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White Paper On the Application of PBPK Models to Carbaryl Risk Assessment

July 24th, 2017

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**List of abbreviations**

AChE Acetylcholinesterase enzyme

BW Body weight

CLh Hepatic clearance

Clint Intrinsic clearance

Cmax Maximum concentration

CYP Cytochrome P450

DDEF Data derived extrapolation factor

EPA Environmental Protection Agency

FA Fraction of absorbed dose

FM Fraction metabolized

FQPA Food Quality Protection Act

Fu Fraction unbound

HPGL Hepatocellularity

ISEF Inter-System Extrapolation Factor

IVIVE *In vitro* to *in vivo* extrapolation

KA Absorption rate constant

Km Michaelis-Menten constant

MC Monte Carlo

MPPGL Microsomal Protein Per Gram Liver

NHANES National Health and Nutrition Examination Survey

PB Air:plasma partition coefficient

PBPK Physiologically Based PharmacoKinetic

PC Tissue to plasma Partition coefficient

PD Pharmacodynamics

PK Pharmacokinetic

PND Postnatal day

POD Point of departure

QAlv Alveolar ventilation rate

RAF Relative activity factor

RBC Red blood cells

SA Sensitivity analysis

SC Sensitivity coefficient

UF Uncertainty factors

UGT Glucuronosyltransferases

**I. Introduction**

**A. Background**

The U.S. EPA 2007 carbaryl Reregistration Eligibility Decision established the points of departure and uncertainty/Food Quality Protection Act (FQPA) safety factors for the product. The RED was amended in 2008 and values remained unchanged. The uncertainty factors used were the default values for inter- and intra-species of 10-fold and 10-fold as well as an FQPA dermal exposure safety factor of 1.8-fold for children. The purpose of uncertainty and exposure factors is to account for the application of animal data and animal life-stage data to the prediction of human risk. The nature of these factors is, as the name implies, default. In the case of the 1.8-fold FQPA factor that is applied to the dermal route of exposure, data exist that identify a 1.8-fold difference in sensitivity between young versus older rats (USEPA, 2007).

The alternative to default factors is to generate data and models that describe the species-specific pharmacokinetics in animals and humans. Therefore, the purpose of this paper is to summarize work that has been done to determine data-derived extrapolation factors (DDEFs) for carbaryl that replace default uncertainty factors, particularly as they apply to life stages. The data and models describe carbaryl’s pharmacokinetics (PK) of absorbed dose and the consequent pharmacodynamics (PD) of cholinesterase inhibition in the species of interest, humans. PK and PD modeling can then demonstrate the relative differences in human populations, *e.g.,* various life stages, thereby deriving biologically relevant DDEFs.

This endeavor follows the guidance from the National Research Council (NRC, 2007) Science and Decisions: Advancing Risk Assessment to, “…continue and expand use of the best, most current science to support and revise default assumptions” and encouragement from the Institute of Medicine to establish specific extrapolation factors (IOM, 2013): “If those factors more accurately reflect the differences between animals and humans than default adjustment factors, the use of such data-derived extrapolation factors would decrease the uncertainty in the risk assessment.”

**B. Physiologically based pharmacokinetic (PBPK) models for risk assessment**

Both the adverse and beneficial responses to compounds are related to the free concentrations of active compounds at the target tissue rather than the amounts of compound at the site of absorption. Therefore, the internal exposure at the target tissue is the appropriate dose metric for use in safety assessment. PBPK modeling offers a scientifically sound framework to integrate mechanistic data for physiological and biochemical processes and serves as a tool to predict internal exposure at the target tissue for a wide range of exposure conditions in animals or humans. PBPK models differ from classical compartmental models in that they include biologically realistic descriptions of tissues and processes involved in exposure, distribution, biotransformation and clearance processes (Clewell and Andersen, 1994). Since physiology and metabolism are described by using physiologically meaningful parameters, a different species can be modeled by simply replacing the appropriate parameters with those for the species of interest. Similarly, the behavior for a different route of administration or exposure scenario can be determined by adding the equations that describe the nature of the input function. The mechanistic basis of PBPK models enhances their predictive power, allowing for various applications of this tool in a risk assessment context. These applications include inter-species extrapolation, route-to-route extrapolation, and high to low dose extrapolation (Clewell and Andersen, 1985), as well as the recent application area of quantitative *in vitro* to *in vivo* extrapolation (IVIVE), supporting the new safety assessment paradigm based on *in vitro* and computational methods (Yoon *et al.,* 2012b; 2016). The advantages of applying PBPK modeling in risk assessment have led to widespread acceptance by regulators (NRC, 1987; Clewell and Clewell, 2008; Loizou *et al.,* 2008). Beyond their applications for quantitative risk assessment, PBPK models can be used to interpret human biomonitoring data (Clewell *et al.,* 2008) and epidemiological data (Wu *et al.,* 2015, Verner *et al..* 2015, Song *et al.,* 2016).

The approach of predicting *in vivo* metabolic clearance on the basis of *in vitro* data using biologically-based scaling processes has gained strong support in recent years (*e.g.,* Yoon *et al.,* 2012b, Houston *et al.,* 2008). By using population-appropriate exposure information, physiological and biochemical parameter values, PBPK models are well-equipped to predict population-specific internal exposure at the target tissue. Because key parameters for PBPK models, *e.g*., metabolism parameters, are provided from *in vitro* assays based on human-derived or human-relevant systems, this modern parameterization approach based on *in vitro* methods provides a high degree of confidence in using the model predictions for human health risk assessment.

**C. Development of PBPK models for carbaryl**

We published a PBPK model for carbaryl in adult humans based on *in vitro* to *in vivo* extrapolation previously (Yoon 2012a and 2015). This model has been used for exposure reconstruction and biomarker interpretation scenarios for carbaryl (Brown *et al.,* 2015; Phillips *et al.,* 2014). To build this model, we used *in vitro* methods to experimentally determine tissue partitioning, plasma protein binding and hepatic metabolism parameters for carbaryl in adult humans (2015). The performance of the model was evaluated by comparing the model-predicted time course curves with the observed time profiles of tissue carbaryl concentrations and RBC acetylcholine esterase (AChE) inhibition in human volunteers (May *et al.,* 1992).

**D. Applications of PBPK models to carbaryl risk assessment**

a. Age-related uncertainty factor or FQPA safety factor

The purpose of the early age dosimetry described in this white paper is to calculate a data-derived extrapolation factor (DDEF) to address age-related PK differences and resulting pharmacodynamic (PD) differences for carbaryl in humans. A PBPK/PD model-based DDEF specific for carbaryl may replace the generic FQPA safety factor. In the case of carbaryl a DDEF is calculated by using age-specific biomarkers of internal exposure and resulting PD effects, *i.e.,* acetylcholinesterase (AChE) inhibition in RBCs and brain, as simulated by the model. In this case, DDEF values for early life exposures can be calculated based on the average or distribution of the maximum inhibition (%) of RBC or Brain AChEs in the juvenile and adult populations. These DDEFs are determined as follows:

* First, the human life stage model is run for the age brackets provided with the EPA exposure scenarios
* For each age bracket in the early life period, the most sensitive age/gender combination is identified at the POD (the dose resulting in 10% AChE inhibition in RBC or brain at its maximum)
* Monte Carlo simulations are performed for the most sensitive juvenile age and 25-year-old (defined as adult) in the corresponding gender, exposed to carbaryl by ingestion at the POD for the adult in both age groups.
* The output of the simulated distributions of the maximum inhibition (%) of AChE in RBC or brain is used for calculation of DDEFs with the following Equation I-1.

**Sensitive Juvenile\_% inhibition50% percentile/Adult\_% inhibition50% percentile  (Eq. I-1)**

Using the PBPK/PD model, DDEFs derived in this way can address both PK and PD differences together. Acute effects of carbaryl are due to the inhibition of AChE by carbaryl, the concentration of which is dependent on age-specific differences in metabolism.

b. Inter-species extrapolation uncertainty factor for human pharmacokinetics

As the point of departure (POD) is the maximum RBC or brain AChE inhibition from carbaryl exposure in humans, inter-species extrapolation is not required. If any PODs from rat studies are to be evaluated for risk assessment in addition to the human POD based on RBC and/or brain AChE inhibition, inter-species extrapolation can be performed using the rat and human PBPK/PD models for carbaryl. Since a PBPK/PD model is used for this process, the inter-species uncertainty factor (UF) could be reduced or eliminated.

c. Intra-species extrapolation uncertainty factor for human PK and PD

The human PBPK/PD model for carbaryl was developed using *in vitro* metabolism data that are relevant for humans together with the human population data on the age-specific enzyme expressions. This provides the key information to simulate realistic inter-individual variability in humans, which is largely attributable to population variability in metabolism resulting from the large variation in metabolizing enzyme expression.

d. Other applications

PBPK models can be used to perform route-to-route extrapolation. Thus, a POD from a certain route of exposure, e.g., oral, can be used to derive a POD for other routes of exposure such as inhalation and dermal.

**II. Modeling approach**

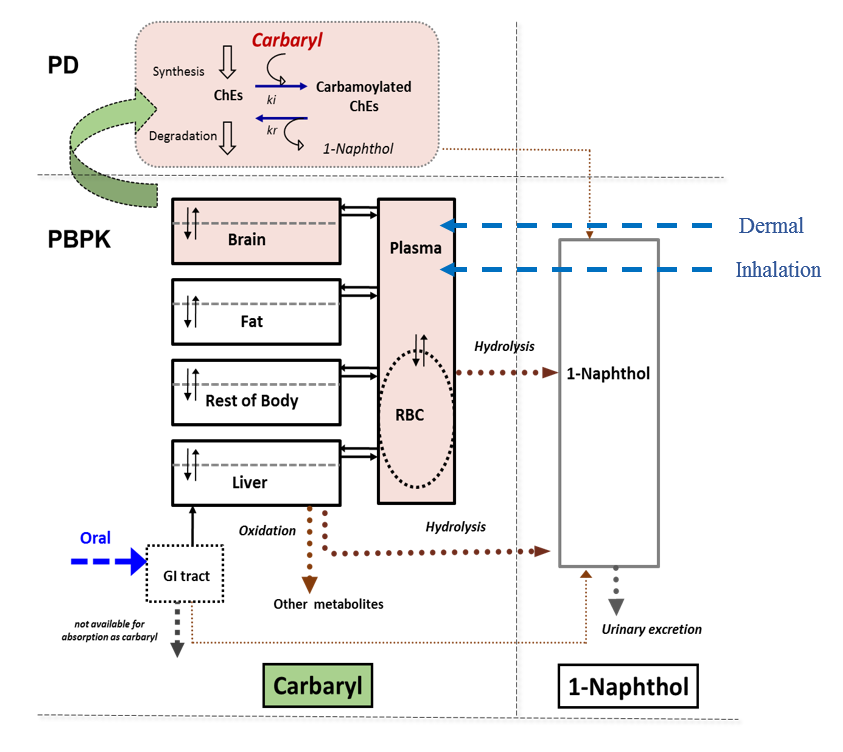
**A. Overview of the structure of the model**

Previously published PBPK/PD models for carbaryl ([Yoon *et al.,* 2012a](http://pubs.acs.org/doi/abs/10.1021/bk-2012-1099.ch020); [Yoon *et al.,* 2015](https://www.ncbi.nlm.nih.gov/pubmed/24863738)) were extended to build the life-stage PBPK model for carbaryl in humans. The life-stage model predicts the disposition of carbaryl and its AChE inhibition dynamics *in vivo* after oral, inhalation and dermal exposure to carbaryl at various ages from birth to adulthood.

Metabolic clearance and AChE inhibition parameters were based on *in vitro* assays as described in the published paper on adult life stages ([Yoon *et al.,* 2015](https://www.ncbi.nlm.nih.gov/pubmed/24863738)). For early ages, the details are described below. These *in vitro* PK and PD parameters were extrapolated to the corresponding parameters in the whole-body PBPK model through biologically appropriate scaling using IVIVE (*In vitro* to *in vivo* extrapolation; [Yoon *et al.,* 2012b](https://www.ncbi.nlm.nih.gov/pubmed/22667820)).

The PBPK model structure is shown in Figure 1. The structure of the current model is an adaptation of the published PBPK/PD model, which was also designed to interpret biomarker data for carbaryl and to support PBPK-based cumulative risk characterization for carbaryl ([Yoon *et al.,* 2015](https://www.ncbi.nlm.nih.gov/pubmed/24863738)). The model has (i) multiple compartments for carbaryl, the active entity for anticholinesterase activity, to describe carbaryl disposition and (ii) one compartment description for 1-naphthol kinetics to simulate the urinary concentration of total 1-naphthol. All tissues were described as diffusion-limited, although depending on the scale of the permeability-area product compared to tissue blood flow, some tissues are essentially flow-limited. Blood was divided into two compartments, RBCs and plasma, and exchange of carbaryl between these two blood compartments is assumed to be rapid. The RBC:plasma partition coefficient determines the ratio of carbaryl between RBC and plasma. In our model, elimination of carbaryl is considered to be through metabolism as it is rapidly metabolized in the body and readily excreted as metabolites in urine and feces (Knaak *et al.,* 2008).

Our model simulates oral, dermal and inhalation exposures to carbaryl at various ages. Both single and multiple daily exposure scenarios can be simulated. Further details of the model structure and parameters are described in the following sections.



**Figure 1. Structure of the life stage carbaryl PBPK/PD model**. **Colored compartments (brain, plasma, and RBC) indicate where inhibition of cholinesterases (ChEs) by carbaryl occurs.**

Note that the PD model describes carbaryl’s interaction with ChEs: (i) AChEs in RBCs and brain, and (ii) butyrylcholinesterases in plasma.

**B. Model parameters**

The physiological parameters of the base model are not chemical-specific, and the values used in the evaluation of an untested compound would be the same as those used for well-characterized compounds. Therefore, physiological parameters were taken from the available literature. Chemical specific parameters other than those that were *in vitro*-derived were taken from the previously published rat models ([Nong *et al.,* 2008](https://www.ncbi.nlm.nih.gov/pubmed/18704829); [Yoon *et al.,* 2015](https://www.ncbi.nlm.nih.gov/pubmed/24863738)). The *in vitro* based parameters were metabolic parameters for carbaryl; and binding and liberation of carbaryl to and from AChEs in the brain and red blood cells (RBCs) and to and from butyrylcholinesterases in plasma. All parameters are provided in the model parameter file ‘MC parameters’ along with their sources. This parameter file also includes the distributions of the parameters for the ages selected as; (i) sensitive gender and age combinations used to calculate DDEFs for oral single dose, (ii) ages used for sensitivity analysis and (iii) scenario specific ages.

1. Life stage parameters

a) Physiological parameters

Age-specific physiological parameters, including body weight (BW), cardiac output, tissue weights (volumes), and tissue blood flows were adapted from published life-stage models ([Wu *et al.,* 2015](https://www.ncbi.nlm.nih.gov/pubmed/26043300); [Song *et al.,* 2016](https://www.ncbi.nlm.nih.gov/pubmed/27429067); [Ruark *et al.,* 2016](https://www.ncbi.nlm.nih.gov/pubmed/27927583)). These published life-stage models use the age-specific physiological parameters of females, which were adapted to represent males based on the most recent National Health and Nutrition Examination Survey (NHANES) (2005-2006) data. Resulting growth curves for males and females are summarized in the MS EXCEL spreadsheet titled “Life stage parameters\_male\_SAP.xlsx” and “Life stage parameters\_female\_SAP.xlsx”. Age-specific values for each parameter were computed and are listed for males and females of age six months to sixty years. If required the equations in the sheet can be used to extrapolate to ages beyond 60 years. The model can simulate both female and male life stages.

b) Enzyme Ontogeny

To predict age-specific metabolic clearance of carbaryl using *in vitro* metabolism data collected in human expressed enzymes, it is critical to obtain the data on the enzyme ontogeny, *i.e.,* the age-dependent changes in the expression profile of an enzyme, for the enzymes responsible for carbaryl metabolism in different ages, is required. The ontogeny curves for the enzymes known to contribute to carbaryl metabolism in humans (Wetmore *et al.,* 2014), CYP1A2, CYP2C9, CYP2C19, CYP2B6, CYP2E1 and UGT1A9, are in the excel worksheets titled ‘CYP1A2’, ‘CYP2C9’, ‘CYP2C19’, ‘CYP2B6’, ‘CYP2E1’ and ‘UGT1A9’ within the master life stage parameter file: “Life stage parameters\_male\_SAP.xlsx” and “Life stage parameters\_female\_SAP.xlsx”.

To derive ontogeny curves for each enzyme listed above, published enzyme protein expression at various ages after birth to young adulthood were used (Hines *et al.,* 2008; Hines *et al.,* 2016, and Song *et al.,* 2016). Nonlinear regression analysis was performed to describe the maturation profiles for each enzyme. Lognormal, hyperbola, allosteric-sigmoidal, dose-response, and Gompertz growth curves were used for fitting. The best fit curve was chosen based on visual inspection and the one with the lowest Akaike information criterion. Then the equation derived from the curve that best describes the ontogeny profile was used to describe age-dependent changes in the expression of each enzyme as a fraction of adult expression.

For UGT1A9, reported expression data (Miyagi *et al.,* 2012) was extracted and used to derive ontogeny curves. For CYPs, available data was for premature and full-term infants, described both in years and in weeks. Nonlinear regression was performed with the age expressed in weeks to accurately capture the ontogeny profiles for early ages. For CYPs, the ages in years in every worksheet were converted into weeks, and 40 weeks were added considering a full-term pregnancy, to use in the derived equations. These ontogeny curves were then utilized for IVIVE as shown in the ‘CL ontogeny’ worksheet.

Our *in vitro* studies, indicated involvement of Ca2+ dependent A-esterase activity such as PON1 in carbaryl metabolism in plasma (Yoon *et al.,* 2015). Smith *et al.* (2011) reported age-dependent hydrolysis of chlorpyrifos oxon in plasma, for which PON1 is primarily responsible. They showed that the capacity of chlopyrifos oxon hydrolysis in plasma is substantially lower in early ages reaching adult levels between age 1 and 10. In our model, age-dependent carbaryl hydrolysis in plasma was described based on PON1 ontogeny inferred from the age-dependent changes in Vmax of oxon-hydrolysis reported by Smith *et al*.. The plasma Clint for ages earlier to 10 years was 27% of the adult plasma Clint.

2. *In vitro* to *in vivo* extrapolation (IVIVE)

*In vitro*-derived parameters were scaled up to *in vivo.* The rationale for extrapolating *in vitro* metabolism parameters to *in vivo* is that the capacity of metabolism (*e.g.,* Vmax) can be related between *in vitro* and *in vivo* by considering the total amount of enzyme present in each system. The affinity of the enzyme for the substrate (*e.g.,* Km) can be related by considering free substrate concentration for enzyme reaction in each system. Therefore, *in vitro*-measured metabolic constants can be ‘scaled-up’ to respective *in vivo* metabolism parameters used in the PBPK models by relating enzyme content *in vitro* (e.g., Vmax per mg protein *in vitro*) to that *in vivo* (*e.g.,* Vmax per g liver *in vivo*). There are several different *in vitro* systems available for metabolism studies and the IVIVE process required for each system varies. Metabolism IVIVE is an accepted concept and has become common practice in drug PBPK models for pediatrics (Johnson *et al.,* 2006).

The chlorpyrifos PBPK model is an example of an IVIVE and life-stage- PBPK model that has been developed in support of risk assessment (Smith *et al.,* 2014). Smith and colleagues (2014) demonstrated the ability of this IVIVE-PBPK model to predict age-specific target tissue exposure to chlorpyrifos and its active metabolite, chlorpyrifos oxon, concentrations in the brain at age 0.5 and 3 in comparison to age 30.

1. *In vitro* data

Biological scaling (*i.e.,* IVIVE) was used to calculate age-specific metabolism parameters based on *in vitro* expressed enzyme data, adult human hepatocyte data, and xenobiotic metabolizing enzyme ontogeny data. This approach accounts for differences between adults and early ages in terms of biochemical processes and physiology that determine hepatic clearance at a given age. This allowed accountability for both changes in growth (liver weight, body weight, biological scaling factors such as microsomal protein content in the liver) and maturation of metabolic clearance mechanisms (enzyme ontogeny).

Published data on carbaryl metabolism rates in human-expressed enzyme panels *in vitro* including both cytochromes (CYPs) and glucuronosyltransferases (UGTs) (Wetmore 2014) were used for IVIVE to estimate the relative contribution of each enzyme to total carbaryl metabolism in the liver. The *in vitro* intrinsic clearance (Clint) values for the enzymes showing metabolic activity toward carbaryl were scaled up to corresponding *in vivo* enzyme Clint values in adults using appropriate scaling factors (described in detail below). To estimate age-specific metabolic clearance of carbaryl, knowledge of the contribution of each specific metabolic clearance pathway in adults and quantitative information regarding the metabolism pathway ontogeny were used (described below). Information on the abundance of each enzyme in adult liver for this calculation was taken from the published meta-analysis of several population studies for CYPs (Achour *et al.,* 2014).

1. Scaling process

In both the *in vitro* and *in vivo* systems, the biochemical principles are the same, only the scale of the system is different between the two. The overall rate of enzyme-catalyzed reactions is directly proportional to the total enzyme present in the system; therefore, the basis of extrapolation of *in vitro* biotransformation parameters to the whole organism is related to the total enzyme content present between the two systems (Kedderis, 2007). The scaling factors used to conduct IVIVE of human-expressed enzyme Clint results for carbaryl are the Inter-System Extrapolation Factor (ISEF), Relative Activity Factor (RAF) and Microsomal Protein Per Gram Liver (MPPGL) as described in the review by Yoon *et al.,* (2012b). The RAF converts the activity level of a specific enzyme in the expressed system to the activity level of this enzyme in the endogenous system without separating the potential sources of system differences (*i.e.* abundance of enzyme per mg of protein or intrinsic activity per unit enzyme). Similarly, the dimensionless ISEF integrates the differences in intrinsic activity and protein expression between two systems. Age-specific liver weights were also incorporated into scaling. Male and female values of ISEF, RAF, MPPGL and liver weight are in the EXCEL sheet titled ‘CL ontogeny’, ‘MPPGL’ and ‘Liver weight’, in the “Life stage parameters\_male\_SAP.xlsx” and “Life stage parameters\_female\_SAP.xlsx”, respectively. ISEF values, listed in the ‘CL ontogeny’ worksheet, are from Wetmore *et al.* (2014) and the age-dependent MPPGL values are from Barter *et al.* (2008).

1. Intrinsic clearance for life stage

The stepwise description for the calculation of intrinsic clearance is as follows:

* Total hepatic intrinsic clearance (Clint) for carbaryl in adults was estimated using the *in vitro* Clint data measured with human adult hepatocytes using IVIVE (Eq 1) as described in Yoon *et al*. (2012b). HPGL, the scaling factor used for this IVIVE, represents hepatocellularity per g liver weight.

**Total Clint, *in vivo* (L/h) = Clint\_*in vitro* × HPGL × Liver weight (Eq. 1)**

* The *in vitro* Clint values for the CYP enzymes showing metabolic activity toward carbaryl were scaled up to corresponding *in vivo* enzyme Clint values in adults, using appropriate scaling factors (described in detail above) into Eq. 2 and 3.

**Clint, *in vivo*\_CYP (L/h) = Clint\_*in vitro*\_CYP × ISEF × MPPGL × Liver weight × CYP abundance in the population (Eq. 2)**

For UGTs, as enzyme abundance data are not yet available to calculate ISEFs for UGTs, RAF was used for extrapolation (Gibson *et al.,* 2013)

**Clint, *in vivo*\_UGT (L/h) = Clint\_*in vitro*\_UGT × RAF × MPPGL × Liver weight**  **(Eq. 3)**

* Then the relative contribution of each enzyme (% of total Clint) to total hepatic Clint for carbaryl in adult liver was calculated as:

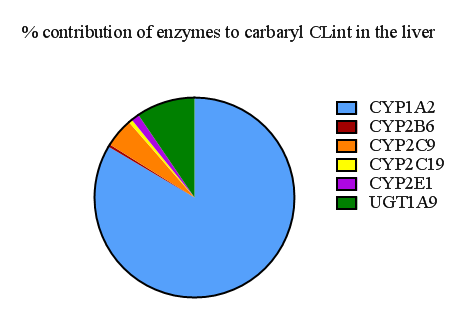
**% contribution = (Clint, *in vivo* for individual enzyme/Total Clint, *in vivo* as sum of all enzymes) \*100 (Eq. 4)**

* The relative contribution (%) values were then used to calculate each CYP or UGT specific Clint, *in vivo* as below (Eq. 5). The sum of all enzyme specific Clint. *i.e.,* Clint, *in vivo* for individual enzyme, is the final total Clint, *in vivo* taken for life stage PBPK modeling. Note that the total Clint, *in vivo* extrapolated from the *in vitro* Clint determined in adult human hepatocytes (from Eq. 1) was used as adult parameter in the model.

**Clint, *in vivo* for individual enzyme = ((% contribution)/100) \* (Total Clint, *in vivo* derived from Eq. 1) (Eq. 5)**

* Enzyme ontogeny was expressed as fractions of adult expression over time (see details above). These fractional expression values were then used to scale Clint values for each enzyme in Eq. 5, *i.e.,* Clint, *in vivo* for an individual enzyme, described above in adult (Eqs 1, 2, 3 and 4) to those at different ages (Figure 2). The estimated Clint values for each enzyme in a given age were then summed to calculate the total hepatic Clint for that age *in vivo*.

The age-dependent changes in carbaryl Clint expressed as L/hr per kg body weight from birth to adulthood is shown in Figure 3. The age-dependent changes for total hepatic clearance (CLh) of carbaryl shows a different developmental trajectory due to the influence of age-dependent changes in physiological factors such as hepatic blood flow and liver weight during development. These factors partially compensate for the lower Clint in early ages, therefore dampening the age-related differences in metabolic clearance for this compound.



**Figure 2. Relative contribution of each enzyme towards carbaryl Clint in adult liver**

 **Figure 3. Age-dependent changes in total Carbaryl CLint and CLh**

1. Other parameters

a) Pharmacodynamic parameters

Inhibition of AChEs by carbaryl is a reversible process, the extent of which is determined by three sequential processes: binding of carbaryl to the enzyme active site, carbamylation of the active site, and subsequent decarbamylation leading to regeneration of enzyme activity (Main *et al.,* 1969). This series of events was incorporated in the model using a bimolecular inhibition rate constant (ki, μM-1·hr-1), which includes both the binding affinity and carbamylation constant (Main *et al.,* 1969) and a first-order rate constant (kr, hr-1) for the decarbamylation process (Yoon *et al.,* 2015). These PD parameters were derived from *in vitro* studies using RBC and brain samples from adult rats and RBC samples from adult humans. For humans, ki and kr for carbaryl interactions with ChEs were determined for RBCs and plasma; RBC values were used for brain. The bimolecular inhibition and decarbamylation rate constants were directly incorporated into the model as measured, *i.e.,* no IVIVE is necessary, since they describe biochemical processes that are not dependent on the amount of enzyme present. As reaction rate is the measure of the change in concentration of the chemical per unit time and dependent on the amount of the chemical present in the reaction, the rate constant for chemical degradation of carbaryl was also directly used in the PBPK model.

Primary and tertiary alignments show that AChEs are highly evolutionarily conserved enzymes (Wiesner *et al.,* 2007). Using the *in vitro* measured bimolecular inhibition rate constants, the model overestimated the AChE inhibition in RBCs and brain (Yoon *et al.,* 2015) when compared to the *in vivo* cholinesterase enzyme depression results in rat (Nong *et al.,* 2008). This is likely due to conformational changes of these ologomeric enzymes which occurred during sample preparation, especially during homogenization and dilution of the tissues, given the complex oligomeric structure that is essential for AChE functions *in vivo* both in animals and humans. Therefore, the *in vitro*-determined ki values were adjusted to be consistent with the observed AChEs inhibition by carbaryl in RBCs and brain in rats (Yoon *et al.,* 2015). *In vitro* determined ki in human RBCs were used for both RBCs and brain with similar adjustments.

Age-related alteration in AChE activity in rats has been reported by Mortensen *et al.,* (1998). In their study, about 54% and 81% of adult brain AChE activity was detected on postnatal day (PND) 4 and PND11 respectively. No such data are available for humans; however, Ecobichon and Stephens (1973) reported that RBC acetylcholinesterase (AChE) levels increase rapidly from term infants to 1 year of age. Similarly, specific activities of RBC AChE in newborns and infants (1 week to 2 months) were found to be 50% and 34% of adult levels, respectively, while 1 year old activities were similar to those for adults (Ecobichon *et al.,* 1973; Karlsen *et al.,* 1981). However, Km values for infants and adult humans were similar indicating that age-related difference is mostly due to quantitative differences in enzyme expression (Ecobichon and Stephens, 1973). Considering that % inhibition of AChE is dependent on the bimolecular inhibition constant and the reactivation rate kr, not the amount of enzyme expressed, the response to carbaryl will be dependent on relative kinetic relationship between these two processes. Therefore, despite the differences in the AChE enzyme expression in different ages, % inhibition will depend on carbaryl’s inherent AChE interaction kinetic (binding and liberation) properties.

Age dependent brain and RBC AChE inhibition was determined experimentally for carbaryl in the rat (Moser *et al.,* 2010). In this study, the remaining brain AChE activity after carbaryl exposure was significantly lower in PND11 rats than that in PND17 and adult rats, whereas the remaining RBC AChE activity was slightly lower in adults compared to the degree of depression of this enzyme activity in PND11 rats. This age-related sensitivity seen in rats is likely due to differences in detoxification, *i.e.,* metabolism, rates for carbaryl. CYP1A2 is the major isoform involved in oxidation of carbaryl (Tang *et al.,* 2002; Wetmore *et al.,* 2014). The expression of the rat equivalent form of this enzyme is about 30% of the adult level in juvenile rats around PND3 and 45% at early puberty (PD42) (Elbarbry *et al.,* 2007). Moreover, hydrolysis capacities including A-esterases in plasma that may be contributing to carbaryl elimination increase as rats mature (Moser *et al.,* 1998). Paraoxonase 1 (PON1), an A-esterase in rats which is believed to be largely responsible for carbaryl hydrolysis, is about 1 -3 % of the adult level both in the liver and plasma in pre-weanling rats (Atterberry *et al.,* 1997). Therefore, the apparent greater sensitivity of younger animals reported in the Moser *et al.,* study is probably due to the lower metabolism, *i.e.,* detoxification ability in the young rats rather than age-dependent changes in AChE sensitivity to the exposure to carbaryl. Indeed, Mortensen and colleagues demonstrated that age-related sensitivity of AChE to carbamate and organophosphates is mainly due to age-related pharmacokinetic differences as shown by similar IC50 values for carbaryl in two age groups, PND4 and adult rat (Mortensen *et al.,* 1998).

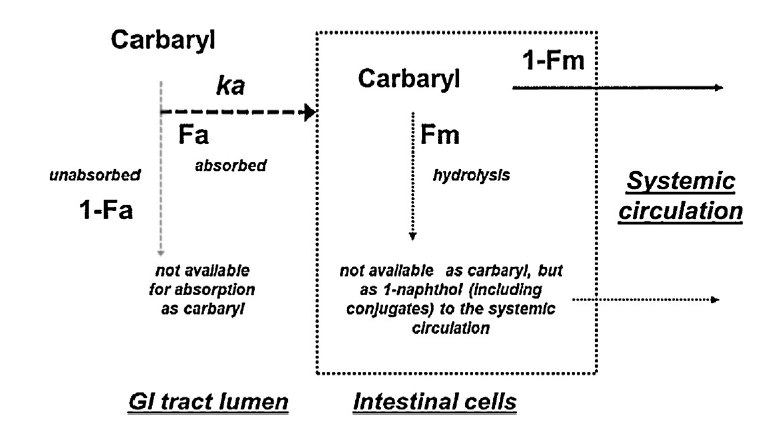
In addition, we have performed a statistical analysis using rat *in vivo* data to determine if there exists an age-dependent susceptibility to carbaryl in the inhibition of brain AChE activity (Appendix C). Data for carbaryl brain concentration and its AChE inhibition measured in the same animals aged post-natal day (PND) 18, 1 month, 4 months, 12 months, and 24 months were available for analysis (Moser *et al*., 2015). Regression analysis of the carbaryl concentration and the corresponding AChE inhibition (% remaining) in brain showed that the best-fit parameters for each age group were not significantly different from one another. Thus, it can be concluded that a single dose-response curve adequately describes the data for all age groups, and that the available evidence does not suggest age-dependent susceptibility to the inhibition of AChE activities by carbaryl in the rat. Therefore, in our life-stage PBPK model, the PD parameter values are assumed to be age-independent.

b) Tissue partitioning and free concentration of carbaryl

Two factors that can significantly affect available carbaryl concentration *in vitro* are (i) the chemical degradation of carbaryl at physiological temperature and pH and (ii) the free (unbound) fraction of carbaryl in the *in vitro* metabolism and cholinesterase interaction assays (Yoon *et al.,* 2015). These factors were determined using the rat tissues and appropriately applied using IVIVE to build the human carbaryl PBPK/PD model. Rat values were adopted for binding and tissue partitioning, based on the similarity in the measured unbound fractions in plasma and RBCs between the rat and human. The unbound fractions (fu) determined in the liver, brain, plasma, and RBCs were used directly in the model to describe free carbaryl concentration available for metabolism and AChE inhibition. The tissue to plasma partition coefficients of carbaryl were calculated using the experimentally measured fu values in plasma and other tissues using a rapid equilibrium dialysis method as described in our published study (Yoon *et al.,* 2015).

c) Intestinal absorption and pre-systemic metabolism

The absorption of carbaryl was described as a first-order process. The absorption rate constant, ka, was from the experimentally determined value (Hwang and Schanker, 1974), which indicates a rapid absorption of carbaryl in the gut. In the rat model, pre-systemic clearance of carbaryl while it is being absorbed in the gut was included based on the observed *in vivo* time course data after a single oral dosing in addition to the feature of less than 100% oral bioavailability. Although evidence for intestinal metabolism of carbaryl is a matter of debate (Houston *et al.,* 1975; Pekas, 1980), the evidence for hydrolysis of esters (Imai *et al.,* 2005) and presence of CYP3A4 (Paine *et al.,* 2006) (one of the CYP isoform responsible for carbaryl metabolism) in the gut, indicates the potential role of intestinal metabolism in carbaryl bioavailability (Figure 4). The pre-systemic clearance and oral bioavailability parameters were estimated in the human model as; a fraction of the dose is absorbed (Fa) as carbaryl passes through the gut lumen, and a fraction of this absorbed carbaryl is metabolized (Fm) in the enterocytes before reaching the systemic circulation.In order to parameterize this gut description, Fa and Fm were fitted to the reported urine concentrations of 1-naphthol metabolites in human volunteers (Knaak *et al.,* 1968). Developmental changes in the intestinal absorption processes including the expression of enzymes that may be responsible for pre-systemic clearance which could potentially alter the bioavailability of chemicals. At this time, these developmental changes have not been completely characterized in human including their impact on the oral bioavailability of chemicals. Therefore, Fa and Fm were assumed to be the same across ages in our model.



**Figure 4. Description of carbaryl absorption in the gut.**

**C. Model Performance**

The performance of the resulting adult human model was evaluated by using published plasma time-concentration data as well as time profiles of AChE depression in RBCs after a single dose of carbaryl (1mg/kg, oral) in human volunteers (May *et al.,* 1992). The model simulations were consistent with the observed carbaryl time-concentration profiles in human plasma (Appendix Figure 1A & B).

**D. Exposure**

The main structural revision of the life stage model is an expansion of the original oral absorption model described in Yoon *et al.,* (2015) to multiple ages and routes of exposure in humans.

1. Oral

Orally dosed carbaryl is rapidly absorbed into the systemic circulation. A first order rate constant, is used to describe the absorption from the gut lumen to systemic circulation. Only a fraction of the dose (Fa) is absorbed into the gut intestinal epithelial cells. In the gut enterocytes, a fraction the absorbed dose is then subsequently metabolized (Fm) before reaching the circulatory system. These parameters were optimized using the human data from Knaak *et al.* (1968).

Both of the oral ingestion scenarios simulated here, carbaryl exposure through food intake and drinking water consumption, are simulated as an oral bolus dose at the beginning of the exposure or as each exposure event in a multiple bolus dose. To calculate the dose through drinking water exposure, total water consumption rate of (i) 0.688557 L/day for infants and children assuming they consume water 6 times a day or (ii) 1.71062 L/day exposure for youth and adult assuming they consume water 4 times a day (provided by EPA) together with drinking water concentration.

2. Inhalation

Inhaled carbaryl aerosol is described as entering the plasma compartment at the rate of alveolar ventilation (QAlv). We assume a rapid and complete absorption of carbaryl in the respiratory tract. To describe a rapid equilibration between air and plasma, a large value for a plasma to air partition coefficient (PB = 1000) is used. Tidal volume and breathing rate differ with activity levels and life stages, and accordingly these two variables determine the QAlv at a given age, where Qalv = (tidal volume-dead space volume) \* breathing rate.

3. Dermal

Dermal exposure was described using conservative assumptions in our model. The dose through this route was considered to be absorbed completely (100%) into the plasma. *In vitro* human dermal absorption studies (EPA-738-R-08-010, RED, 2008) report absorption rates from 0.41 to 3 µg/cm2/hr (low to high dose).

For dermal exposure, zero-order absorption (0.41 µg/ cm2/hr) of the compound from exposed skin to plasma was assumed. Therefore, the exposure time determines the total dose absorbed via dermal exposure. For reverse dosimetry, the exposure time to result in the maximum AChE inhibition at 10% is estimated in each exposure scenario as the dermal dose applied at time zero is an unknown variable. Then the dermal dose (µg/ cm2) is calculated using Eq.6.

**Dermal dose (µg/cm2) = (dermal absorption rate (µg/ cm2/hr) \* exposure time (hr) \* exposure area (cm2))/exposure area (cm2) (Eq. 6)**

The ‘exposure time’ in this equation is not ‘exposure duration’ given in the exposure scenarios provided by the EPA, but an ‘exposure time to achieve the maximum AChE inhibition of 10%’ when the dose is given as bolus at time zero to the surface of the skin exposure site. For some exposure scenarios, the exposure duration in the scenarios was not long enough to achieve the 10% inhibition of AChE using the absorption rate of 0.41 µg/ cm2/hr within the given exposure duration. In such cases, the zero-order absorption rate was increased to a value until 10% inhibition could be achieved during the given exposure duration. If the given exposure duration in the scenario is sufficient to see 10% inhibition within the given exposure window, then the model was run with the default absorption rate of 0.41 µg/ cm2/hr.

For instance, in one of the worker (mixer loader) scenarios (# 1 in Table 2), EPA suggests maximum exposure duration to be 8 hr, however with zero order rate of absorption of 0.41 µg/ cm2/hr, 10% AChE inhibition in RBC is achieved in 0.9 hr, an outcome due to the conservative assumptions we made; 1) 10% inhibition must occur during the given exposure duration and 2) the dermal dose is given as a bolus on the skin surface at the beginning of exposure before it is washed away In realistic human exposure scenarios, neither of these would be true and the model results indicate that it is unrealistic to reach 10% AChE inhibition with dermal exposure regardless of exposure scenario unless very conservative assumptions are made. In residential handler scenario (# 7 in Table 2), EPA provides 1 hr exposure duration, but with 0.41 µg/cm2/hr absorption rate, it was not possible to achieve 10 % inhibition. Therefore, rate of absorption was increased from 0.41 to 1.6 µg/cm2/hr to achieve 10% AChE inhibition within the EPA provided total dermal dose exposure duration of 1 hr.

To use the model for forward dosimetry for risk assessment, a dermal dose can be specified as the dose (µg/cm2) or as the exposure time in conjunction with the zero-order absorption rate (µg/ cm2/hr). In the model code, rate of carbaryl dosing to plasma after dermal exposure is as Eq.7.

**Rate of dermal dosing to plasma (µg/hr) = dermal absorption rate (µg/ cm2/hr) \* exposure area (cm2) Eq. 7**

Note that simulation time control functions are used to turn on or off dermal dosing depending on the exposure duration in the scenario or the calculated dermal dose (µg/ cm2), so that the dermal exposure can be appropriately terminated when the bolus dose is all consumed or when the time for termination of exposure (EPA assumes wash out of dermal dose on the skin at the end of exposure duration) is reached.

**III. Exposure scenarios**

**A. Scenarios**

Table1 provides exposure scenarios to be used in the risk assessment of carbaryl.

**Table. 1: Exposure scenarios in the risk assessment of carbaryl**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Exposure Scenario** | **Exposure Duration** | **Exposure Frequency** | **Body weight (age bracket)** | **Other factors** |
| **ORAL** | | | | |
| Food | 1 day | 1 dose/day everyday | 4.8 kg (< 1 year old) | NA |
| Food | 1 day | 1 dose/day everyday | 12.6 kg (Children 1-2 years) | NA |
| Food | 1 day | 1 dose/day everyday | 18.7 kg (Children 3-5 years) | NA |
| Food | 1 day | 1 dose/day everyday | 37.1 kg (Children 6-12 years) | NA |
| Food | 1 day | 1 dose/day everyday | 67.3 kg (Youth 13-19 years) | NA |
| Food | 1 day | 1 dose/day everyday | 81.5 kg (Adults 20-49 years) | NA |
| Food | 1 day | 1 dose/day everyday | 81.2 kg (Adults 50-99 years) | NA |
| Food | 1 day | 1 dose/day everyday | 72.9 kg (Females 13-49 years) | NA |
| Drinking Water | 1 day | 6 doses/day everyday | 4.8 kg (< 1 year old) | Total water consumption rate= 0.688557 L/day (0.1147595 L/exposure event) |
| Drinking Water | 1 day | 6 doses/day everyday | 12.6 kg (Children 1-2 years) | Total water consumption rate= 0.688557 L/day (0.1147595 L/exposure event) |
| Drinking Water | 1 day | 6 doses/day everyday | 18.7 kg (Children 3-5 years) | Total water consumption rate= 0.688557 L/day (0.1147595 L/exposure event) |
| Drinking Water | 1 day | 6 doses/day everyday | 37.1 kg (Children 6-12 years) | Total water consumption rate= 0.688557 L/day (0.1147595 L/exposure event) |
| Drinking Water | 1 day | 4 doses/day everyday | 67.3 kg (Youth 13-19 years) | Total water consumption rate=1.71062 L/day (0.427655 L/exposure event) |
| Drinking Water | 1 day | 4 doses/day everyday | 81.5 kg (Adults 20-49 years) | Total water consumption rate=1.71062 L/day (0.427655 L/exposure event) |
| Drinking Water | 1 day | 4 doses/day everyday | 81.2 kg (Adults 50-99 years) | Total water consumption rate=1.71062 L/day (0.427655 L/exposure event) |
| Drinking Water | 1 day | 4 doses/day everyday | 72.9 kg (Females 13-49 years) | Total water consumption rate=1.71062 L/day (0.427655 L/exposure event) |
| Oral (turf) | 180 days | 1.5 hrs/day; 4 replenishment/ hour (6 replenishments/day) | 11 kg (Standard Child 1<2) | NA |
| **INHALATION** | | | | |
| Inhalation | 180 days | 8 h/day | 80 kg (Standard Adult 16<81) | breathing rate = 1.73 m3/hr |
| Inhalation | 180 days | 8 h/day | 80 kg (Standard Adult 16<81) | breathing rate = 1.73 m3/hr |
| Inhalation | 180 days | 8 h/day | 80 kg (Standard Adult 16<81) | breathing rate = 1.73 m3/hr |
| Inhalation | 180 days | 1 hr/day | 80 kg (Standard Adult 16<81) | breathing rate = 0.64 m3/hr |
| **DERMAL** | | | | |
| Dermal | 180 days | 8 h/day | 80 kg (Standard Adult 16<81) | Skin contact = 100% |
| Dermal | 180 days | 8 h/day | 80 kg (Standard Adult 16<81) | Skin contact = 100% |
| Dermal | 180 days | 8 h/day | 80 kg (Standard Adult 16<81) | Skin contact = 100% |
| Dermal | 180 days | 1 hr/day | 80 kg (Standard Adult 16<81) | Skin contact = 50% |
| Dermal (turf) | 180 days | 1.5 hr/day | 80 kg (Standard Adult 16<81) | Skin contact = 50% |
| Dermal (turf) | 180 days | 1.5 hr/day | 57 kg (Standard Child 11<16) | Skin contact = 50% |
| Dermal (turf) | 180 days | 1.5 hr/day | 32 kg (Standard Child 6<11) | Skin contact = 50% |
| Dermal (turf) | 180 days | 1.5 hr/day | 11 kg (Standard Child 1<2) | Skin contact = 50% |
| Dermal (gardens/trees) | 180 days | 2.2 hr/day | 80 kg (Standard Adult 16<81) | Skin contact = 50% |
| Dermal (gardens/trees) | 180 days | 1.1 hr/day | 32 kg (Standard Child 6<11) | Skin contact = 50% |

**B. Point of departure (POD)**

A POD is a dose estimate developed from a chemical’s dose-response curve that aids extrapolation of anticipated risks associated with the exposure levels in the human population. EPA has suggested the use of a POD for a risk assessment of carbaryl based on AChE inhibition in RBCs and brain. RBC AChE inhibition provides an indirect indication of effects on the nervous system (EPA, 2000 and 2016). In the current analysis, the dose resulting in the maximum inhibition of AChEs at 10% in RBCs or brain was used as a POD as recommended by the EPA.

Our model can predict RBC and brain AChE inhibition over a period of time for various ages. Thus, PODs based on 10% maximum RBC and brain AChE inhibition can be predicted for various exposure scenarios across ages and body weights.

**IV. Model outputs**

**A. Simulation of exposure scenarios**

We ran the model with various exposure scenarios provided by EPA. The model incorporated appropriate ages for each exposure scenario to simulate life-stage internal exposure levels and resulting AChEs inhibitions in RBCs and brain. Table 2 below lists the POD for 10% AChE maximum inhibition in RBCs and brain in different exposure scenarios over 180 days as required in EPA exposure scenarios.

**Table. 2: PODs for PBPK based exposure scenarios.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **#** | **Exposure scenarios** | **Route, exposure days** | **Exposure frequency and other factors** | **Gender** | **Sensitive Age (years)** | **10% AChE RBC inhibition** | | **10% AChE Brain inhibition** | |
| **TLEN (only for dermal route)** | **POD** | **TLEN (only for dermal route)** | **POD** |
| 1 | Worker (mixer loader); 80kg | Dermal; 180 days | 8h/day; skin contact=100%; Rate = 0.41 ug/cm2/hr | Female | 16Y | 0.86 hr | 0.361 ug/ cm2 | 2.84 hr | 1.1644 ug/ cm2 |
| 2 | Worker (applicator); 80kg | Dermal; 180 days | 8h/day; skin contact=100%; Rate = 0.41 ug/cm2/hr | Female | 16Y | 0.86 hr | 0.361 ug/ cm2 | 2.84 hr | 1.1644 ug/ cm2 |
| 3 | Worker (PHED combo); 80kg | Dermal; 180 days | 8h/day; skin contact=100%; Rate = 0.41 ug/cm2/hr | Female | 16Y | 0.86 hr | 0.361 ug/ cm2 | 2.84 hr | 1.1644 ug/ cm2 |
| 4 | Worker (mixer loader); 80kg | Inhalation; 180 days | 8h/day, RESPR = 1730L/hr | Male | 25Y | NA | 0.205 ppm | NA | 0.417 ppm |
| 5 | Worker (applicator); 80kg | Inhalation; 180 days | 8h/day, RESPR = 1730L/hr | Male | 25Y | NA | 0.205 ppm | NA | 0.417 ppm |
| 6 | Worker (PHED combo); 80kg | Inhalation; 180 days | 8h/day, RESPR = 1730L/hr | Male | 25Y | NA | 0.205 ppm | NA | 0.417 ppm |
| 7 | Residential Handler; 80kg | Dermal; 180 days | 1h/day; skin contact=50%, Rate = 1.6 ug/ cm2/hr | Female | 16Y | 0.41 hr | 0.656 ug/cm2 | 1 hr | 1.6 ug/cm2 |
| 8 | Worker (PHED combo); 80kg | Inhalation; 180 days | 1 hr/day, RESPR = 640 L/hr | Male | 25Y | NA | 3.22 ppm | NA | 7.1 ppm |
| 9 | Residential Post-app; 80 kg | Dermal turf; 180 days | 1.5h/day; skin contact=50%, Rate = 1.17 ug/cm2/hr | Female | 16Y | 0.575 hr | 0.6727 ug/cm2 | 1.45 hr | 1.696 ug/cm2 |
| 10 | Residential Post-app; 57 kg | Dermal (turf); 180 days | 1.5h/day; skin contact=50%, Rate = 1.17 ug/cm2/hr | Female | 13Y | 0.56 hr | 0.655 ug/cm2 | 1.4 hr | 1.638 ug/cm2 |
| 11 | Residential Post-app; 32 kg | Dermal (turf); 180 days | 1.5h/day; skin contact=50%, Rate = 1.17 ug/cm2/hr | Female | 6Y | 0.367 hr | 0.4294 ug/cm2 | 0.8 hr | 0.936 ug/cm2 |
| 12 | Residential Post-app; 11 kg | Dermal (turf); 180 days | 1.5h/day; skin contact=50%, Rate = 1.17 ug/cm2/hr | Male | 1Y | 0.29 hr | 0.3393 ug/cm2 | 0.635 hr | 0.743 ug/cm2 |
| 13 | Residential Post-app, 11kg | Oral (turf); 180 days | 1.5 hrs/day; 4 replenishment/per hour (6 replenishments per day) | Male | 1Y | NA | 0.465 mg/kg | NA | 0.97 mg/kg |
| 14 | Residential Post-app, 80kg | Dermal (gardens/trees); 180 days | 2.2 h/day; skin contact=50%, Rate = 0.935 ug/cm2/hr | Female | 16Y | 0.735 hr | 0.687 ug/cm2 | 2.12 hr | 1.982 ug/cm2 |
| 15 | Residential Post-app, 80kg | Dermal (gardens/trees); 180 days | 1.1 h/day; skin contact=50%, Rate = 1.48 ug/cm2/hr | Female | 16Y | 0.45 hr | 0.666 ug/cm2 | 1.08 hr | 1.598 ug/cm2 |

Body Weight (BW): Standard adult age range 16<60 (80 kg)

Standard child age range 1<2 (11 kg)

Standard child age range 3<6 (19 kg)

Standard child age range 6<11 (32 kg)

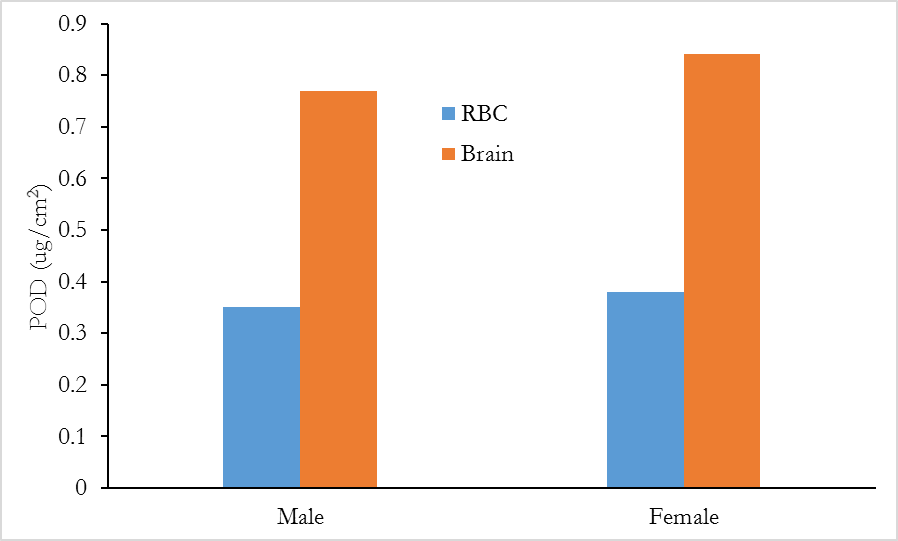
For each exposure scenario (Table 1), an age range and a representative BW were assessed. We simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW indicated by the EPA. Based on the output, the most sensitive age to be simulated for each exposure scenario was selected.

*Inhalation exposure specific PODs:* For inhalation exposure, adult male (25-year-old) was determined to be the most sensitive age and gender combination. Pulmonary parameters are largely responsible for this combination to be the most sensitive as shown in our sensitivity analysis results.

*Dermal exposure specific PODs:* Female adults were shown to be more sensitive than males for dermal exposure under the same exposure condition when we selected the most sensitive gender and age combination, whereas in younger ages, males were slightly more sensitive (Table 2 and Figure 7). This is likely due to the difference in distribution as the relative fat volume is greater in adult females, which is opposite in the younger ages. In addition, exposure duration plays a role in determining dermal route specific PODs. As shown in Figure 5, female workers are more sensitive compared to female residential post-applicators in the given scenarios when exposed to carbaryl dermally, likely due to longer exposure hours and more skin contact in workers (Exposure duration: 8 h/day vs 1 h/day; Skin contact: 100% vs 50%). Simulation of the dermal exposure scenarios for resident post-applicators (Table 2. Scenarios 9, 10, 11, 12 & 14) to obtain exposure -specific PODs shows that females with lesser body weight (lower ages) have lower PODs than adults (Figure 6) indicating younger-age females are more sensitive than adults for the same exposure scenario. Note that as discussed earlier, dermal dose is determined by both the dose per unit exposure area and the % of body surface area exposed specified in the exposure scenarios. As the younger organism has relatively larger body surface area per unit weight than adults, PODs expressed in µg/cm2 would be lower for the young under the same exposure condition.

**Figure 5: Comparative dermal route POD (µg/cm2) in female worker vs residential post-applicators**

**Figure 6: Body weight specific dermal route POD (µg/cm2) in male residential post-applicators exposed for 1.5h/day.**

****

**Figure 7: Dermal route POD (µg/cm2) for Scenario 12 in male vs female residential post-applicators.**

*Oral exposure specific PODs:* Dietary based exposure output shows very minimal gender differences toward sensitivity. Carbaryl exposure through food results in similar POD across different ages from 6 months upto 49 years. However, with the drinking water scenario, the early ages have lower PODs compared to adults. This difference in observation with two different methods of oral exposure, is mostly due to the difference in carbaryl dose per bodyweight. Carbaryl through food is dosed as mg/kg so all ages receive similar amount according to bodyweight. In drinking water scenario, PODs are in the unit of drinking water concentration (mg/L) resulting in age-dependent changes in dose in mg/kg due to the age-related difference in the exposure factor, water consumption rate in this case.

**Table. 3: PODs for Dietary based exposure scenarios provided by EPA**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Scenario | BW; Age | Exposure route | Conditions | Sensitive gender and age | POD | |
| RBC | Brain |
| 1 | All Infants (< 1 year old); 4.8kg | Food; Single dose per day everyday | NA | Female, 0.5Y | 0.44  mg/kg | 0.92  mg/kg |
| 2 | All Infants (< 1 year old); 4.8kg | Drinking water; Oral, 6 doses/day | Total water consumption rate= 0.688557 L/day (0.1147595 L/exposure event) | Female, 0.5Y | 17.55  mg/L | 36.55  mg/L |
| 3 | Children 1-2 years old, 12.6 kg | Food; Single dose per day everyday | NA | Female, 1Y | 0.425  mg/kg | 0.92  mg/kg |
| 4 | Children 1-2 years old, 12.6 kg | Drinking water; Oral, 6 doses/day | Total water consumption rate= 0.688557 L/day (0.1147595 L/exposure event) | Male, 1Y | 24  mg/L | 50  mg/L |
| 5 | Children 3-5 years old, 18.7 kg | Food; Single dose per day everyday | NA | Male, 3Y | 0.42  mg/kg | 0.9  mg/kg |
| 6 | Children 3-5 years old, 18.7 kg | Drinking water; Oral, 6 doses/day | Total water consumption rate= 0.688557 L/day (0.1147595 L/exposure event) | Female, 3Y | 36.6  mg/L | 76.25  mg/L |
| 7 | Children 6-12 years old, 37.1 kg | Food; Single dose per day everyday | NA | Female, 6Y | 0.45  mg/kg | 0.96  mg/kg |
| 8 | Children 6-12 years old, 37.1 kg | Drinking water; Oral, 6 doses/day | Total water consumption rate= 0.688557 L/day (0.1147595 L/exposure event) | Male, 6Y | 65  mg/L | 138  mg/L |
| 9 | Youth 13-19 years old, 67.3 kg | Food; Single dose per day everyday | NA | Female, 19Y | 0.465  mg/kg | 1  mg/kg |
| 10 | Youth 13-19 years old, 67.3 kg | Drinking water; Oral, 4 doses/day | Total water consumption rate=1.71062 L/day (0.427655 L/exposure event) | Female, 13Y | 53.25  mg/L | 115  mg/L |
| 11 | Adults 20-49 years old, 81.5 kg | Food; Single dose per day everyday | NA | Female, 49Y | 0.426  mg/kg | 0.95  mg/kg |
| 12 | Adults 20-49 years old, 81.5 kg | Drinking water; Oral, 4 doses/day | Total water consumption rate=1.71062 L/day (0.427655 L/exposure event) | Female, 20Y | 64  mg/L | 140  mg/L |
| 13 | Adults 50-99 years old, 81.2 kg | Food; Single dose per day everyday | NA | Female, 60Y | 0.43  mg/kg | 0.94  mg/kg |
| 14 | Adults 50-99 years old, 81.2 kg | Drinking water; Oral, 4 doses/day | Total water consumption rate=1.71062 L/day (0.427655 L/exposure event) | Female, 60Y | 68  mg/L | 148  mg/L |
| 15 | Youth 13-19 years old, 67.3 kg | Food; Single dose per day everyday | NA | Female, 19Y | 0.465  mg/kg | 1  mg/kg |
| 16 | Youth 13-19 years old, 67.3 kg | Drinking water; Oral, 4 doses/day | Total water consumption rate=1.71062 L/day (0.427655 L/exposure event) | Female, 13Y | 53.25  mg/L | 115  mg/L |

Body Weight (BW): Standard adult age range 16<60 (80 kg)

Standard child age range 1<2 (11 kg)

Standard child age range 3<6 (19 kg)

Standard child age range 6<11 (32 kg)

**B. DDEF derivation**

Monte Carlo (MC) simulations were conducted with 1000 iterations to perform population-level simulations, at which the convergence was achieved. It is often difficult to decide when it is safe to terminate MC simulation and conclude that the results represent the distribution to the greatest possible degree. Therefore, MC was performed with a further increase in the number of iterations to 5000 and 10000. The output was not substantially different, confirming that 1000 iterations was sufficient, and 1000 runs were thus used for MC simulation to generate the distributions of the maximum AChE inhibition (%) in RBCs and brain for DDEF derivations as described in Section I.

Briefly, the first initial mean simulation output was used to select the most sensitive gender and age combination for comparison to the adult for DDEF calculation (Appendix 1). Males were found to be more sensitive than females and the sensitive juvenile age was 6 months. Then, based on the sensitivity analyses results, parameters that influenced brain carbaryl Cmax were varied in MC simulations for the most sensitive age and the adult at the same oral dose (the POD for the adult to reach 10% AChE inhibition maximum). PD parameters were not included in the MC simulations as the variability in the key PD parameters (ki and kr) are not known in humans of different ages.

Then the resulting 50% percentile values of the RBC or brain AChE inhibition distribution in each age group were then used to calculate DDEF (Eq. I-1) (Table.2). The ratio of the two median % AChE inhibition values from different ages are less than 1, resulting in a DDEF for age-related PK difference of less than 1 for carbaryl. This indicates that there is no additional adjustment factor required for age-related PK differences for carbaryl. Model parameters and their distributions, those varied for the MC analysis, are listed in the EXCEL file named ‘MC Parameters\_Male\_SAP.xlsx’.

**Table 4. Calculated DDEF of Carbaryl.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Dose** | **Age** | **DDEF** | |
| **%AChE RBC inhibition** | **%AChE Brain inhibition** |
| **POD** | 0.5 vs. 25Y | 0.988 | 0.989 |
| **+ 10-fold POD** | 0.988 | 0.988 |
| **- 10-fold POD** | 0.989 | 0.988 |

+ 10-fold POD indicates the use of a 10-fold higher dose than the POD; - 10-fold POD indicates the use of a 10-fold lower dose than the POD.

**V. Sensitivity analysis**

To evaluate the relative influence of each of the model parameters on simulated brain Cmax values after three different routes of dosing (oral, inhalation and dermal), a sensitivity analysis of the human carbaryl model parameters was performed. To perform sensitivity analysis, initial simulations were performed to pick a few representative exposure levels for each exposure route as follows.

*Oral route:*

• The PoD for 25Y adult (male and female) resulting in 10% AChE inhibition in RBCs and brain inhibition, whichever lower, was picked as high dose for the sensitivity analysis. This allowed for the choice of the sensitive gender as well.

• This high dose was used to select the most sensitive early age to use in this analysis (age that resulted in highest % AChE inhibition in early life period). Then, the low dose was selected to result in 10% AChE RBC and brain inhibition, whichever is lower, for this sensitive age. The sensitivity analysis was conducted at two doses representative of the adult POD for 10% AChE inhibition and the young age POD for 10% AChE inhibition, which are indicated as high- and low-dose, respectively.

*Dermal and inhalation route:*

Similar steps were used to select high and low doses for use in sensitivity analysis. For dermal scenario-1 from Table 2 and for inhalation, scenario-4 from Table 2 were used for this selection process for sensitive age and dose for sensitivity analysis (Note: The doses used in the sensitive analysis is not in the Table 2 for these scenarios. The most sensitive age in the given adult age bracket, which is a 16-year-old, was used).

Each parameter was individually increased by 1% of their original value with all the other parameters held constant to determine the influence of small changes in the parameters to the model output on the internal dose metric of choice, in this case brain Cmax. The sensitivity coefficient (SC) was calculated as below (Yoon *et al.,* 2009):

**SC = Fractional change in model output/ Fractional change in parameter (Eq. 6)**

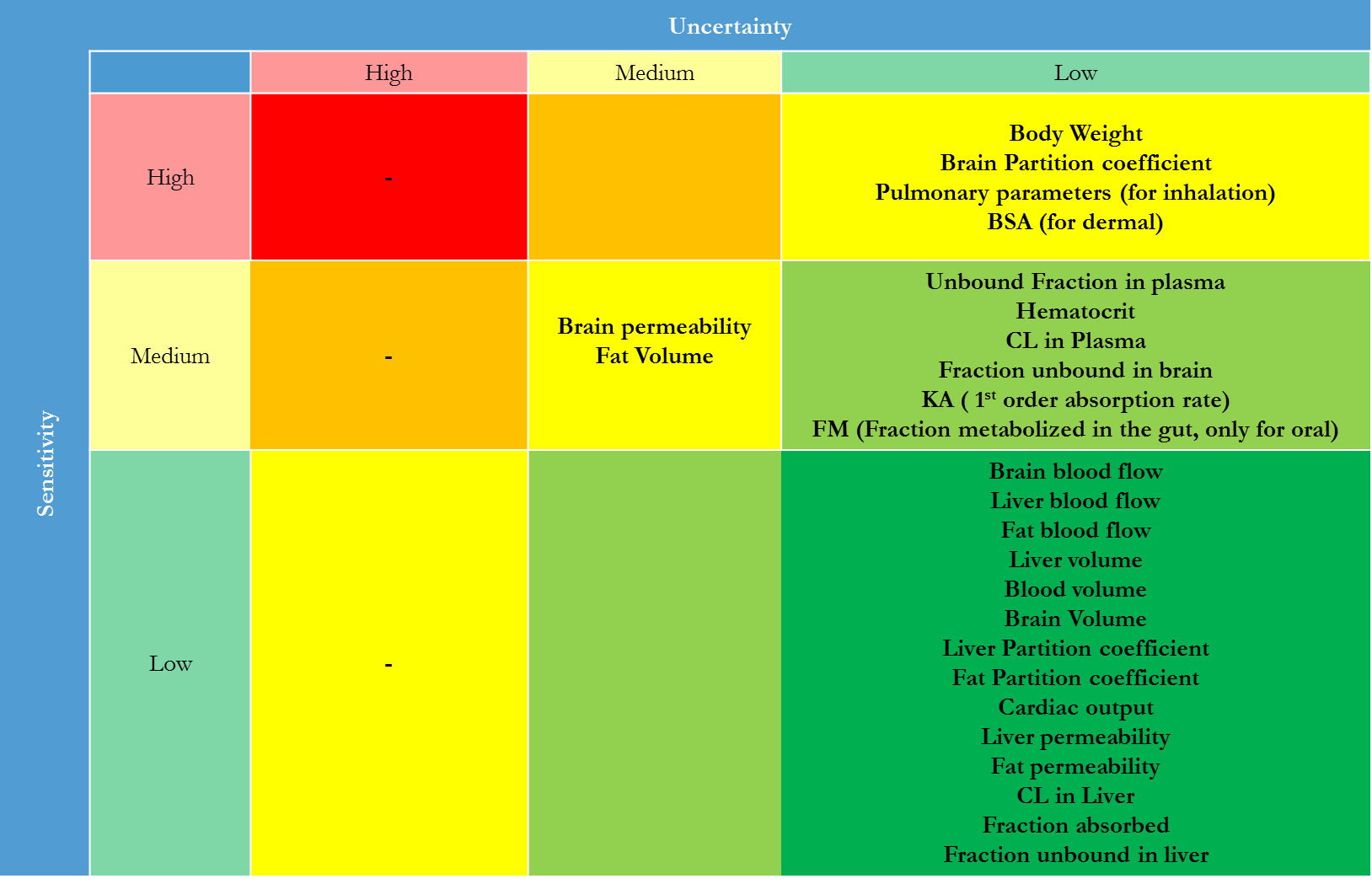
The larger the absolute value of the sensitivity coefficient, the more important the parameter. An SC of 1 represents 1:1 relationship between the change in the parameter and an increase the internal dose metric of choice. A negative SC indicates the given parameter influences the dose metric in an inverse (opposite) direction. The SCs are grouped in three categories, high (absolute values greater than or equal to 0.5), medium (absolute values greater than or equal to 0.2 but less than 0.5), or low (absolute values greater than or equal to 0.1 but less than 0.2), according to the IPCS guideline (World Health Organization, 2010).

|  |  |  |
| --- | --- | --- |
| High | >= 0.5 |  |
| Medium | >= 0.2 < 0.5 |  |
| Low | >= 0.1 < 0.2 |  |

Results from the sensitivity analysis are in the EXCEL file titled ‘Sensitivity\_analysis\_Carbaryl.xlsx’. In general, modeled brain Cmax is more sensitive to the parameters responsible for carbaryl distribution than the metabolic clearance related parameters. This indicates that around the time of peak concentration, the brain concentration is largely dependent on the distribution characteristics in addition to the metabolic clearance, whereas the overall clearance of carbaryl for specific ages is determined by metabolism. This emphasizes the importance of accurately capturing the age-related physiological parameters to increase confidence in the internal dose prediction for carbaryl across ages.

In addition to evaluating the sensitivity of model parameters to outputs, we also evaluated the uncertainty for each parameter. To check the reliability of the model outputs, it’s important to evaluate the sensitivity of the model predictions to the input parameters and also uncertainty of the parameter values. Emphasis is mostly placed on the extent of the knowledge about the values of a model parameters when determining parameter uncertainty (Table 5). Overall, sensitive parameters have low uncertainty, reflecting good level of confidence in our model prediction on the internal exposure at the target tissue (brain).

**Table 5. IPCS Sensitivity/Uncertainty matrix**

****

**VI. Discussion**

The current life-stage model has the capability of simulating oral, inhalation and dermal exposure to carbaryl at various ages from birth to adulthood. Model outputs from the current models include carbaryl concentrations in the tissue and plasma and acetylcholinesterase inhibitions in the brain (target tissue) and red blood cells in different ages under different exposure scenarios. We used a modern parameterization approach of *in vitro* to *in vivo* extrapolation for PBPK/PD model development for different human populations (life stages).

Since internal exposure at the target tissue can vary depending on route of exposure, PBPK modeling provides a scientifically sound method of route-to-route extrapolation. Children can potentially be more sensitive than adults to chemicals due to numerous factors, including the relatively larger body surface area with respect to their BW in children compared to adults, air intake per body weight and hand-to-mouth behavior. Importantly, childhood metabolic pathways are immature so their ability to metabolize chemicals may be different from an adult’s. Knowledge and consideration of the ontogeny of metabolizing enzymes is thus a critical factor in determining internal dose and risk of chemical exposure to children in comparison to adults. We therefore evaluated various datasets and published information to establish the enzyme ontogeny for many CYP isoforms, which bolsters our IVIVE and improves PK/PD predictions for environmental chemicals such as carbaryl. Use of age-specific physiological and biochemical parameters in our PBPK/PD model allow route-specific simulation of different scenarios for different ages and body weights.

Oral carbaryl exposures in rats (Moser, 1998 and Moser *et al.,* 2010) have reported that brain AChE inhibition in postnatal day (PND) 11 rats was greater than in PND17 or adult rats. In contrast, our human simulations with various exposure scenarios for different ages predict no significant differences in either internal carbaryl exposure or % AChE inhibition. This difference between rats and humans can be ascribed to two factors: (1) differences in the ontogeny of the enzymes responsible for the clearance of carbaryl in rats and humans (Saghir *et al.,* 2012), and (2) differences in the clearance of carbaryl at the much higher doses used in the animal studies as compared to expected human exposures, leading to a potential for saturation of metabolism in young rats that results in higher internal exposure to carbaryl, leading to the appearance of an early life sensitivity that is not relevant to the much lower human exposures (Sheets *et al.,* 1994).

The ultimate goal of the IVIVE-PBPK modeling presented here is to support risk assessments for human life stages. We have demonstrated the validity and utility of the carbaryl PBPK models, parameterized with *in vitro* metabolism data, in predicting target tissue and plasma concentrations of carbaryl and the consequent effect on AChE in different ages of humans under various exposure conditions. The model indicates that the FQPA DDEF should be 1 for carbaryl.

**VII. List of files submitted**

* Life stage parameters ‘Life stage parameters\_male\_SAP.xlsx’ and ‘Life stage parameters\_female\_SAP.xlsx’. These EXCEL files contain the current preliminary descriptions of age-dependent physiological parameters, non-linear regression analysis of the enzyme ontogeny data, scaling factors, and IVIVE calculations.
* MC parameter distribution table ‘MC Parameters\_SAP.xlsx’. This EXCEL file contains the parameter table used for MC simulation to derive DDEF.
* Sensitivity analysis table ‘Sensitivity\_analysis\_Carbaryl

This EXCEL file contains the sensitivity coefficient for the parameters use in the PBPK model for DLM and CPM in males and females.

* Model folder “R submission”

1. **Installing R, R studio and packages needed to run models**

Installing R

1. In a web-browser, navigate to https://cran.r-project.org/

2. Select “Download R for Windows” under “Download and Install R”

3. Select “Install R for the first time”

4. Select “Download R 3.(version) for Windows”

5. Save and run the installer file

Installing Rstudio

1. In a web-browser navigate to https://www.rstudio.com/

2. On the homepage select Download Rstudio

3. Download the open source license version of Rstudio (first from left)

4. Select windows installer (Under Installers -> RStudio 1.(ver) – Windows Vista /7/8/10

5. Save and Run the executable file

Installing Packages:

1. Run Rstudio

2. Select Packages tab from the bottom right panel in RStudio

3. Select Install in the packages tab

4. Under packages enter deSolve and select install

This will setup the environment needed to run the models

1. **Model folder “R submission**

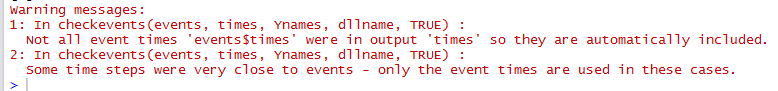
This is the current version of the Carbaryl human model in R as of July 28th, 2017. Model file (model. R) is saved in the folder named ‘Model’ and parameter files for Carbaryl simulations are saved in the folders named ‘Carbaryl’. R files are included in the folder named ‘Scenarios’ to simulate POD (10% AChE inhibition in RBC and brain) for Carbaryl at a given exposure scenario in human at specific ages using pre-populated default parameters. Any parameters can be changed to examine the impact of the change on model outputs. All together there are a total of 57 model files, and their file extensions have been changed to .txt. In order to run these model files in R, the file extensions need to be changed back to R:

1. Copy the whole model folder (R submission) on your computer.
2. **Important: The name of the folders where you will copy the files from the docket is very important to run the PBPK model in RStudio, therefore after copying all the R files, they need to be organized into subfolders as suggested below:**
   1. Copy the whole model folder (R submission) on your computer
   2. Make 3 subfolders under this main folder as “Model”, “Carbaryl” and “Scenarios”.
   3. Save the “model-new-annotated” as “.R” in a folder named “model”. Note as this file is provided as txt, therefore need to be saved as R to be able to run in R-studio.
   4. Save all parameter files names starting with “params” in folder called “Carbaryl” by “.R”. Note as these files are provided as txt, therefore need to be saved as R to be able to run in R-studio.
   5. Save all scenario files in folder called “Scenarios” by “.R”. Note as these files are provided as txt, therefore need to be saved as R to be able to run in R-studio.
3. **Running Scenarios in “R”**

To run the scenarios, click on “Source”, not “run” in RStudio. There are 29 files for scenario (Note 2) simulations and 1file named “Oral-ParaHuman-standard” to simulate single oral dose of 1mg/kg for human carbaryl model validation with May et al. data.

* 1. Exposure values need to be changed (as per cited in table 2 and 3 in white paper) in respective scenario files to simulate either 10% AChE inhibition in RBC or brain.
  2. If different scenario is desired to be simulated, then you need to change the chemical, the gender and the age in #get paramFile accordingly with the scenario needed. If you want to change parameters that are in the parameter files, don’t change them in these parameter files but in the scenario files. Copy this new line: params[["NAME OF THE PARAMETER"]] <- x.

1. **Note 1:**
2. While running the R version of the models, in case of few scenarios, you may come across the message below. The first message indicates that some dosing events were triggered at times that were originally not a part of timepoints that the model was asked to output. So, they were automatically added to the output vector. The second message indicates that in this simulation the dosing events occurred very close to a model timepoint, and so the model time point was ignored (eg. Dosing began at 0.09 hr and model was supposed to print an output at 0.1 hr. In this case only the output at 0.09 hr was printed)



1. The exposure related parameters in scenario files are setup such that the result provides a POD for brain. To obtain the correct POD value for RBC, please update the parameters to match the values provided in this report.
2. **Note 2: Description of each scenario in the R Scenario folder**

|  |  |
| --- | --- |
| **Exposure scenarios** | **Description** |
|
| Dermal-Residential Handler-80kg-half SKcontact-1hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 16Y Female, exposed to carbaryl through dermal route at a rate of 0.41 ug/cm2/hr for 180 days and 100% of skin comes in contact to the carbaryl exposure. Note: EPA provided BW as 80 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 16Y Female was most sensitive, we report the corresponding PODs in Table-2 (Scenario #1) |
| Dermal-Residential post-app-11kg-half SKcontact-1\_5hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 1Y Male, exposed to carbaryl through dermal route at a rate of 1.17 ug/cm2/hr for 180 days and 50% of skin comes in contact to the carbaryl exposure. Note: EPA provided BW as 11 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 1Y Male was most sensitive, we report the corresponding PODs in Table-2 (Scenario #12) |
| Dermal-Residential post-app-32kg-half SKcontact-1\_5hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 6Y Female, exposed to carbaryl through dermal route at a rate of 1.17 ug/cm2/hr for 180 days and 50% of skin comes in contact to the carbaryl exposure. Note: EPA provided BW as 32 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 6Y Female was most sensitive, we report the corresponding PODs in Table-2 (Scenario #11) |
| Dermal-Residential post-app-57kg-half SKcontact-1\_5hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 13Y Female, exposed to carbaryl through dermal route at a rate of 1.17 ug/cm2/hr for 180 days and 50% of skin comes in contact to the carbaryl exposure. Note: EPA provided BW as 57 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 13Y Female was most sensitive, we report the corresponding PODs in Table-2 (Scenario #10) |
| Dermal-Residential post-app-80kg-half SKcontact-1\_1hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 16Y Female, exposed to carbaryl through dermal route at a rate of 1.48 ug/cm2/hr for 180 days and 50% of skin comes in contact to the carbaryl exposure. Note: EPA provided BW as 80 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 16Y Female was most sensitive, we report the corresponding PODs in Table-2 (Scenario #15) |
| Dermal-Residential post-app-80kg-half SKcontact-1\_5hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 16Y Female, exposed to carbaryl through dermal route at a rate of 1.17 ug/cm2/hr for 180 days and 50% of skin comes in contact to the carbaryl exposure. Note: EPA provided BW as 80 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 16Y Female was most sensitive, we report the corresponding PODs in Table-2 (Scenario #9) |
| Dermal-Residential post-app-80kg-half SKcontact-2\_2hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 16Y Female, exposed to carbaryl through dermal route at a rate of 1.17 ug/cm2/hr for 180 days and 50% of skin comes in contact to the carbaryl exposure. Note: EPA provided BW as 80 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 16Y Female was most sensitive, we report the corresponding PODs in Table-2 (Scenario #14) |
| Dermal-Worker-Applicator-80kg-full SKcontact-8hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 16Y Female, exposed to carbaryl through dermal route at a rate of 0.41 ug/cm2/hr for 180 days and 100% of skin comes in contact to the carbaryl exposure. Note: EPA provided BW as 80 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 16Y Female was most sensitive, we report the corresponding PODs in Table-2 (Scenario #2) |
| Dermal-Worker-mix loader-80kg-full SKcontact-8hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 16Y Female, exposed to carbaryl through dermal route at a rate of 0.41 ug/cm2/hr for 180 days and 100% of skin comes in contact to the carbaryl exposure. Note: EPA provided BW as 80 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 16Y Female was most sensitive, we report the corresponding PODs in Table-2 (Scenario #1) |
| Dermal-Worker-PHED-80kg-full SKcontact-8hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 16Y Female, exposed to carbaryl through dermal route at a rate of 0.41 ug/cm2/hr for 180 days and 100% of skin comes in contact to the carbaryl exposure. Note: EPA provided BW as 80 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 16Y Female was most sensitive, we report the corresponding PODs in Table-2 (Scenario #3) |
| Inhalation-Worker-Applicator-80kg-1730RESPR-8hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 25Y Male, exposed to carbaryl through inhalation for 8hr/day over 180 days at breathing rate of 1730L/hr (1.73 m3/hr). Note: EPA provided BW as 80 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 25Y Male was most sensitive, we report the corresponding PODs in Table-2 (Scenario #5) |
| Inhalation-Worker-Loader-80kg-1730RESPR-8hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 25Y Male, exposed to carbaryl through inhalation for 8hr/day over 180 days at breathing rate of 1730L/hr (1.73 m3/hr). Note: EPA provided BW as 80 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 25Y Male was most sensitive, we report the corresponding PODs in Table-2 (Scenario #4) |
| Inhalation-Worker-PHED-80kg-640RESPR-1hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 25Y Male, exposed to carbaryl through inhalation for 8hr/day over 180 days at breathing rate of 640L/hr (0.64 m3/hr). Note: EPA provided BW as 80 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 25Y Male was most sensitive, we report the corresponding PODs in Table-2 (Scenario #8) |
| Inhalation-Worker-PHED-80kg-1730RESPR-8hr | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 25Y Male, exposed to carbaryl through inhalation for 8hr/day over 180 days at breathing rate of 1730L/hr (1.73 m3/hr). Note: EPA provided BW as 80 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 25Y Male was most sensitive, we report the corresponding PODs in Table-2 (Scenario #6) |
| Oral turf-Residential Post-app-11kg | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 1Y Male, exposed to carbaryl orally for 1.5 hrs as 6 replenishments per day over 180 days. Note: EPA provided BW as 11kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 1Y Male was most sensitive, we report the corresponding PODs in Table-2 (Scenario #13) |
| Drinking water-4\_8Kg-6 doses | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 6 months Female, exposed to carbaryl through drinking water. Total water consumption is 0.7L/day as 6 times a day. Note: EPA provided BW as 4.8 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 6-month Female was most sensitive, we report the corresponding PODs in Table-3 (Scenario #2). |
| Drinking water-12\_6Kg-6 doses | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 1Y Male, exposed to carbaryl through drinking water. Total water consumption is 0.7L/day as 6 times a day. Note: EPA provided BW as 12.6 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 1Y male was most sensitive, we report the corresponding PODs in Table-3 (Scenario #4). |
| Drinking water-18\_7Kg-6 doses | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 3Y female, exposed to carbaryl through drinking water. Total water consumption is 0.7L/day as 6 times a day. Note: EPA provided BW as 18.7 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 3Y female was most sensitive, we report the corresponding PODs in Table-3 (Scenario #6). |
| Drinking water-37\_1Kg-6 doses | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 6Y male, exposed to carbaryl through drinking water. Total water consumption is 0.7L/day as 6 times a day. Note: EPA provided BW as 37.1 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 6Y male was most sensitive, we report the corresponding PODs in Table-3 (Scenario #8). |
| Drinking water-67\_3Kg-4 doses | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 13Y female, exposed to carbaryl through drinking water. Total water consumption is 1.7L/day as 4 times a day. Note: EPA provided BW as 67.3 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 13Y Female was most sensitive, we report the corresponding PODs in Table-3 (Scenario #10). |
| Drinking water-81\_2Kg-60Y-4 doses | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 60Y female, exposed to carbaryl through drinking water. Total water consumption is 1.7L/day as 4 times a day. Note: EPA provided BW as 81.2 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 60Y Female was most sensitive, we report the corresponding PODs in Table-3 (Scenario #14). |
| Drinking water-81\_5Kg-4 doses | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 20Y female, exposed to carbaryl through drinking water. Total water consumption is 1.7L/day as 4 times a day. Note: EPA provided BW as 81.5 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 20Y Female was most sensitive, we report the corresponding PODs in Table-3 (Scenario #12). |
| Food-Single-4\_8Kg | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 6 months Female, exposed to carbaryl through food once a day. Note: EPA provided BW as 4.8 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 6-month Female was most sensitive, we report the corresponding PODs in Table-3 (Scenario #1). |
| Food-Single-12\_6 kg | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in1Y Female, exposed to carbaryl through food once a day. Note: EPA provided BW as 12.6 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 1Y Female was most sensitive, we report the corresponding PODs in Table-3 (Scenario #3). |
| Food-Single-18\_7 kg | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 3Y Male, exposed to carbaryl through food once a day. Note: EPA provided BW as 18.7 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 3Y Male was most sensitive, we report the corresponding PODs in Table-3 (Scenario #5). |
| Food-Single-37\_1 kg | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 6Y Female, exposed to carbaryl through food once a day. Note: EPA provided BW as 37.1 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 6Y Female was most sensitive, we report the corresponding PODs in Table-3 (Scenario #7). |
| Food-Single-67\_3 kg | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 19Y Female, exposed to carbaryl through food once a day. Note: EPA provided BW as 67.3 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 19Y Female was most sensitive, we report the corresponding PODs in Table-3 (Scenario #9). |
| Food-Single-81\_2 kg - 60Y | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 60Y Female, exposed to carbaryl through food once a day. Note: EPA provided BW as 81.2 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 60Y Female was most sensitive, we report the corresponding PODs in Table-3 (Scenario #13). |
| Food-Single-81\_5 kg | This scenario is used to generate the Carbaryl PODs (10% AChE inhibition in RBC and Brain) in 49Y Female, exposed to carbaryl through food once a day. Note: EPA provided BW as 81.5 kg, so we simulated the lower and upper end of the age range in addition to the age corresponding to the representative BW for both gender. As 49Y Female was most sensitive, we report the corresponding PODs in Table-3 (Scenario #11). |
| Oral-ParaHuman-standard | This scenario is used to generate the time profiles after a single oral dose of carbaryl (1mg/kg) as evaluation of the adult PBPK model performance using May *et al*. data |

**VIII. Link to PLETHEM Interface**

The PLETHEM interface for human carbaryl model is a standalone web application that is designed to provide a user-friendly interface to run the submitted R models. The interface holds all the scenario and parameter sets required to run the R models listed above. These R files are all available for download from the PLETHEM site. To access and run the models in PLETHEM, please follow the link below. Please request username and password to access the site.

<https://scitovation.shinyapps.io/carabarylmodel/>

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**X. Appendix**

**Appendix A. Mean simulation outputs for DDEF calculation**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | | | | |
| Details | | % AChE RBC Inhibition | | % AChE Brain Inhibition | |
| Female | Male | Female | Male |
| POD (mg/kg) | | 0.46 | 0.48 | 0.99 | 1.05 |
| Adult | 25Y | 10.07 | 9.99 | 9.93 | 10.09 |
| All Infants (< 1 year old) | 6 months | 10.49 | 17.02 | 10.68 | 17.60 |
| Children 1-2 years old | 1Y | 10.66 | 11.11 | 10.81 | 11.44 |
| 2Y | 10.50 | 11.21 | 10.60 | 11.44 |
| Children 3-5 years old | 3Y | 10.14 | 11.34 | 10.29 | 11.53 |
| 5Y | 10.20 | 10.82 | 10.33 | 11.11 |
| Children 6-12 years old | 6Y | 10.14 | 10.70 | 10.30 | 11.00 |
| 12Y | 9.35 | 9.45 | 9.30 | 9.60 |
| Youth 13-19 years old | 13Y | 9.49 | 9.53 | 9.43 | 9.68 |
| 19Y | 9.95 | 9.86 | 9.84 | 9.99 |
| Adults 20-49 years old | 20Y | 9.98 | 9.92 | 9.86 | 10.04 |
| 49Y | 10.63 | 10.53 | 10.46 | 10.62 |

Appendix Table 1. Mean simulation outputs for various ages to selected the sensitive age and gender combination for DDEF calculation.

**Appendix B. Evaluation of the adult PBPK model performance using May *et al.* data**





Appendix Figure 1. Time profiles after a single oral dose of carbaryl (1mg/kg). (A) Plasma time-concentration profile and (B) RBC time-AChE inhibition profile.

**Appendix C. The analysis of carbaryl AChE inhibition by age**

**Methods**

A statistical analysis was performed using rat *in vivo* data to determine if there exists an age-dependent susceptibility to carbaryl inhibition of brain avetylcholinesterase (AChE) activity. Data for rats aged post-natal day (PND) 18, 1 month, 4 months, 12 months, and 24 months were available for analysis (Moser *et al*., 2015). Concentration-response data were analyzed using the Curve Fitting toolbox within MATLAB (R2016a, Version 9.0.0.341360, Natick, MA). Data were fit using nonlinear regression to a hyperbolic function (see Eq. 1), or to a linear curve (see Eq. 2), where *C* is the concentration of carbaryl in the brain in μM and activity is normalized to the untreated controls.

Eq. 1

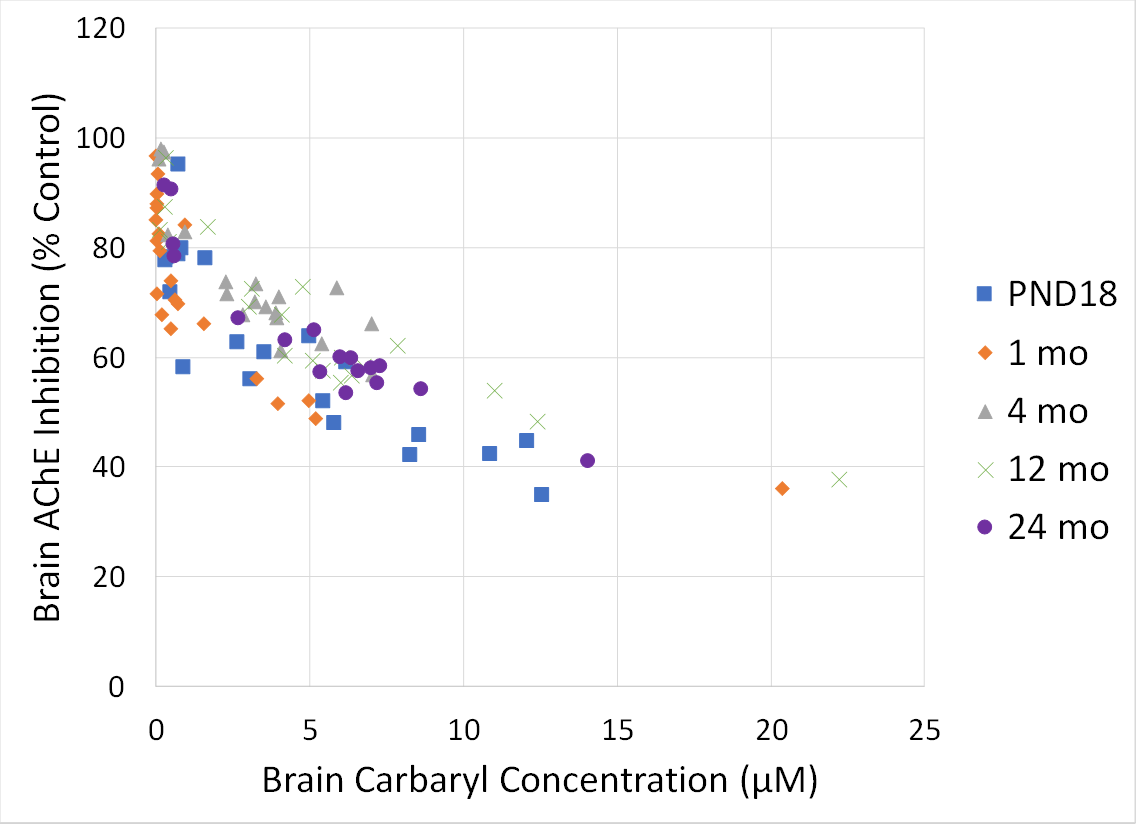
Eq. 2

**Results**

Inhibition of AChE activity by age group overlapped one another (Figure 1). In addition, there was no age-related trend in the best fit parameters for either Michaelis-Menten (Table 1) or linear (Table 2) kinetics.

**Conclusions**

A single concentration-response curve adequately describes the data for all age groups. The available evidence does not suggest any age-dependent susceptibility to AChE inhibition by carbaryl in the rat.

****

Appendix Figure 2. Inhibition of brain AChE as a function of age and dose (mg/kg/day).

Appendix Table 2. Best fit parameters & 5-95% confidence intervals (CI) for M-M inhibition (Eq. 1) by age.

|  |  |  |  |
| --- | --- | --- | --- |
| **Age** | ***V*max [5, 95] %CI** | ***K*m [5, 95] %CI** | **Adj. R2** |
| PND 18 | 61.02 [49.84, 72.21] | 1.359 [0.4416, 2.277] | 0.6921 |
| 1 month | 46.73 [37.63, 55.83] | 0.1993 [0.03895, 0.3596]a | 0.6406 |
| 4 months | 42.89 [34.59, 51.2] | 1.295 [0.3854, 2.205] | 0.8816 |
| 12 months | 66.4 [50.49, 82.3] | 3.785 [1.377, 6.192] | 0.8206 |
| 24 months | 54.56 [47.11, 62.01] | 1.672 [0.744, 2.599] | 0.8999 |
| a The value for *K*m for the 1 month group was significantly lower than for the other four age groups; however, this was not part of a trend in this parameter by age | | | |

Appendix Table 3. Best fit parameters & 5-95% confidence intervals (CI) for linear inhibition (Eq. 2) by age.

|  |  |  |  |
| --- | --- | --- | --- |
| **Age** | ***m* [5, 95] %CI** | ***b* [5, 95] %CI** | **Adj. R2** |
| PND 18 | -3.432 [-4.403, -2.461] | 76.92 [71.06, 82.77] | 0.7404 |
| 1 month | -2.761 [-3.937, -1.585] | 78.84 [73.37, 84.32] | 0.5094 |
| 4 months | -4.619 [-6.127, -3.111] | 88.88 [83.09, 94.67]a | 0.6936 |
| 12 months | -2.422 [-3.145, -1.698] | 79.69 [74.26, 85.11] | 0.7183 |
| 24 months | -3.524 [-4.324, -2.724] | 82.57 [77.55, 87.58] | 0.8449 |
| a The value of the intercept for the 4 month group was significantly higher than for the PND 18 group, but was not significantly different than for the other three age groups | | | |