.

b) Please comment on the appropriateness of using the chlorpyrifos PBPK/PD model structure, but with dimethoate and omethoate metabolic parameters, to describe the PK and PD properties of dimethoate.
c) Please comment on the correctness of mathematical equations used to describe the model structure, and implementation of these equations in the programming language acslX. Please comment on the appropriateness of the integration of algorithm and integration internals selected for this model. Please comment on the mass balance of the model.
d) Please comment on the selection and/or estimation of values and distributions for all model parameters, including age- and gender-dependent physiological parameters, tissue/blood partition coefficients, metabolic constants, and pharmacodynamic parameters that determine the binding of omethoate to cholinesterases active site. Please comment on the correctness of allometric scaling of parameters.
e) Please comment on the *in vitro* studies that are conducted by Mississippi State University to inform dimethoate and omethoate metabolism, as well as omethoate inhibition of AChE. Please comment on the appropriateness of methods used to extrapolate *in vitro*-derived data (e.g., metabolism constants) to an *in vivo* system.
f) Please comment on the strategy used to calibrate the PBPK/PD model, by first fitting the model simulations to time-concentration data for urinary metabolites and to time profiles of AChE inhibition in RBC obtained from rats exposed to omethoate.
g) Keeping in mind the need to conduct a refined human health risk assessment in the near future, please provide advice on an appropriate model fitting strategy or on additional studies critical for improving the model predictions of time profiles of AChE inhibition in RBC obtained from rats exposed to dimethoate.