

**TITLE:**  
**Physiologically-based Pharmacokinetic/Pharmacodynamic  
(PBPK/PD) Models for Malathion**

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## STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

This report is a review of existing data. Good Laboratory Practice Standards, 40 CFR Part 160, are not applicable to this submission.

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## Executive Summary

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This report describes the ongoing development of rat and human physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models for malathion. These models may be useful in the upcoming human health risk assessment for malathion by allowing the estimation of data-derived extrapolation factors (DDEFs) in place of standard, default uncertainty factors.

The metabolism of malathion is a key process to understand in order to build a PBPK/PD model. There is a large body of literature on malathion metabolism and FMC Corporation (FMC) has conducted rat and human metabolism studies that identify levels of key metabolites in urine. The key processes include:

- Activation of malathion to malaoxon via P450s.
- Metabolism of malathion via a catalytic reaction with carboxylesterase, including tracking the formation of mono-carboxylic acid (MCA) and di-carboxylic acid (DCA) of malathion.
- Metabolism of malaoxon via a catalytic reaction with carboxylesterase.
- Metabolism of malaoxon via a stoichiometric reaction with carboxylesterase, including reactivation of carboxylesterase.
- Stoichiometric reactions of malaoxon with other esterases including butyrylcholinesterase and acetylcholinesterase (AChE), including esterase reactivation and aging.

The later process, as it relates to AChE in red blood cells, is the key indicator of toxicity.

The PBPK/PD models being developed for malathion describe all of these processes. The activation of malathion to malaoxon, and catalytic metabolism of malathion and malaoxon are described with Michaelis-Menten kinetics. The stoichiometric reactions are modeled using a biomolecular rate constant and by dynamically tracking esterase levels. Metabolism primarily occurs in the liver, though metabolism is also modeled in the kidney, plasma, and brain. Inhibition of esterases is calculated in the model.

To quantify the rates of many of these processes, an *in vitro* testing program is underway at the laboratory of Dr. Janice Chambers at Mississippi State University (MSU). The program includes the following data collections:

- Estimation of  $V_{max}$  and  $K_m$  for malathion to malaoxon activation in liver microsomes for adult rats and humans of different ages.



- Estimation of  $V_{max}$  and  $K_m$  for malathion detoxication via carboxylesterase in liver microsomes for adult rats and humans of different ages.
- Estimation of  $IC_{50}$  values for malaoxon detoxication via carboxylesterase in liver microsomes for adult rats and humans of different ages.
- Estimation of the bimolecular rate constant for inhibition of malaoxon with AChE for rats and humans.

The *in vitro* testing program at MSU is not completed yet, thus, a final model has not yet been produced. Therefore, this paper presents a conceptual model for the PBPK/PD rat and human models, the current versions of the code, describes all of the available data including the methods for data still to be collected, and presents some preliminary results of the PBPK/PD rat model.

## Introduction

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Malathion is a commonly used organophosphate (OP) insecticide with both agricultural and residential uses, and is considered an important public health pesticide for controlling mosquito vectored diseases in humans and animals. It is activated *in vivo* to malaoxon, which is the active toxic moiety. Malathion and malaoxon are primarily metabolized by carboxylesterase. Malaoxon also inhibits carboxylesterase, which complicates metabolism, at least at high doses. Malaoxon inhibits acetylcholinesterase (AChE), which is assumed to be the most sensitive indication of toxicity.

The United States (U.S.) Environmental Protection Agency (EPA) has used a human physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) model in order to modify uncertainty factors (UFs) used in the human health risk assessment of chlorpyrifos (USEPA 2014). FMC Corporation (FMC) is undertaking an effort to construct PBPK/PD models for malathion that can be used to develop pharmacokinetic (PK) and pharmacodynamic (PD) data-derived extrapolation factors (DDEFs) to inform the upcoming human health risk assessment of malathion. This document outlines the model structures, the data used in model development, and how the models can be used.

EPA developed a rat PBPK model for malathion about a decade ago that was specifically oriented towards the head lice use (USEPA 2007). It was primarily parameterized using urinary metabolite data and built using AcsIX software. The relevant kinetic parameters for malathion and malaoxon metabolism were estimated by the rate of metabolite generation detection in urine using a rat metabolism study. Therefore, the starting point of the current process was this EPA-based model. We could not obtain the AcsIX code and therefore built a version similar to the EPA model and an additional pharmacodynamic (PD) component. We then built a corresponding human PBPK/PD model.

After examining the chlorpyrifos model and the associated reviews by EPA and the Agency's Science Advisory Panel (SAP), it was determined that additional *in vitro* testing data were needed to estimate kinetic parameters for malathion activation to malaoxon, malathion and malaoxon detoxication, and inhibition of AChE by malaoxon. Therefore, experiments were designed and the laboratory of Dr. Janice Chambers at Mississippi State University (MSU) was contracted to conduct these experiments. The experiments include:

- Estimation of  $V_{max}$  and  $K_m$  for malathion to malaoxon activation in liver microsomes for adult rats and humans of different ages.
- Estimation of  $V_{max}$  and  $K_m$  for malathion detoxication via carboxylesterase in liver microsomes for adult rats and humans of different ages.

- Estimation of  $IC_{50}$  values for malaoxon detoxication via carboxylesterase in liver microsomes for adult rats and humans of different ages.
- Estimation of the bimolecular rate constant for inhibition of malaoxon with AChE for rats and humans.

Note that a  $V_{max}$  and  $K_m$  were not estimated for malaoxon detoxication because there are two separate processes, including a reaction where malaoxon acts as a substrate for carboxylesterase and one where malaoxon is inhibited by carboxylesterase. Separating these two processes is difficult, particularly given that carboxylesterase is consumed in the inhibition reaction.  $IC_{50}$  values were collected to inform potential differences in rats versus humans.

Given the collection of the *in vitro* data, it was also decided that a model could be built that was similar to the chlorpyrifos model that was already accepted by EPA. Therefore, the rat and human PBPK/PD models for malathion are similar in structure (compartments, metabolism, and other processes included) to the previously published PBPK/PD model for chlorpyrifos (Timchalk et al. 2002; Smith et al. 2014). The rat and human models for malathion describe the pharmacokinetics (absorption, distribution, metabolism, excretion) of malathion and the oxon metabolite and the subsequent inhibition of AChE by the oxon, and they can be used to predict varying levels of AChE inhibition resulting from oral, dermal (human only), or inhalation (human only) exposure to malathion or the oxon. Comparison of rat and human pharmacokinetic (PK) and PD with use of the models allows for development of interspecies PK and PD DDEFs, which can potentially modify the interspecies 10X UF. The human model is a life stage model and is coded to run simulations with Monte Carlo analysis; this model will allow for development of intraspecies PK and PD DDEFs.

The *in vitro* testing program is not completed yet, thus, a final model cannot be produced. Therefore, this paper presents a conceptual model for the PBPK/PD rat and human models, the current versions of the code, describes all of the available data including the methods for data still to be collected, and presents some preliminary results of the PBPK/PD rat model.

We are seeking advice from the SAP on methods to refine the model. Specifically, we are interested in the SAP's views on:

- Whether the model structure captures all relevant processes needed to simulate malathion AChE inhibition.
- Our methodologies for estimating parameters using a mix of *in vitro* testing data and model fitting.
- The ability of the model to simulate age-related differences in metabolism.

# Metabolism

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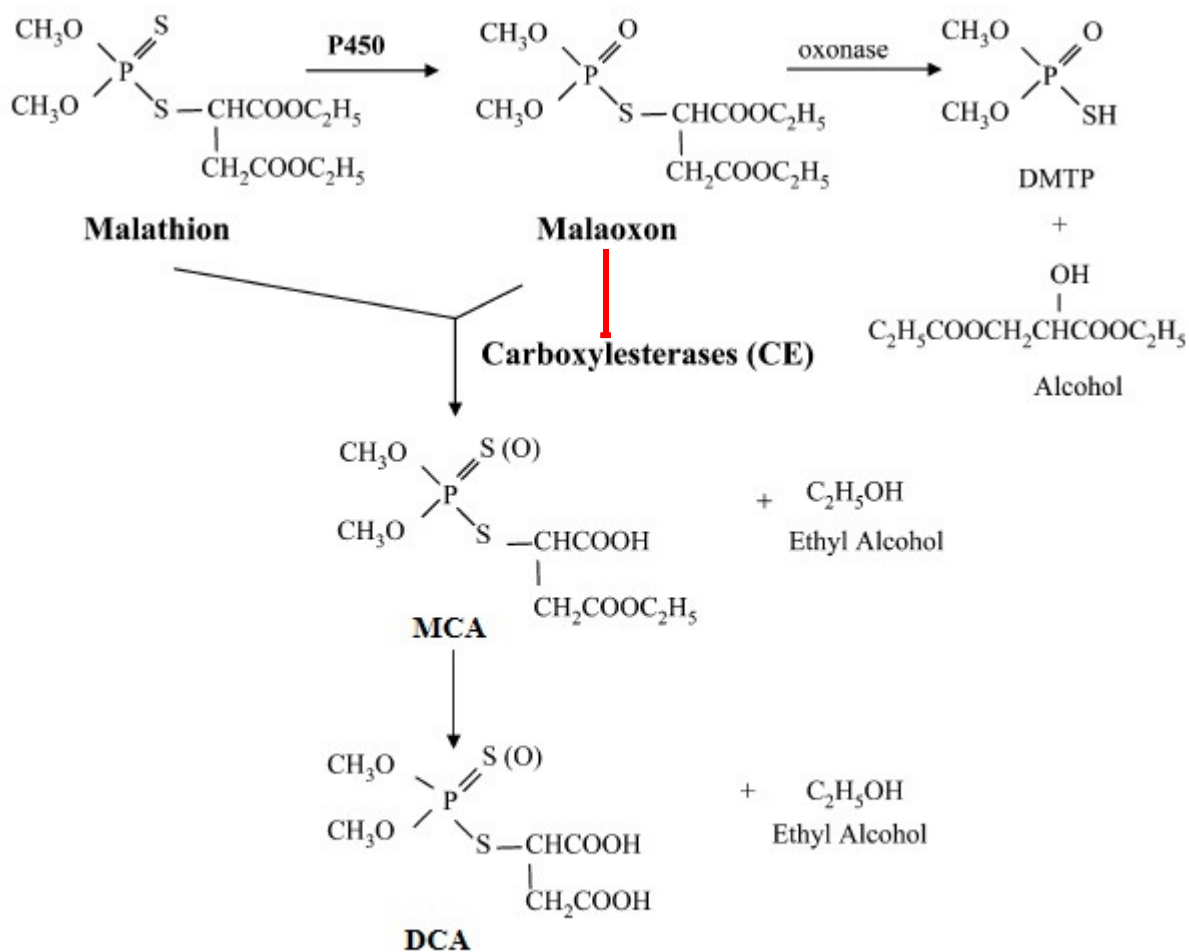
## General Metabolism Scheme

Understanding the metabolism of malathion is key to building a successful PBPK/PD model. Malathion metabolism occurs via at least two pathways (Figure 1, adapted from Buratti and Testai 2005). The primary “detoxication” pathway subjects malathion to rapid hydrolysis by the enzyme carboxylesterase (Sato and Hosokawa 1998; Buratti and Testai 2005).

Carboxylesterases process malathion at either or both of its two carboxyester moieties to give alpha- and beta- malathion monocarboxylic acid (MCA) and malathion dicarboxylic acid (DCA), non-AChE inhibitors, leading to a low toxicological profile for malathion (Main and Braid 1962; ATSDR 2003; Buratti and Testai 2005; USEPA 2007). Excreted malathion MCA and DCA have been considered specific biomarkers of malathion exposure (ATSDR 2003; Tarhoni et al. 2008). The MCA and DCA metabolites may undergo further hydrolytic processing to form the phosphoric derivatives, dimethyl dithiophosphate (DMDTP), dimethyl thiophosphate (DMTP), and dimethyl phosphate (DMP), which are considered nonspecific biomarkers of malathion exposure (Fenske and Leffingwell 1989). These dimethylphosphates may also form directly from malathion and malaoxon, sometimes referred to as an oxanase pathway. Tissue-specific concentrations of human carboxylesterases are greatest in the liver, the gastrointestinal tract, and the brain, and unlike rats, very little carboxylesterase activity is found in the plasma of humans (Talcott 1979; Buratti and Testai 2005). In female Holtzman rats administered a 73  $\mu\text{mol/kg}$  body weight dose of malathion, the downstream products of malathion hydrolysis (MCA, DCA, DMDTP, DMTP, and DMP) accounted for about 70% of the total excreted dose in urine (Chen et al. 2013). Note that plants metabolize malathion similarly; thus, these metabolites are not always useful for estimating direct exposure to malathion expect under controlled conditions (Chen et al., 2013).

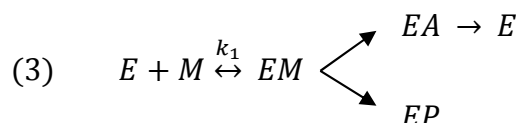
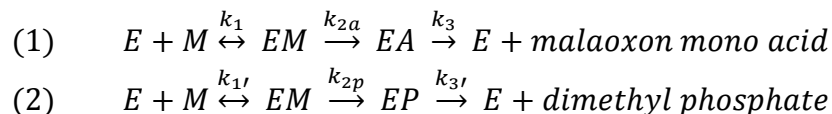
The substrate hydrolysis of malathion by carboxylesterase competes with the desulfurization reaction of malathion by cytochrome P450 (CYP) enzymes to give the oxidation product, malaoxon (Figure 1, top). Similar to other pentavalent organophosphate pesticide metabolism pathways, the bioactivation reaction produces a toxic oxon metabolite that inhibits AChE (Buratti *et al.* 2004). Through activity studies between malathion and human liver microsomes, it has been shown that at low malathion concentrations, the CYP isoforms CYP1A2 most efficiently catalyzes malaoxon formation, and the isoforms CYP3A4, CYP1A1, CYP2C8, and CYP2C9 contribute negligibly (Buratti and Testai 2007). Similar to malathion, malaoxon binds with carboxylesterases, and hydrolysis occurs to give analogous MCA and DCA products. Hydrolysis of the oxon metabolite also competes with processing by oxonases; however, it has been shown that this pathway only accounts for a relatively small proportion of the metabolized malaoxon (see discussion below).

**Figure 1. Biotransformation pathway for malathion. Across the top: desulfurization reaction of malathion by cytochrome P450 into malaoxon. Top to bottom: substrate hydrolysis reaction of malathion or malaoxon with carboxylesterase. Red: inhibition pathway for malaoxon acting on carboxylesterase. (Adapted from Buratti & Testai 2005).**



Carboxylesterase hydrolysis of malaoxon is further complicated by a second outcome of their binding: malaoxon also acts as an inhibitor of carboxylesterase (Main and Braid 1962). The inhibition scheme initially involves reversible competitive binding of malaoxon to carboxylesterase, and irreversible inhibition of the enzyme likely occurs via phosphorylation (Main and Iverson 1966). This simultaneous inhibition reaction between malaoxon and carboxylesterase has been demonstrated, and it is understood that bound malaoxon may act as a

substrate or inhibitor, but not both. However, it is unknown whether the substrate reactions occur independently or involve a shared reversible binding complex (Main and Dauterman 1966), as characterized in the equations 1-3 below:



E = carboxylesterase

M = malaoxon

EM = carboxylesterase-malaoxon binding complex

EA = acetylated hydrolysis complex

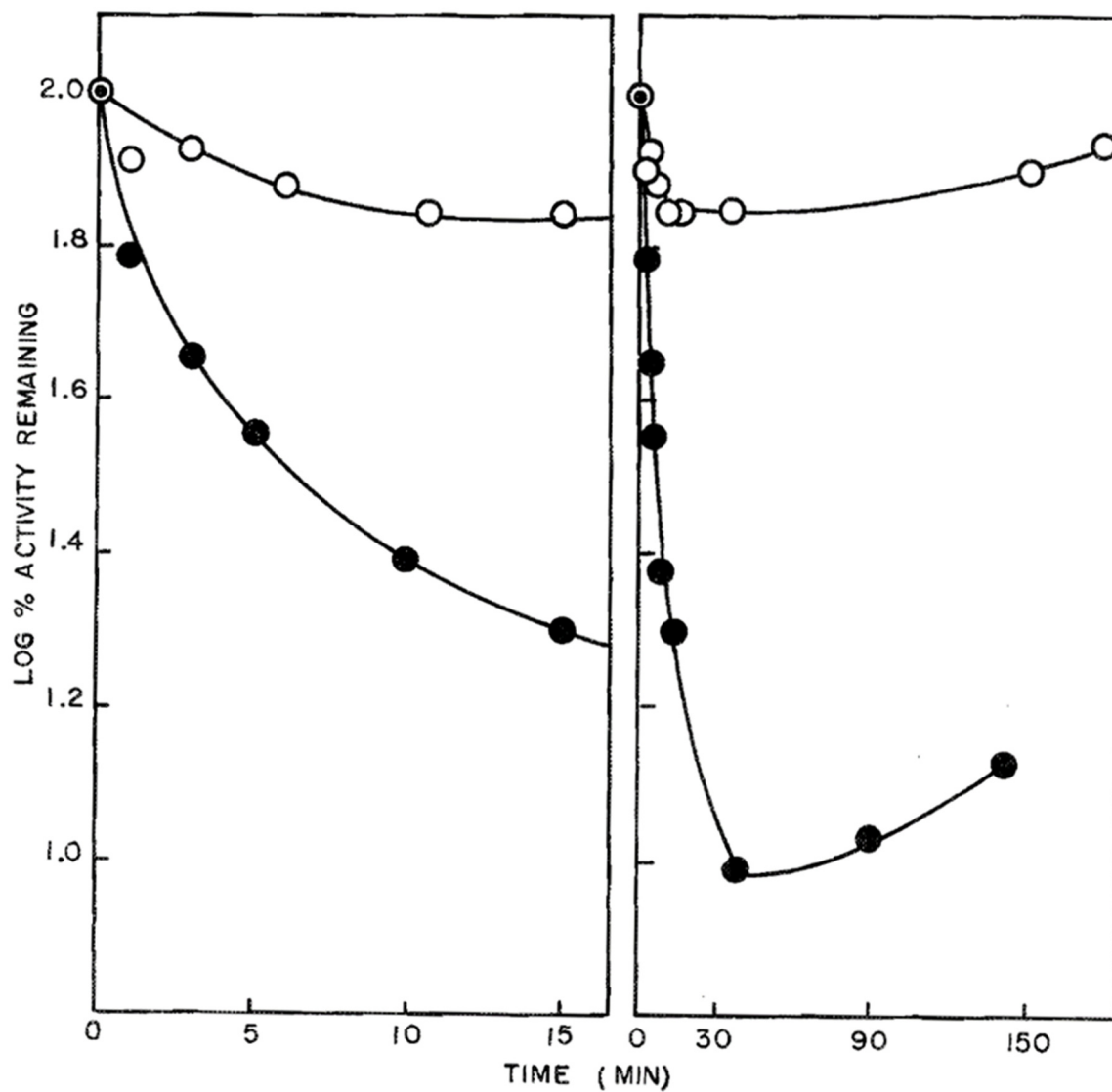
EP = phosphorylated inhibition complex

(Schema adapted from Main and Dauterman 1966)

The first scheme illustrates the malaoxon substrate reaction (1) and inhibition reaction (2) as independent pathways, whereas the second scheme (3) illustrates a shared pathway post-binding. Using the above equations, and by measuring the malaoxon inhibition of carboxylesterase in the presence of a second substrate, the bimolecular rate constant,  $k_i$ , has been calculated (Main and Dauterman 1966). Toxicity differences in optically active isomers of malaoxon have been observed, with the *d*-isomer more toxic than its analogous *l*-isomer, suggesting a specificity of substrate binding (Hassan and Dauterman 1968), which makes the first scheme more likely.

From a kinetic standpoint, malaoxon inhibition of carboxylesterase acts irreversibly; however, some inhibited carboxylesterases exhibit reactivation (Main and Dauterman 1966). This behavior has been demonstrated via the recovery of carboxylesterase activity *in vivo* (Figure 2), and would be analogous to reactivation of acetylcholinesterases inhibited by malathion and other organophosphates (Wallace and Herzberg 1988; Mason et al. 1993; Carr and Chambers 1996; Mason et al. 2000).

**Figure 2. Log of activity of 230  $\mu\text{g/L}$  rat liver carboxylesterase incubated with  $1 \times 10^{-5}$  M (blank circles) or  $1 \times 10^{-4}$  M (filled circles) malaoxon over time. At  $t > 30$  min, recovery of carboxylesterase activity is observed. (Figure adapted from Main and Dauterman 1967).**



## Malathion and Malaoxon Metabolism in Rats

A method for detecting and evaluating excreted malathion metabolites in rats post-exposure was developed by Bradway and Shafik (1977) (Bradway and Shafik 1977). Here, male Sprague-Dawley rats were dosed at five different concentrations, three times over three days, and their urine was collected before, during, and two days post-dosing. Metabolites were identified and measured via gas chromatography, using a procedure previously described (Shafik and Enos 1969). Notably, the carboxylic acid products of malathion hydrolysis, malathion MCA and DCA, constituted the primary metabolites recovered in urine (Table 1 and 2). Other downstream products (DMTP, DMDTP, DMP) further accounted for a nontrivial percent of total excreted dose.

**Table 1. Malathion metabolite excretion levels in male Sprague-Dawley rat urine for two levels of exposure, expressed as percent of total dose (calculated from Bradway and Shafik 1977, Table II).**

Dose ( $\mu$ M)	Percent of total dose excreted (%)					Percent of total dose recovered
	DMTP	DMDTP	DMP	MCA	DCA	
627	5.90	4.51	0.608	31.6	48.5	91.12
6.27	7.12	3.73	6.70	6.44	44.7	68.66

Limited data for malathion metabolism is also available from a rat metabolism study sponsored by the Malathion Task Force, in which Sprague-Dawley rats were dosed with low (40 mg/kg body weight), high (8 mg/kg body weight) or successive (40 mg/kg body weight over 15 days) doses of  $^{14}\text{C}$ -radiolabeled malathion to observe urine metabolites (Reddy et al. 1989). The study supports the trend that the predominant HPLC-detected metabolites excreted in the urine were malathion MCA and DCA; however, this study did not create a complete metabolism profile, and thus was not included in development of the present model.



**Table 2. Cumulative excretion of malathion metabolites in male Sprague-Dawley rats (Bradway and Shafik, 1977)**

Dose	Day	time (h)	DMT ( $\mu\text{mol}$ )	DMDTP ( $\mu\text{mol}$ )	DMP ( $\mu\text{mol}$ )	MCA ( $\mu\text{mol}$ )	DCA ( $\mu\text{mol}$ )	MCA+DCA combined ( $\mu\text{mol}$ )	DMPs combined ( $\mu\text{mol}$ )
69 mg	0	0	0	0	0	0	0	0	0
	1	24	7.6	3.5	0.8	47	71	118	11.9
	1-2	48	16.4	7.8	2	112	157	269	26.2
	1-3	72	36.4	26.8	2.88	195	297	492	66.08
	1-4	96	36.81	27.9	3.01	196.9	302	498.9	67.72
	1-5	120	36.97	28.27	3.81	197.86	304.4	502.26	69.05
6.9 mg	0	0	0	0	0	0	0	0	0
	1	24	1.8	0.44	0.63	3.9	16	19.9	2.87
	1-2	48	3.2	1	1	6.7	29	35.7	5.2
	1-3	72	5.8	1.92	1.72	9.7	46	55.7	9.44
	1-4	96		2.05			46.1		11.49
	1-5	120							
0.69 mg	0	0	0	0	0	0	0	0	0
	1	24	0.092	0.077	0.15	0.2	0.91	1.11	0.319
	1-2	48	0.202	0.128	0.27	0.33	1.76	2.09	0.6
	1-3	72	0.432	0.206	0.42	0.404	2.67	3.074	1.058
	1-4	96	0.447	0.234			2.8		1.739

## Human Urinary Excretion Profile

A study sponsored by Cheminova Agro A/S, Denmark included an analysis of urine metabolites in male and female human volunteers administered 0.5, 1.5, 10, or 15 mg/kg single oral doses of malathion (Aston 2000; Gillies and Dickson 2000). The fractional distribution of malathion MCA, malathion DCA, and phosphoric derivatives (DMDTP, DMTP, DMP) detected is given below as percent of administered oral dose (Table 3).

**Table 3. Observed urinary metabolites excreted in male and female human volunteers, expressed as percent of administered oral dose (adapted from Bouchard *et al.* 2003, Table 3).**

Malathion metabolite	Urine collection period (h)	0.5 mg/kg (male)	1.5 mg/kg (male)	10.0 mg/kg (male)	15.0 mg/kg (male)	15.0 mg/kg (female)	Mean
MCA	0-12	39.5	33.6	30.7	41.3	29.6	35.0
	0-24	39.7	34.0	31.5	42.6	30.1	35.6
	0-48	39.9	34.0	31.6	42.7	30.3	35.7
DCA	0-12	11.4	3.7	3.4	10.0	3.9	6.5
	0-24	13.1	7.1	4.8	11.9	4.4	8.3
	0-48	15.9	7.3	5.0	12.1	4.6	9.0
Phosphoric derivatives	0-12	15.0	21.7	18.0	24.8	15.9	20.1
	0-24	15.9	24.4	19.5	26.6	16.9	20.6
	0-48	17.2	24.7	20.0	26.9	17.5	21.3

## Plasma/Red Blood Cell Measurements

A recent study collected measurements in plasma and red blood cells (RBCs) following gavage doses in rats of 800 and 1200 mg/kg (Libberton 2017). Malathion was not detectable with a detection limit of about 9  $\mu\text{mole/L}$ . The MCA levels were 37.2  $\mu\text{mole/L}$  (plasma at 800 mg/kg), 30.4  $\mu\text{mole/L}$  (RBCs at 800 mg/kg), 16.4  $\mu\text{mole/L}$  (plasma at 1200 mg/kg), and 11.8  $\mu\text{mole/L}$  (RBC at 1200 mg/kg). The DCA levels were 367.5  $\mu\text{mole/L}$  (plasma at 800 mg/kg), 143.5  $\mu\text{mole/L}$  (RBCs at 800 mg/kg), 355.6  $\mu\text{mole/L}$  (plasma at 1200 mg/kg), and 100.8  $\mu\text{mole/L}$  (RBC at 1200 mg/kg).

Another older study by Ryan and Fukuto (1985) measured malathion, malaoxon, and metabolite levels following a 250 mg/kg gavage dose (Ryan and Fukuto 1985). Detectable levels of malathion and malaoxon were found. However, there is reason to be skeptical of these data. The malaoxon levels averaged about 0.5  $\mu\text{mole/L}$  over the first hour. In the PBPK/PD model, the estimated AChE baseline activity in RBCs is  $1.6 \times 10^{-5}$   $\mu\text{moles}$  for a 200 g rat. Given the inhibition rate constant of 16.2 L/  $\mu\text{mole/hr}$  (see below), a 0.5  $\mu\text{mole/L}$  malaoxon concentration

would substantially inhibit all of the RBC AChE in much less than an hour. Yet, the *in vivo* data show that RBC AChE inhibition for a 250 mg/kg adult rat is actually fairly modest.

## Metabolism Summary

The current version of the model includes pathways for (1) activation of malathion to malaoxon, (2) malathion detoxication via carboxylesterase, (3) malaoxon detoxication via carboxylesterase, and (4) malaoxon inhibition of carboxylesterase.

There is an additional pathway that detoxifies malathion directly to dimethylphosphates, perhaps via P450s. The pathway is considered minor and not included in the model. Bradway and Shafik (1977) recovered 91% of the malathion mass in urine from DMTP, DMDTP, DMP, MCA, and DCA in the experiment with the highest dose. The carboxylic acids accounted for 88% of the recovered mass and the dimethylphosphates for 12% of the recovered mass. Further, Chen et al. (2013) directly dosed rats with each of the five metabolites. Some MCA and DCA were converted to the dimethylphosphates, showing that some of the recovered dimethylphosphates are from carboxylesterase-related metabolism.

In the human metabolism study, the percentage of MCA and DCA recovered was somewhat lower. The mean recovery of MCA/DCA was 68% of the total recovered mass. However, the total recovery was lower at only 66% of the total malathion ingested. Chen et al. (2013) recovered 70% of ingested malathion at a very low doses in rats. When MCA and DCA were directly dosed to rats, the recovery was only 32% (MCA) and 46% (DCA). Thus, MCA and DCA may be further metabolized in some cases, which could explain the lower total recovered mass in the human study.

Given these data, it was decided that the detoxication pathway leading to dimethylphosphates would not be included in the model given that it is relatively small and the need for model efficiency.

## Available *in vivo* Cholinesterase Inhibition Data

There are extensive *in vivo* cholinesterase inhibition data for malathion and malaoxon as summarized in Table 4. All of the studies include RBC and brain AChE inhibition, while a few studies also include plasma cholinesterase inhibition. The data are provided in AcsIX files (see below).

Barnett (2008a) performed a time-to-peak effect study for malathion with PND11 pups (Barnett 2008a). The exposure was 150 mg/kg via gavage and RBC and brain cholinesterase inhibition was measured. The time to peak effect was at 1 hour. These data are useful for estimating the time course of cholinesterase inhibition.

**Table 4. Cholinesterase data for malathion and malaoxon**

Study	Chemical	Ages	Doses	Cholinesterase Compartments	Datasets
Barnett (2008a)	malathion	PND11 pups	Gavage (150 mg/kg)	RBC and brain inhibition	<ul style="list-style-type: none"> <li>Time to peak effect for PND11 pups resulting from high single oral dose (ChE measurements taken from 0.5 to 2.5 hr postdose)</li> </ul>
Barnett (2008b)	Malathion; malaoxon	PND11 pups	Gavage: malathion—10, 25, 50, 100, or 150 mg/kg; oxon—1.0, 3.5, 7.0, 10, or 12.5 mg/kg	RBC and brain inhibition	<ul style="list-style-type: none"> <li>Measurements taken 60 min following single oral dose</li> </ul>
Fulcher (2006)	Malathion	PND11 pups	Gavage (150 mg/kg)	RBC and brain inhibition	<ul style="list-style-type: none"> <li>Measurements taken from 0.5 to 6 hr postdose following single oral dose</li> </ul>

Study	Chemical	Ages	Doses	Cholinesterase Compartments	Datasets
Fulcher (2001)	Malathion	Adults and PND11 pups	Gavage (5, 50, 150, or 450 mg/kg)	RBC, Plasma, and Brain inhibition	<ul style="list-style-type: none"> <li>Measurements taken 2 hr postdose following single oral dose in adults and PND11 rats</li> </ul>
Fulcher (2001)	Malathion	Adults and PND11-PND21 pups	Gavage (5, 50, or 150 mg/kg)	RBC, Plasma, and Brain inhibition	<ul style="list-style-type: none"> <li>Measurements taken 2 hr postdose following 11 oral doses in adults and PND21 rats</li> </ul>
Barnett (2012a)	Malathion	Adults	Dietary exposure for 90 days (100, 500, 5000, or 10000 ppm)	RBC and brain	<ul style="list-style-type: none"> <li>Measurements taken on last day of dietary exposure</li> </ul>
Barnett (2012b)	Malathion	Adults	Dietary exposure for 28 days (100, 500, 5000, or 10000 ppm)	RBC and brain	<ul style="list-style-type: none"> <li>Measurements taken on last day of dietary exposure</li> </ul>
Daly (1996)	Malaoxon	Adults	Dietary exposure for 90 days (20, 1000, or 2000 ppm)	RBC and brain	<ul style="list-style-type: none"> <li>Measurements taken on last day of dietary exposure</li> </ul>

Barnett (2008b) conducted an acute gavage study with malathion and malaoxon for PND11 pups (Barnett 2008b). The BMD<sub>10S</sub> for RBC AChE inhibition were 13.3 mg/kg for males and 13.1 mg/kg for females for malathion (USEPA 2016). EPA could not estimate a BMD<sub>10</sub> for males (poor fit) for malaoxon, but estimated a BMD<sub>10</sub> of 0.60 mg/kg for females.

Barnett (2012a) and Barnett (2012b) were 90-day and 28-day dietary exposure studies, respectively, for malathion (Barnett 2012a; Barnett 2012b). BMD<sub>10S</sub> for RBC AChE inhibition ranged from 13-18 mg/kg/day. There was no significant difference between the 28-day and 90-day exposures, showing that steady-state was reached by 28 days.

Given the comparison between BMD<sub>10S</sub> in the acute gavage and 28- and 90-day dietary studies, it is tempting to conclude that there is no difference in inhibition for acute and chronic exposures. However, the series of studies by Barnett did not include an acute exposure to adults. A study by Fulcher (2001) included acute exposure to adults (Fulcher 2001) and the BMD<sub>10S</sub> for acute exposures for RBC AChE inhibition were 158 and 491 mg/kg for males and females, respectively (USEPA 2005). Thus, the AChE inhibition for acute exposures to adults is much less than for pups, likely due to differences in carboxylesterase activity.

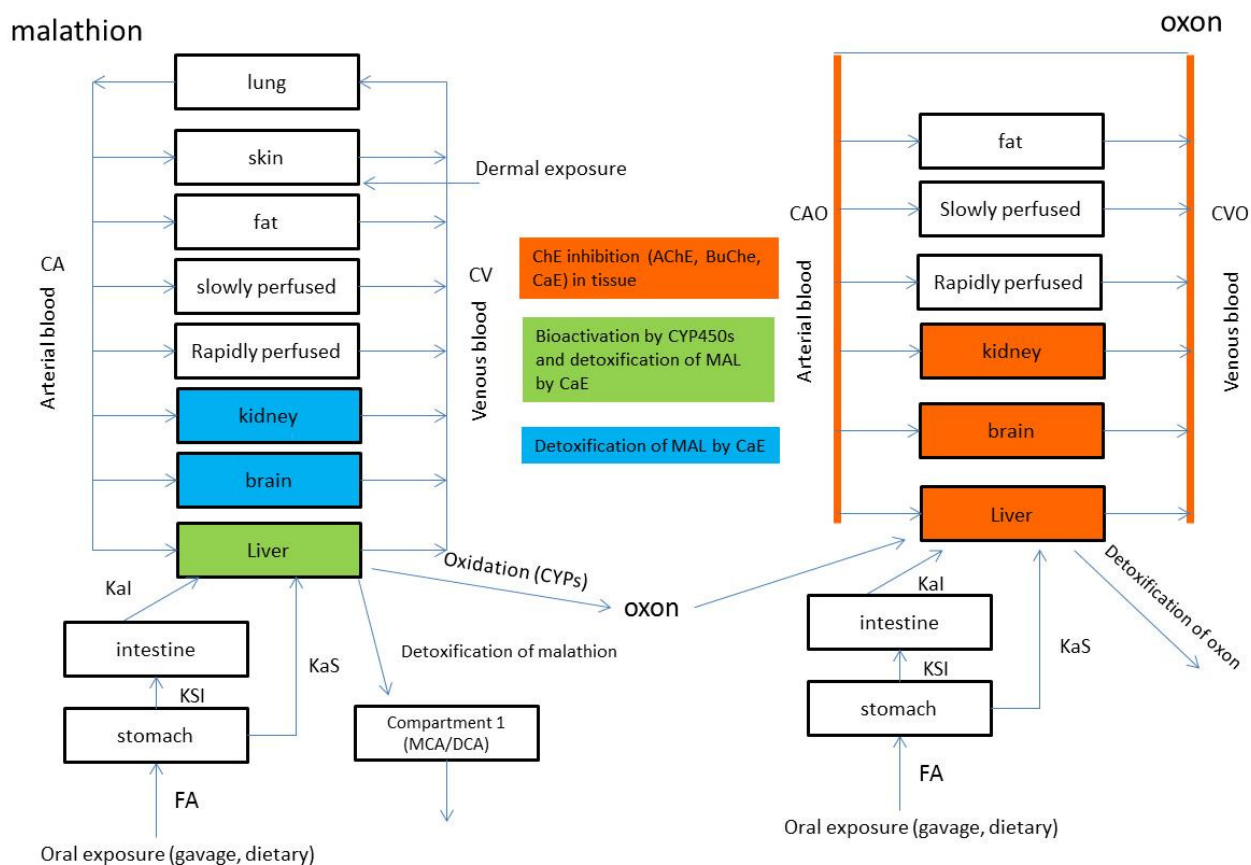
Overall, these data provide a robust dataset for validation and fitting of the PBPK/PD model given that there are acute and chronic exposures for different life stages and there is direct dosing data for malaoxon.

# Malathion Model Structures—Rat and Human

## Model Structure

The model structure for the rat and human PBPK/PD models is shown in Figure 3. Both the rat and human models describe the time course of absorption, distribution, metabolism, and excretion of malathion and malaoxon as well as AChE and B-esterase, including butyrylcholinesterase (BuChE) and carboxylesterase (CaE) inhibition by malaoxon. Timchalk et al. (2002) provide a detailed mathematical basis for the model. The data suggest that pharmacokinetics and metabolism are similar in both species (Bradway and Shafik 1977; Reddy et al. 1989; Gillies and Dickson 2000); the model structures are thus very similar (compartments included, metabolic processes).

**Figure 3. PBPK model structure for rat and human malathion models. Exposure to malathion occurs by the oral, dermal, or inhalation route (oral exposure only for rat).**



Oral absorption is modeled as a two-compartment model. The rate of change in the stomach is modeled as:

$$\frac{dStom}{dt} = -K_a S * Stom - K_s I * Stom$$

where *Stom* is the mass of malathion in the stomach,  $K_a S$  is the transfer rate from the stomach to the liver and  $K_s I$  is the transfer rate from the stomach to the intestine. In the intestine, the rate of change is modeled as:

$$\frac{dInst}{dt} = K_s I * Stom - K_a I * Inst$$

where *Inst* is the mass of malathion in the intestine,  $K_a I$  is the transfer rate from the intestine to liver. The total rate of oral absorption is:

$$\frac{dOral}{dt} = K_s S * Stom + K_a I * Inst$$

The metabolism of malathion was discussed previously. In both rats and humans, malathion is metabolized by both a hydrolytic pathway (carboxylesterase-dependent) and an oxidative pathway (CYP450 isoforms) (Talcott 1979; Buratti et al. 2005; Buratti and Testai 2005; Moser and Padilla 2016). Urine analyses of metabolites in both species indicate that hydrolysis is the predominant pathway, resulting in formation of malathion MCA and malathion DCA, which are the major metabolites. In addition to being detoxified by hydrolysis, a minor metabolic pathway is oxidative desulfuration to the toxic oxon form (malaoxon). Urinary data in both rats and humans indicate that malathion MCA and DCA are the major urinary metabolites and account for most of the administered dose; dimethyl phosphates (DMP, DMTP, and DMDTP), malathion, and malaoxon are present in only small amounts (Bradway and Shafik 1977; Gillies and Dickson 2000). DMDTP, DMTP, and DMP have all been used as biomarkers of malathion exposure; however, these are not specific to malathion as other OPs produce these urinary metabolites as well (USEPA 2015). Thus, metabolism of malathion described in the model is hydrolysis of malathion to MCA+DCA and activation of malathion to the active oxon form.

In the liver, the rate of change of malathion is modeled as:

$$\frac{dMal_{liver}}{dt} = QH * (CAM - CVM) + \frac{dOral}{dt} - \frac{dAct}{dt} - \frac{dCaEDetox_{parent}}{dt}$$



where  $Mal_{liver}$  is the mass of malathion in the liver,  $QH$  is the blood flow to the liver,  $CAM$  is the malathion concentration in arterial blood,  $CVM$  is the malathion concentration in the venous blood leaving the liver,  $dAct/dt$  is the rate of malathion activation to malaoxon:

$$\frac{dAct}{dt} = \frac{V_{max,act}[Mal]}{[Mal] + K_{m,act}}$$

where  $V_{max,act}$  is the  $V_{max}$  for malathion activation to malaoxon,  $[Mal]$  is the concentration of malathion in the liver, and  $K_{m,act}$  is the  $K_m$  for activation to the oxon.  $dCaEDetox_{parent}/dt$  is the rate of catalytic malathion detoxication via carboxylesterase:

$$\frac{dCaEDetox_{parent}}{dt} = \frac{V_{max,mal}[Mal]}{[Mal] + K_{m,adj,mal}}$$

where  $V_{max,mal}$  is the  $V_{max}$  for malathion detoxication via CaE,  $[Mal]$  is the concentration of malathion in the liver, and  $K_{m,adj,mal}$  is the  $K_m$  for parent detoxication via CaE adjusted for competitive inhibition. As the malaoxon inhibits CaE, this process will inhibit malathion detoxication as well as catalytic detoxication of the malaoxon by CaE. Thus, competitive inhibition of catalytic detoxication of the malaoxon is described using the standard competitive inhibition equation for the effect on  $K_m$ :

$$K_{m,adj} = K_m \left( 1 + \frac{C_{oxon,liver}}{K_D} \right)$$

where  $K_{m,adj}$  is the adjusted  $K_m$  to account for reduced carboxylesterase activity and  $K_D$  is the dissociation constant of malaoxon and carboxylesterase. An analogous equation is used for the malaoxon substrate reaction. This treatment is assumed in all tissues in which malaoxon-mediated CaE inhibition occurs (liver, kidney, brain, plasma).

Similarly, the rate of change of malaoxon in the liver is modeled as:

$$\frac{dOxon}{dt} = QH * (CAO - CVO) + \frac{dAct}{dt} - \frac{dCaEDetox_{oxon}}{dt} - \sum_{i=1}^3 \left( \frac{dInhib_i}{dt} \right)$$

Where  $Oxon$  is the mass of oxon in the liver,  $CAO$  is the malaoxon concentration in arterial blood,  $CVO$  is the malaoxon concentration in venous blood leaving the liver,  $dCaEDetox_{oxon}/dt$  is the rate of malaoxon detoxication via carboxylesterase, and  $dInhib/dt$  is the rate of esterase inhibition reaction with the oxon, and  $i=1$  is AChE,  $i=2$  is BuChE, and  $i=3$  is CaE. Oxon detoxication via carboxylesterase is modeled as a Michaelis-Menton process:

$$\frac{dCaEDetox_{oxon}}{dt} = \frac{V_{max,oxon}[Oxon]}{[Oxon] + K_{m,adj,oxon}}$$

where  $V_{max,oxon}$  is the  $V_{max}$  for oxon detoxication via CaE,  $[Oxon]$  is the concentration in the liver, and  $K_{m,adj,oxon}$  is the  $K_m$  for oxon detoxication via CaE adjusted for competitive inhibition.

Note that for the catalytic detoxication of malathion and malaoxon via CaE, there will be a competitive process for enzyme sites at high doses. There is not a straightforward mathematical formulation for this competitive process. Therefore, it is not included in the model structure and is assumed to be insignificant at doses relevant to risk assessment.

Esterase reactions are modeled as:

$$\frac{dInhib_i}{dt} = Esterase_i * k_i * C_{oxon,liver}$$

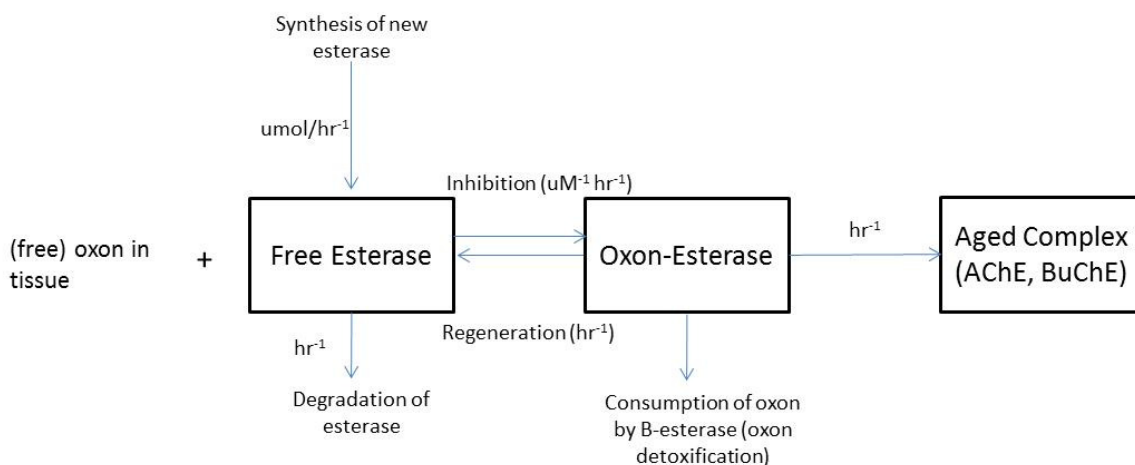
where  $Esterase$  is the esterase available mass,  $k_i$  is the bimolecular rate constant, and  $C_{oxon,liver}$  is the concentration of oxon in the liver. This treatment is assumed in all tissues in which oxon-mediated CaE inhibition occurs (liver, kidney, brain, plasma).

The kinetics of malathion MCA+DCA resulting from hydrolysis of the parent malathion are modeled using a simple one-compartment analysis to describe urinary elimination. As MCA metabolizes to DCA *in vivo*, MDA+DCA are considered as a lumped constituent. Although A-esterases (i.e., PON1) play an important role in the detoxification of several OPs, such as chlorpyrifos, PON1 does not appear to appreciably detoxify malaoxon; hydrolysis by carboxylesterases is thought to be more important in detoxification of malaoxon (Sams and Mason 1999; Moser and Padilla 2016). This was confirmed in the recent *in vitro* testing. As the other dialkyl phosphate metabolites are found to be excreted in smaller amounts and are not specific to malathion exposure, these are not explicitly described in the current models. Although some organophosphorus insecticides, such as chlorpyrifos, exhibit a high degree of plasma protein binding, studies with rat and human albumin did not indicate that malathion or malaoxon bind albumin to an appreciable extent (Tarhoni et al. 2008); thus, plasma protein binding is assumed to be negligible in both the rat and human models.

In addition to describing the pharmacokinetics of malathion and malaoxon, the models include a description of AChE inhibition by malaoxon, as this is the toxicity endpoint of interest. AChE inhibition as well as inhibition of other B-esterases (butylcholinesterase; BuChE and carboxylesterase; CaE) by the oxon is included in these models. B-esterases can detoxify the oxon by hydrolysis; however, the oxon becomes irreversibly bound, inhibiting and thus

inactivating the enzyme. For AChE and BuChE, the oxon-esterase complex can age, preventing reactivation of the enzyme. Figure 4 illustrates the model description of oxon binding and subsequent enzyme inhibition.

**Figure 4. B-esterase (AChE, BuChE, and CaE) inhibition in the malaoxon model.**  
**Inhibition of CaE by the oxon will inhibit metabolism of malathion to MCA/DCA and detoxification of the oxon. Note only AChE and BuChE are assumed to age; CaE is not assumed to age.**



Esterase levels are modeled as:

$$\frac{dEsterase}{dt} = K_s - Esterase * (K_d + K_i * C_{oxon,tissue}) + InActive * K_r$$

where  $K_s$  is the zero-order synthesis rate,  $K_d$  is the enzyme degradation rate,  $InActive$  is the mass of inactive enzyme, and  $K_r$  is enzyme reactivation rate. The inactive enzyme is modeled as:

$$\frac{dInActive}{dt} = Esterase * K_i * C_{oxon,tissue} - InActive * (K_a + K_r)$$

where  $K_a$  is the enzyme aging rate.

In the current human model, exposure to malathion can occur through the oral, dermal, and inhalation routes (dermal and inhalation are in the human model only; these routes are not described for the rat). Direct exposure to malaoxon is through the oral route only in the PBPK model. The adult rat and human models assume that PK responses are independent of gender, as this is consistent with results of rat and human studies (Reddy et al. 1989; Gillies and Dickson 2000). Both the rat and human models include a model for the parent malathion and a submodel for the metabolite malaoxon; both describe absorption, distribution, metabolism, and

excretion of the parent and oxon. The model structure includes compartments for fat, kidney, brain, liver, and lumped compartments for slowly and rapidly perfused tissues. A two-compartment model (stomach and intestine) is used in order to model uptake and absorption from the oral route (gavage and dietary exposure).

Dermal uptake is modeled using a skin compartment that uses information on the surface area exposed and the skin permeability coefficient of malathion. A standard  $K_p$ -based formulation is used (e.g., Corley et al., 1994). Uptake by inhalation is based on alveolar ventilation rate, the concentration of chemical in the air, and the fraction available to the deep lung. Malathion is highly metabolized to both the inactive metabolites and the active oxon form. Most metabolism (both hydrolysis and oxidation) occurs in the liver; however, hydrolysis by carboxylesterase to malathion MCA has also been found to occur in the kidney and brain as well as in the blood of rats (Talcott 1979; Rabovsky and Brown 1993; Buratti et al. 2005; Buratti and Testai 2005). Carboxylesterase is also present in the intestine (Satoh and Hosokawa 2006; Hatfield et al. 2011). In humans the predominant form in the intestine is CES-2 while the predominant form in the liver is CES-1; however, CES-2 does not appear to hydrolyze organophosphate insecticides and thus metabolism by carboxylesterase is not included in the intestine in the current models.

In this model, carboxylesterase-mediated (hydrolysis) metabolism of malathion to the inactive malathion MCA+DCA is described in the liver, kidney, and brain in both the human and rat models. Hydrolysis of malathion by carboxylesterases is also described in the blood in the rat model; humans lack carboxylesterase in blood (Main and Braid 1962; Talcott 1979). CYP-mediated (oxidative) metabolism of malathion to malaoxon is described in liver. In addition to detoxification of malathion, CaE also detoxifies the malaoxon; this process is described in the liver, brain, kidney, and plasma (rat only). All metabolism processes are described as saturable processes (i.e., with Michaelis-Menten kinetics), except for the malaoxon detoxification by binding to B-esterases with subsequent inhibition of the enzyme, which is modeled as a stoichiometric (non-catalytic) process.

The malathion model is linked to the oxon model through the liver compartment. As stated above, kinetics of malathion MCA + DCA resulting from hydrolysis of malathion is modeled using a simple one-compartment model to describe urinary elimination of these metabolites.

AChE, BuChE, and CaE inhibition by malaoxon occur in the liver, kidney, brain, RBCs, and plasma. Esterase inhibition kinetics (illustrated by Figure 4) are described by the same methods used in the chlorpyrifos PBPK/PD model (Timchalk et al. 2002; Smith et al. 2014). Kinetics of esterase inhibition are determined by the inhibition rate, aging rate, and reactivation rates of the enzymes. AChE and BuChE are assumed to undergo aging; however, evidence suggests that CaE is not subject to the aging process (Maxwell et al. 1994; Hemmert et al. 2010).

## Extension of Adult Human and Rat Models

In order to address any potential life stage sensitivities in PK or PD, the adult human model has been extended to a life stage model and thus incorporates the necessary changes in physiology, metabolism, and enzyme activities that occur over an individual's lifetime (birth to adult). The adult human model was modified for a life-stage model using the same methods used in the chlorpyrifos life stage model by incorporating age-specific body weight prediction, which is used to scale tissue volumes, blood flows, metabolism rates, and enzyme activities and synthesis rates (Smith et al. 2014). Thus, compartment volumes and blood flows vary with age and body weight, and metabolic rates and enzyme activity/synthesis rates are scaled based on tissue size. The  $V_{max}$  and  $K_m$  estimates for malathion activation to malaoxon from the *in vitro* metabolism experiments demonstrated that there was no apparent effect of age. The *in vitro* AChE inhibition experiments that are underway will provide data on any age-specific pharmacodynamic differences, and these data will be incorporated into the model. Model simulations with the life stage model allow for comparison of blood and tissue levels of malathion and malaoxon as well as brain and RBC AChE inhibition from birth to adulthood to examine any possible effects of age on PK or PD.

The human life stage model can also be run to do a Monte Carlo (MC) analysis. The initial life stage model will provide a single (mean) estimate of response at any one dose at any given age and for given exposure scenarios; the MC model provides a range of responses to a single dose that take into account variation in physiology, metabolism, and physical activity levels. The most sensitive model parameters (a sensitivity analysis will be performed to determine these) in the human life stage model (i.e., those model parameters that have the largest impact on RBC and brain AChE inhibition) will be varied in the MC model. As MC analysis considers variability in physiological and metabolic parameters and enzyme activity, this can aid in the derivation of an intraspecies DDEF.

## Postnatal Rat Model

The adult rat model was extended to describe the PK and PD of malathion and malaoxon in postnatal rats, as studies have demonstrated that juvenile animals can be more sensitive to the acute effects of some OP insecticides (Timchalk et al. 2007). Age-dependent sensitivity may be associated with differences in CYP450 (bioactivation) or B-esterase (detoxification) activity. Carboxylesterases are largely responsible for detoxification of both malathion and malaoxon, and age-dependent expression and/or activity differences in both carboxylesterase 1 (CES1) and carboxylesterase 2 (CES2) have been reported for both rats and humans (Karanth and Pope 2000; Yang et al. 2009; Zhu et al. 2009; Shi et al. 2011; Ohura et al. 2013; Hines et al. 2016). The adult rat model was extended to describe the postnatal period using the same methods used by Timchalk et al in the pre-weanling rat chlorpyrifos model (Timchalk et al. 2007). To

simulate pharmacokinetics and pharmacodynamics in the neonatal rat, the adult rat PBPK/PD model parameters associated with metabolism and esterase activity were scaled as function of tissue volume, which is a function of BW that is scaled to age. AChE inhibition data in neonatal rats resulting from malathion exposure are available (Stannard 2006; Barnett 2006a; Barnett 2006b; Barnett 2008a; Barnett 2008b) and will be used for model testing and validation. Simulations with the adult and neonatal rat models will allow for comparison of blood and tissue levels of malathion and malaoxon as well as brain and RBC AChE inhibition.

## Model Parameters

Several types of data were needed to develop these models, which included physiological constants, partition coefficients, biochemical parameters to describe metabolism, and parameters to describe B-esterase inhibition by the oxon. Data were obtained from the literature, from experiments, or by fitting (optimization) to available kinetic or inhibition data. The models were coded and run in acslX version 3.1.4.2. Parameter values, sources, and assumptions are provided in Table 5. The bases for these values are reviewed in this section.

**Table 5. Model parameter values for rats and humans**

Parameter	Rat Value	Source	Human Value	Source
Cardiac output (L/hr/kg BW <sup>0.75</sup> )	14	Brown et al 1997	363 (L/hr)	Calculated as sum of all other blood flows
<b>Fractional blood flows (as % of cardiac output)</b>			<b>Blood flow (L/hr/kg tissue)</b>	
Fat	0.09	Timchalk et al 2002	1.45	Poet et al 2014
Liver	0.25	Timchalk et al 2002	50.4	Poet et al 2014
Rapidly perfused tissues	0.43	Timchalk et al 2002	61.8	Poet et al 2014
Brain	0.03	Timchalk et al 2002	30.6	Poet et al 2014
Kidney	0.14	Brown et al 1997	200	Brown et al 1997; Luecke et al 2007
Alveolar ventilation rate (L/hr/kg BW)	NA		4.3	USEPA 1988
Body weight (kg)	Varies with study		Varies based on life stage; adult BW = 69 kg	
<b>Fractional volumes (as % of BW)</b>			<b>Tissue volume (L; in adults):</b> Calculated from Luecke et al 2007 and Young et al 2009 equations; same as Smith et al 2014	
Blood	0.06	Timchalk et al 2002	4.7	
Fat	0.07	Timchalk et al 2002	13.8	
Liver	0.04	Timchalk et al 2002	1.6	
Brain	0.012	Timchalk et al 2002	1.4	
Kidney	0.015	Brown et al 1997	0.29	
Slowly perfused tissues	0.78	Timchalk et al 2002	29.3	
Rapidly perfused tissues	0.04	Timchalk et al 2002	1.72	
Lung	NA		0.02	
Intestine	0.022	Brown et al 1997	0.63	
Skin	NA		2.4	
Hematocrit	0.46	Timchalk et al 2002	0.45	Timchalk et al 2002
<b>Tissue:blood partition coefficients--malathion</b>				

Parameter	Rat Value	Source	Human Value	Source
Fat	10	U.S. EPA, 2007	10	U.S. EPA, 2007
Rapidly perfused	5.2	U.S. EPA, 2007	5.2	U.S. EPA, 2007
Liver	8	U.S. EPA, 2007	8	U.S. EPA, 2007
Kidney	4.9	U.S. EPA, 2007	4.9	U.S. EPA, 2007
Brain	12.8	U.S. EPA, 2007	12.8	U.S. EPA, 2007
Slowly perfused	5	U.S. EPA, 2007	5	U.S. EPA, 2007
Lung	Not applicable for rat		1.25	U.S. EPA, 2007
Skin	Not applicable for rat			estimated
Lung:air	Not applicable for rat			Estimated
<b>Tissue:blood partition coefficients--oxon</b>				
fat	0.63	U.S. EPA, 2007	0.63	U.S. EPA, 2007
Rapidly perfused	1.0	U.S. EPA, 2007	1.0	U.S. EPA, 2007
Liver	1.17	U.S. EPA, 2007	1.17	U.S. EPA, 2007
Kidney	1.04	U.S. EPA, 2007	1.04	U.S. EPA, 2007
Brain	1.45	U.S. EPA, 2007	1.45	U.S. EPA, 2007
Slowly perfused	1.02	U.S. EPA, 2007	1.02	U.S. EPA, 2007
<b>MCA/DCA compartment parameters</b>				
Elimination rate constant (/hr)	0.2	fitted	0.2	Same as rat
Volume of distribution (L)	3.6	Fitted	3.6	Same as rat
<b>Maximum rates of metabolism (μmol/hr/kg tissue)</b>				
Malathion detox in liver	23382	Chambers and Meek 2017	34848	Chambers and Meek 2017
Malathion detox in plasma	17303	74% of liver, based on Talcott 1979	25788	74% of liver, based on Talcott 1979
Malathion detox in brain	1169	5% of liver, fitted	1742	5% of liver, fitted
Malathion detox in kidney	14029	60% of liver, based on Talcott 1979	20909	60% of liver, based on Talcott 1979
Malathion oxidation to oxon in liver	16628	Chambers and Meek 2017	46314	Chambers and Meek 2017
Oxon detox in liver	2000	Fitted to oxon inhibition data	2000	Same as rat
Oxon detox in plasma	1480	74% of liver, based on Talcott 1979	1480	74% of liver, based on Talcott 1979
Oxon detox in brain	100	5% of liver, fitted	100	5% of liver, fitted
Oxon detox in kidney	1200	60% of liver, based on Talcott 1979	1200	60% of liver, based on Talcott 1979
<b>Affinity constants (umol/L)</b>				
Malathion detox in liver	0.334	Chambers and Meek 2017	0.492	Chambers and Meek 2017



Parameter	Rat Value	Source	Human Value	Source
Malathion detox in plasma	0.334	Same as liver	0.492	Same as liver
Malathion detox in brain	0.334	Same as liver	0.492	Same as liver
Malathion detox in kidney	0.334	Same as liver	0.492	Same as liver
Malathion oxidation to oxon in liver	2730	Chambers and Meek 2017	2320	Chambers and Meek 2017
Oxon detox in liver	0.5	Fitted to oxon inhibition data	0.5	Same as rat
Oxon detox in plasma	0.5	Same as liver	0.5	Same as liver
Oxon detox in brain	0.5	Same as liver	0.5	Same as liver
Oxon detox in kidney	0.5	Same as liver	0.5	Same as liver
Dissociation constant	0.56	Krstic et al 2008	0.56	Krstic et al 2008
<b>Transfer rate constants (/hr)--malathion</b>				
Stomach to liver	1.2	Fitted	1.2	Same as rat
Stomach to intestine	0.2	Fitted	0.2	Same as rat
Intestine to liver	0.2	Fitted	0.2	Same as rat
Fractional absorption	1	Assumed all is absorbed	1	Assumed all is absorbed
Fraction into deep lung (inhalation exposure only)	NA			Estimated
Transfer rate constant from lung to GI (/hr; inhalation only)	NA			Estimated
Skin permeability coefficient (cm/hr; dermal exposure only)	NA		$2.35 \times 10^{-6}$	Bogen & Singhal 2017
<b>Transfer rate constants (/hr)--oxon</b>				
stomach to liver	1.5	Fitted	1.5	Same as rat
stomach to intestine	0.3	Fitted	0.3	Same as rat
Intestine to liver	0.4	Fitted	0.4	Same as rat
Fractional absorption	1	Assumed all is absorbed	1	Assumed all is absorbed
Molecular weight—malathion (g/mol)	330.4	U.S. EPA, 2007	330.4	U.S. EPA, 2007
Molecular weight—oxon (g/mol)	314.3	U.S. EPA, 2007	314.3	U.S. EPA, 2007
<b>Enzyme turnover rates (/h)</b>				
AChE	1.17E+07	Maxwell et al 1987	1.17E+07	Maxwell et al 1987

Parameter	Rat Value	Source	Human Value	Source
BuChE	3.66E+06	Maxwell et al 1987	3.66E+06	Maxwell et al 1987
CaE	1.09E+05	Maxwell et al 1987	1.09E+05	Maxwell et al 1987
<b>Enzyme degradation rates (/h)</b>				
AChE (liver, brain, plasma, RBCs, kidney)	3.00E-03	Timchalk et al 2002	0.01	Timchalk et al 2002
BuChE (liver, brain, plasma, kidney)	1.00E-02	Timchalk et al 2002	0.0024	Timchalk et al 2002
CaE (brain)	7.54E-04	Timchalk et al 2002	7.54E-04	Timchalk et al 2002
CaE (liver, plasma, kidney)	1.00E-03	Timchalk et al 2002	1.00E-03	Timchalk et al 2002
<b>Enzyme activity (umol/hr/kg tissue)--AChE</b>				
Brain	4.40E+05	Maxwell et al 1987	4.40E+05	Maxwell et al 1987
Plasma	2.33E+04	Timchalk et al 2002	NA	
Liver	1.02E+04	Maxwell et al 1987	1.02E+04	Maxwell et al 1987
RBCs	3.39E+04	Poet et al 2014	4.27E+05	Poet et al 2014
Kidney	5.40E+03	Maxwell et al 1987	5.40E+03	Maxwell et al 1987
<b>Enzyme activity (umol/hr/kg tissue)--BuChE</b>				
Brain	4.68E+04	Maxwell et al 1987	4.68E+04	Maxwell et al 1987
Plasma	7.85E+03	Poet et al 2014	2.63E+05	Poet et al 2014
Liver	3.00E+04	Maxwell et al 1987	3.00E+04	Maxwell et al 1987
Kidney	1.02E+04	Maxwell et al 1987	1.02E+04	Maxwell et al 1987
<b>Enzyme activity (umol/hr/kg tissue)--CaE</b>				
Brain	2.88E+05	Poet et al 2014	2.88E+05	Poet et al 2014
Plasma	8.40E+05	Poet et al 2014	NA	
Liver	1.94E+06	Poet et al 2014	1.27E+06	Poet et al 2014
Kidney	1.79E+06	Maxwell et al 1987	1.79E+06	Maxwell et al 1987
<b>Bimolecular inhibition rate constant (L/umol/hr)</b>				
AChE (liver, brain, plasma, RBCs, kidney)	16.2	Herzprung et al. 1992; bovine RBC AChE	16.2	Herzprung et al 1992; bovine RBC AChE
BuChE (liver, brain, plasma, kidney)	1.26	Herzprung et al. 1992; human plasma BuChE	1.26	Herzprung et al 1992; human plasma BuChE
CaE (liver, brain, plasma, kidney)	1.02	Hassan and Dauterman 1968; rat liver CaE	1.02	Hassan and Dauterman 1968; rat liver CaE
<b>Reactivation rate constant (/hr)</b>				
AChE (liver, brain, plasma, RBCs, kidney)	0.018	Mason et al. 2000; human RBC AChE for dimethoxy OPs	0.018	Mason et al 2000; human RBC AChE for dimethoxy OPs
BuChE (liver, brain, plasma, kidney)	0.03	Mason et al 2000; human plasma BuChE for dimethoxy OPs	0.03	Mason et al 2000; human plasma BuChE for dimethoxy OPs

Parameter	Rat Value	Source	Human Value	Source
CaE (liver, brain, plasma, kidney)	0.018	Should be similar to AChE	0.018	Should be similar to AChE
<b>Aging rate constant (/hr)</b>				
AChE (liver, brain, plasma, RBCs, kidney)	0.022	Mason et al 2000; human RBC AChE for dimethoxy OPs	0.022	Mason et al 2000; human RBC AChE for dimethoxy OPs
BuChE (liver, brain, plasma, kidney)	0.116	Mason et al 2000; human plasma BuChE for dimethoxy OPs	0.116	Mason et al 2000; human plasma BuChE for dimethoxy OPs
CaE (liver, brain, plasma, kidney)	0.00	Assume no aging of CaE	0.0	Assume no aging of CaE

## Physiological Constants and Partition Coefficients

Physiological parameters (tissue volumes, blood flows) for both the rat and human are from the chlorpyrifos rat and human models (Timchalk et al. 2002; Poet et al. 2014) or obtained from the literature (Brown et al. 1997). Tissue:blood partition coefficients for malathion and malaoxon have not been experimentally measured in rat or human tissues. In the current models, tissue:blood partition coefficients for both the parent and oxon were adapted from the USEPA assessment of malathion used as a lice treatment (USEPA 2007), which were derived using the Poulin and Theil algorithm (Poulin and Thiel 2000). The same values are used in both the rat and human models as partition coefficients are generally thought to be consistent across species. Although some organophosphorus insecticides, such as chlorpyrifos, exhibit a high degree of plasma protein binding, malathion and malaoxon do not bind albumin to an appreciable extent (Tarhoni et al. 2008). Thus, in both the rat and human models, both malathion and malaoxon are assumed to be unbound in plasma.

## Biochemical Constants

### Activation of Malathion to Malaoxon via P450

In a recent study sponsored by FMC and performed by Dr. Janice Chambers at Mississippi State University, malathion activation by cytochrome P450 (CYP) to malaoxon was measured in hepatic microsomes from rats and humans (Chambers and Meek 2017). Rat hepatic microsomes were obtained from homogenized livers extracted from adult Sprague Dawley-derived rats of both sexes (6 per sex, 70 days old, 250-300 g). Human hepatic microsomes from humans of both sexes and several races and ethnicities were obtained from XenoTech, LLC (27 total human sources) with ages ranging from 0.04 to 75 years. Thus, this dataset allows us to estimate age-specific rates.

The study aimed to first quantify malathion bioactivation via inhibition of purified exogenous AChE (obtained from electric eel) following incubation of hepatic microsomes with malathion. Cholinesterase inhibition was used as an analytical tool to serve as an indirect measure of malaoxon concentration. The hypothesis was that the AChE active metabolite, malaoxon, could be quantified with high sensitivity and before appreciable degradation due to its potency against AChE, which acts as a “trap” to remove oxon from the system to measure how much was formed. From this reaction, the study aimed to calculate an apparent affinity constant ( $K_{mapp}$ ) and a maximum rate of metabolism ( $V_{max}$ ) that could be used in PBPK modeling. These values were determined through analyses of Lineweaver-Burk plots (inverse of malathion concentration versus inverse of reaction velocity) for rat and human hepatic microsomes, as denoted below in Tables 6 and 7.

**Table 6.  $K_{mapp}$ ,  $V_{max}$ , and  $V_{max}/K_{mapp}$  for malathion bioactivation in adult Sprague Dawley-derived rat liver microsomes.**

Subject #	Age (days)	$K_{mapp}$ (mM)	$V_{max}$ (nmoles oxon/min/mg P)	$V_{max}/K_{mapp}$	$R^2$
Male 1	70	6.907	7.369	1.07	0.987
Male 2	70	2.169	4.037	1.86	0.947
Male 3	70	2.253	5.844	2.59	0.984
Male 4	70	2.926	2.488	0.85	0.922
Male 5	70	8.185	5.73	0.70	0.851
Male 6	70	0.393	5.847	14.88	0.974
Female 1	70	2.299	10.05	4.37	0.992
Female 2	70	1.469	7.575	5.16	0.987
Female 3	70	0.761	3.195	4.20	0.926
Female 4	70	1.456	4.214	2.89	0.903
Female 5	70	2.298	10.01	4.36	0.993
Female 6	70	1.69	7.547	4.46568	0.93

**Table 7.  $K_{mapp}$ ,  $V_{max}$ , and  $V_{max}/K_{mapp}$  for malathion bioactivation in human hepatic microsomes of both sexes and several races and ethnicities**

Subject #	Sex	Race/Ethnicity	Age (years)	$K_{mapp}$ (mM)	$V_{max}$ (nmoles oxon/min/mg P)	$V_{max}/K_{mapp}$	$R^2$
354	Female	Caucasian	0.04	8.15	46.42	5.70	0.942
845	Male	Caucasian	0.08	0.358	19.92	55.64	0.987
268	Male	Caucasian	0.343	18.6	59.241	3.19	0.948
270	Male	Caucasian	0.42	0.664	5.69	8.57	0.887
395	Male	Caucasian	0.42	1.19	48.16	40.47	0.986

Subject #	Sex	Race/ Ethnicity	Age (years)	$K_{mapp}$ (mM)	$V_{max}$ (nmoles oxon/min/mg P)	$V_{max}/$ $K_{mapp}$	$R^2$
825	Male	Caucasian	0.92	0.437	13.66	31.26	0.93
322	Male	Hispanic	1	5.361	24.71	4.61	0.88
57	Female	Caucasian	2	0.445	10.3	23.15	0.876
551	Male	Caucasian	2	0.327	3.445	10.54	0.865
852	Male	Hispanic	2	0.327	3.443	10.53	0.868
346	Male	Caucasian	3	0.281	19.6	69.75	0.947
792	Male	American Indian	4	0.756	9.099	12.04	0.971
215	Male	Caucasian	6	2.1	13.21	6.29	0.938
59	Male	Caucasian	9	0.175	4.23	24.17	0.956
485	Male	Caucasian	10	0.381	21.43	56.25	0.979
410	Male	Caucasian	11	2.067	26.68	12.91	0.954
133	Female	Caucasian	17	1.8	15.4	8.56	0.97
236	Male	Asian	17	0.535	12.19	22.79	0.897
25	Female	Caucasian	30	0.248	10.88	43.87	0.853
393	Female	Caucasian	30	1.19	48.16	40.47	0.986
36	Male	African American	37	1.15	25.12	21.84	0.904
420	Male	Caucasian	42	2.29	30.99	13.53	0.979
177	Female	Caucasian	45	1.51	22.82	15.11	0.94
115	Female	Caucasian	48	1.49	17.75	11.91	0.935
201	Male	Hispanic	58	4.72	57.9	12.27	0.96
355	Female	Caucasian	71	5.83	46.26	7.93	0.971
203	Male	Caucasian	75	0.239	14.85	62.13	0.897

There were no statistically significant correlations between any of the measured parameters and age, sex or race/ethnicity. The slope for  $V_{max}$  vs. Age was positive, but the p-value was only 0.17. Therefore, there appears to be no significant age-or sex-related differences in activation.

### Malathion Hydrolysis by Carboxylesterases

Hydrolysis of malathion to malathion MCA and DCA by carboxylesterases is described in these models using Michaelis-Menten kinetics.  $V_{max}$  and  $K_m$  for hydrolysis of malathion by carboxylesterases have been reported for human liver microsomes (Buratti and Testai 2005). Three single human liver microsome samples (HLM<sub>1-3</sub>), and a sample from a five-donor pool (HLM<sub>4</sub>), were incubated with substrate malathion (0.25-400  $\mu$ M). All HLM preparations exhibited typical saturation curves (activity versus malathion substrate concentration), and each Eadie-Hofstee plot ( $V$  versus  $V/S$ ) produced two straight lines indicative of two different liver components that bind and catalyze malathion at different affinities. Each of the two slopes corresponds to a different  $K_m$  and  $V_{max}$ ; thus, apparent  $K_m$ ,  $V_{max}$ , and  $CL_i$  values were determined

for the different phases of affinity (Table 8). It was assumed that the low affinity carboxylesterase component (with  $V_{max2}$ ) should contribute negligibly to malathion detoxication at low malathion concentrations, while the high affinity components (with  $V_{max1}$ ) should dominate at either low or high malathion concentrations. Thus, low affinity biochemical constants were recalculated to exclude the high affinity phase contribution, and the combined kinetic parameters ( $V_{max\ tot}$ ) were included.

**Table 8. Kinetic parameters of malathion detoxication for four human liver microsome preparations at separate and combined phases of affinity (adapted from Buratti and Testai, 2005).**

Affinity Phase	HLM Number	$V_{max1}$ (nmol malathion hydrolyzed / mg protein / min)	$K_{m1}$ ( $\mu$ M)	$CL_{i1}$ (nmol malathion hydrolyzed / mg protein / min / $\mu$ M)
High	HLM1	$9.1 \pm 0.3$	$0.25 \pm 0.02$	$36.4 \pm 0.1$
	HLM2	$9.4 \pm 0.4$	$0.31 \pm 0.03$	$30.9 \pm 0.1$
	HLM3	$16.9 \pm 0.4$	$0.69 \pm 0.04$	$24.6 \pm 0.01$
	HLM4	$10.5 \pm 0.5$	$0.5 \pm 0.1$	$19.7 \pm 0.1$
Low		$V_{max2}$	$K_{m2}$	$CL_{i2}$
	HLM1	$16.1 \pm 1.4$	$10.3 \pm 2.3$	$1.6 \pm 0.2$
	HLM2	$6.4 \pm 0.4$	$15.8 \pm 3.2$	$0.4 \pm 0.2$
	HLM3	$44.4 \pm 2.8$	$26.8 \pm 4.2$	$1.7 \pm 0.2$
	HLM4	$20.7 \pm 1.3$	$24 \pm 4$	$0.9 \pm 0.2$
Combined		$V_{max\ tot}$	$K_{m\ tot}$	$CL_{i\ tot}$
	HLM1	$24.0 \pm 0.6$	$3.9 \pm 0.3$	$6.4 \pm 0.1$
	HLM2	$15.6 \pm 0.2$	$1.7 \pm 0.1$	$9.4 \pm 0.1$
	HLM3	$58.9 \pm 0.7$	$12.4 \pm 0.3$	$4.7 \pm 0.03$
	HLM4	$30.1 \pm 0.6$	$4.5 \pm 0.3$	$6.7 \pm 0.1$

$V_{max}$  and  $K_m$  for hydrolysis of malathion by carboxylesterases have also been reported for rat liver, kidney, and plasma (Talcott 1979). Carboxylesterase activity was measured via colorimetric assay, in which the carboxylesterase-mediated hydrolysis of malathion was stoichiometrically equivalent to reduction of the dye, *p*-iodonitrophenyltetrazolium violet. Each test site (liver homogenate, liver microsomes, blood serum, kidney microsomes) was incubated with concentrations of malathion ranging from 5-300  $\mu$ M. Plots of activity versus malathion concentration yielded kinetic parameters, denoted in Table 9. The  $K_m$  value for rat liver microsomes is significantly higher than that expected for human liver microsomes, suggesting that human liver detoxication of malathion is more efficient than rat liver detoxication.

**Table 9. Kinetic Parameters for Malathion Hydrolysis by Carboxylesterase in Female Sprague-Dawley rats (adapted from Talcott 1979, Table 3).**

Enzyme Source	Apparent $K_m$ (mM)	Apparent $V_{max}$ (nmol/min/mg protein)
Liver homogenate <sup>1</sup>	0.062 ± 0.01	12.0 ± 0.73
Liver microsomes <sup>2</sup>	0.070 ± 0.003	34.2 ± 0.56
Kidney homogenate <sup>1</sup>	0.091 ± 0.018	20.4 ± 1.75
Serum	0.020 ± 0.003	25.2 ± 1.27

<sup>1</sup>250 µg protein/mL in incubation mixture

<sup>2</sup>From homogenate; 250 µg protein/mL

<sup>3</sup>From orbital sinus; 1 part/400 parts

Although the liver is the primary site of metabolism, hydrolysis has been found to occur in kidney (and in the blood in the rat), and carboxylesterases are known to be present in the intestine and brain, though intestinal carboxylesterase is mostly CES-2 whereas OPs are thought to be primarily metabolized by CES-1. CES-2 is not thought to play a significant role in OP detoxication. In addition to the existing *in vitro* data, *in vitro* experiments with rat and human liver microsomes were recently completed as described previously. These new data were implemented in the models through use of *in vitro* to *in vivo* extrapolation (IVIVE), and hydrolysis in extrahepatic tissues (kidney, intestine, brain, and rat blood) was to be scaled to a percentage of that of the liver based on the rat data from Talcott 1979.

As we were validating the PBPK model, it became clear that the model was underestimating detoxication based on the limited literature data. Therefore, we worked with MSU to develop a way to estimate malathion detoxication kinetics. This presented certain challenges given that the MSU test system can only measure the oxon. To date, these assays are not yet completed.

## Malaoxon Detoxication

Attempts were also made to measure malaoxon detoxication. However, it was not possible to isolate the enzyme systems potentially involved in detoxication; therefore, only IC<sub>50</sub> values could be estimated. These IC<sub>50</sub> values for malaoxon inhibition of AChE are given below for rat and human hepatic microsomes in Tables 10 and 11, respectively. IC<sub>50</sub> values for rats and humans were generally similar. While the IC<sub>50</sub> values cannot be directly used in the PBPK model, the similarity over a 30-minute incubation provides some evidence that rat and human detoxication is similar.

**Table 10. Rat hepatic microsome malaoxon detoxication. Hepatic microsomes extracted from livers from adult Sprague Dawley-derived rats.**

Subject #	Age (days)	AChE IC <sub>50</sub> (nM)
Male 1	70	234.72
Male 2	70	137.74
Male 3	70	185.34
Male 4	70	173.69
Male 5	70	128.21
Male 6	70	139.04
Female 1	70	114.30
Female 2	70	142.75
Female 3	70	84.74
Female 4	70	98.15
Female 5	70	91.98
Female 6	70	103.65

**Table 11. Human hepatic microsome malaoxon detoxication. Hepatic microsomes extracted from livers from human hepatic microsomes of both sexes and various races.**

Subject #	Sex	Race/ Ethnicity	Age (years)	AChE IC <sub>50</sub> (nM)
354	Female	Caucasian	0.04	282.10
845	Male	Caucasian	0.08	132.75
268	Male	Caucasian	0.343	124.65
395	Male	Caucasian	0.42	61.05
270	Male	Caucasian	0.42	154.39
825	Male	Caucasian	0.92	254.17
322	Male	Hispanic	1	78.51
852	Female	Caucasian	2	82.21
551	Male	Caucasian	2	157.17
57	Male	Hispanic	2	136.35
346	Male	Caucasian	3	159.60
792	Male	American Indian	4	121.39
215	Male	Caucasian	6	83.63



Subject #	Sex	Race/ Ethnicity	Age (years)	AChE IC <sub>50</sub> (nM)
59	Male	Caucasian	9	144.25
485	Male	Caucasian	10	99.07
410	Male	Caucasian	11	118.36
133	Female	Caucasian	17	115.43
236	Male	Asian	17	133.86
25	Female	Caucasian	30	59.49
393	Female	Caucasian	30	125.92
36	Male	African American	37	100.45
420	Male	Caucasian	42	137.34
177	Female	Caucasian	45	145.71
115	Female	Caucasian	48	57.93
201	Male	Hispanic	58	94.94
355	Female	Caucasian	71	112.47
203	Male	Caucasian	75	266.09

### Carboxylesterase Inhibition by Malaoxon

The bimolecular rate constant ( $k_i$ ) for the inhibition of carboxylesterase activity by malaoxon has been previously investigated (Main and Dauterman 1966). Here, inhibition rates were measured by observing the effect of malaoxon on carboxylesterase in the presence of a second substrate, dibutyl malate, which has a known  $K_m$  ( $0.25 \pm 0.043$  mM) and  $V_{max}$  ( $32.5 \pm 2.9$   $\mu$ mol/mg protein/min). The equation for  $k_i$ , in the presence of dibutyl malate as a second substrate, was derived from equations (1) and (2) (discussed previously) and is as follows:

$$\frac{1}{\rho} = \frac{1}{ik_i} \left( 1 + \frac{s}{K_m'} \right) + \frac{1}{k_{2p}} \left( 1 + \frac{K_a}{K_m} \right)$$

in which  $\rho$  is the term:  $2.3\Delta\log v / \Delta t$ ;  $i$  and  $s$  are the concentrations of malaoxon and dibutyl malate, respectively;  $K_m$  and  $K_m'$  are affinity constants for malaoxon and dibutyl malate, respectively;  $k_{2p}$  is the rate constant for irreversible inhibition of the enzyme-substrate complex, as in equation (2); and  $K_D$  is the dissociation constant for carboxylesterase and malaoxon.

Carboxylesterase activity in the presence of this second substrate at different concentrations of malaoxon (0.125 to 1.10 mM) was monitored via radiometer pH-stat, which provides a measurement of hydrolysis. Regression of the plot of hydrolysis over time yielded the term  $2.3\Delta\log v / \Delta t$ , which was subsequently plotted against the following term:

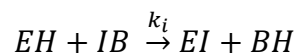
$$\frac{1}{i} \left( 1 + \frac{s}{K_{m'}} \right) mM^{-1}$$

The slope of the preceding plot equals  $\frac{1}{k_i}$  and  $k_i$  was calculated to be  $1.70 \pm 0.14 \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$ . The  $k_i$ , adjusted to account for the initial velocity of the carboxylesterase-dibutyl malate reaction just before malaoxon was added, was calculated to be  $1.74 \pm 0.12 \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$ , indicating that malaoxon hydrolysis did not appreciably affect the reaction with dibutyl malate.  $k_i$  values remained consistent when the reaction was retested with a different concentration of dibutyl malate, suggesting that the second substrate did not introduce secondary effects to the malaoxon inhibition rate.

## Esterase inhibition

Inhibition of AChE, BuChE, and CaE by malaoxon is described by the same methods used in the chlorpyrifos PBPK/PD model (Timchalk et al. 2002; Smith et al. 2014), and both chemical-specific and non-chemical specific parameters are needed. Non-chemical specific parameters include baseline B-esterase activity levels in tissues, enzyme turnover rates, and degradation rates, which are based on Maxwell et al and Timchalk et al (Maxwell et al. 1987; Timchalk et al. 2002). Chemical-specific parameters include the bimolecular inhibition rate constants ( $k_i$ ) and enzyme reactivation and aging rates; chemical-specific data was incorporated wherever possible. *In vitro* studies are currently underway at MSU to determine the  $k_i$ s for the inhibition of human and rat RBC AChE for a number of organophosphate oxons, including malaoxon; the  $k_i$ s measured for rat and human RBC AChE will then be used for the AChE  $k_i$  in all tissues for which AChE inhibition occurs in the respective models.

While the data are being collected on bimolecular rate constants, interim data from a study by Herzsprung *et al.* 1992 are being used (Herzsprung et al. 1992). An AChE inhibition reaction, combined with the following scheme, allowed for determination of the bimolecular rate constant,  $k_i$ :



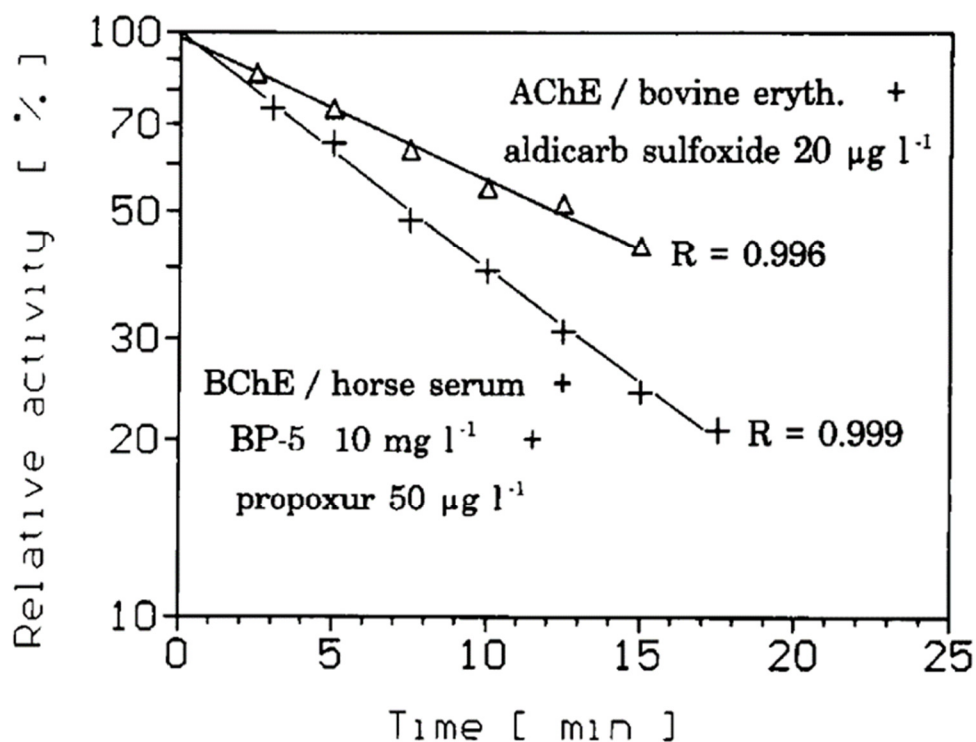
$$k_i = \frac{\ln 2}{t_{0.5} \times [IB]}$$

Using the preceding formulae, in which  $[EH]$  is active enzyme concentration and  $[IB]$  is active inhibitor concentration,  $k_i$  and  $t_{0.5}$  (the reaction half time) were calculated via a plot of relative activity (activity after inhibition time / initial inhibited activity) versus time and via linear regression analysis to give:

$$k_i = \frac{\ln 2 \times b}{\log 2 \times [IB]}$$

in which  $b$  is the coefficient to the slope of the plot of relative activity versus time. An example plot for the reaction of 20 µg/L aldicarb sulfoxide with bovine erythrocyte AChE is given in Figure 3. Malaoxon was subjected to similar mathematical treatment in reactions with eel AChE, bovine AChE, human BuChE, and horse BuChE bimolecular rate constants are given in Table 12.

**Figure 5. Inactivation of bovine erythrocyte AChE over time by 20 µg/L aldicarb sulfoxide (adapted from Herzsprung *et al.* 1992 Figure 1).**



**Table 12. Bimolecular rate constant  $k_i$  for malaoxon and four cholinesterases (adapted from Herzprung *et al.* 1992 Table 3)**

$k_i$ [L * mol <sup>-1</sup> * min <sup>-1</sup> ] ± ( $\Delta k_i/k_i$ ) * 100% (n)			
AChE eel	AChE bovine	BuChE human	BuChE horse
$1.7 \times 10^6 \pm 6\%$	$2.7 \times 10^5 \pm 4\%$	$2.1 \times 10^4 \pm 5\%$	$1.1 \times 10^4 \pm 6\%$

$\Delta k_i$ : Confidence interval (0.95, n)

n: Number of determination of  $\Delta E$  min<sup>-1</sup> as a function of inhibition time  $t$

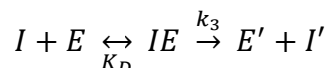
Aging and reactivation rate constants for rat brain AChE, human RBC AChE, human plasma BuChE, and rat plasma cholinesterase (rat plasma BuChE activity consists of both AChE and BuChE activity) have been determined for various dimethoxy OP oxon-cholinesterase complexes (Skrinjaric-Spoljar *et al.* 1973; Wallace and Herzberg 1988; Worek *et al.* 1999; Mason *et al.* 2000). Although only one study measured these constants specifically for malaoxon (Wallace and Herzberg 1988), the part of the OP compound that remains bound to the enzyme is the same for dimethoxy OPs, so the dimethoxy OP-oxon complexes would be expected to behave similarly with respect to aging and reactivation (Carr and Chambers 1996; Poet *et al.* 2004).

The only data specific to malaoxon was from Wallace and Herzberg (1988). These authors measured a reactivation rate of 0.158 hr<sup>-1</sup> (15.8% per hour) for malaoxon. This is an extremely high reactivation rate for an OP; this fast of a rate would be more expected for a carbamate. Given the rapid clearance of malathion, one would not expect significant differences in inhibition between acute and chronic exposures with a reactivation rate this high. However, it is clear from the adult data that there is a large difference in inhibition between single and repeated doses. Therefore, these data were not used. Instead, the data from Mason *et al.* (2000) were considered the most reliable. They measured a reactivation rate of 0.018 hr<sup>-1</sup> and an aging rate of 0.022 hr<sup>-1</sup> for azinphos-methyl (another dimethoxy OP) and AChE.

## Dissociation Constant

The dissociation constant  $K_D$  for the malaoxon-AChE enzyme-inhibitor complex has been previously determined (Krstic *et al.* 2008) and is needed to estimate the effects of competitive inhibition of malaoxon (see below for more details). In this paper, both free and immobilized AChE were reacted with malathion and malaoxon, over concentrations ranging from  $1 \times 10^{-9}$  to  $1 \times 10^{-1}$  M, in the presence of a second substrate, acetylthiocholine iodide. Enzyme activity was monitored by measuring hydrolysis: the hydrolysis product of acetylthiocholine, thiocholine,

reacts with 5,5'-dithio-bis-2-nitrobenzoic acid to form thio-2-nitrobenzoate, which was subsequently measured via UV/VIS spectrophotometer. Inhibition rates were monitored via sample withdrawal at several time intervals following the initial reaction (Kitz and Wilson 1962). Inhibition levels were shown to be dependent on inhibitor concentrations, for both malathion and malaoxon, and as expected, a lesser concentration of malaoxon was needed to inhibit AChE compared to malathion. An expression for irreversible enzyme inhibition, from a previously defined reaction scheme (Kitz and Wilson 1962):



was derived as follows:

$$\ln \frac{E}{E_0} = \frac{k_3 t}{1 + \frac{K_D}{[I]}}$$

in which  $E/E_0$  is the proportion of active enzyme (E) normalized to initial activity ( $E_0$ ),  $k_3$  is the rate constant for irreversible inhibition of enzyme, and  $I$  and  $I'$  are the inhibitor and inhibition product, respectively. Further, the slope of the above equation was expressed below, assuming the satisfied condition that  $I \gg E_0$ :

$$\frac{1}{k_{app}} = \frac{1}{k_3} + \frac{K_D}{k_3} \times \frac{1}{[I]}$$

in which  $k_{app}$  is defined as  $\frac{k_3}{1 + \frac{K_D}{[I]}}$ . In order to determine inhibition parameters, slopes and intercepts for plots of the above equations were calculated; these values are listed in Table 13.

**Table 13. Kinetic and inhibition constants for the inhibition of bovine AChE by malathion and malaoxon (adapted from Krstić *et al.* 2008, Table 1).**

Inhibitor	Free AChE				Bound AChE
	IC <sub>50</sub> (M)	K <sub>D</sub> (M <sup>-1</sup> )	k <sub>3</sub> (s <sup>-1</sup> )	k <sub>3</sub> /K <sub>D</sub> (mol <sup>-1</sup> s <sup>-1</sup> )	IC <sub>50</sub> (M)
Malathion	(3.7 ± 0.2) × 10 <sup>-4</sup>	1.3 × 10 <sup>-4</sup>	2.1 ± 0.2	78.1	(1.6 ± 0.1) × 10 <sup>-4</sup>
Malaoxon	(2.4 ± 0.3) × 10 <sup>-6</sup>	5.6 × 10 <sup>-6</sup>	2.1 ± 0.2	3.6 × 10 <sup>3</sup>	(3.4 ± 0.1) × 10 <sup>-6</sup>

The calculated bimolecular rate constant  $k_i$  for malaoxon inhibition of bovine AChE in per-minute ( $2.16 \times 10^5 \text{ mol}^{-1} \text{ min}^{-1}$ ), which contains the term  $K_D$ , is in near-agreement with the value determined by Herzprung *et al* ( $2.7 \times 10^5 \text{ mol}^{-1} \text{ min}^{-1}$ ). Additionally, the bimolecular rate constant from Dauterman and Main for malaoxon-carboxylesterase inhibition ( $1.7 \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$ ) is about an order of magnitude smaller than the values for malaoxon-AChE inhibition. Assuming that the nature of malaoxon-AChE binding is analogous to malaoxon-carboxylesterase binding, for the purposes of this model, the dissociation constant  $K_D$  for malaoxon-carboxylesterase binding will be approximated to the  $K_D$  for malaoxon-AChE binding. Because the bimolecular rate constant for malaoxon-carboxylesterase inhibition is about an order of magnitude smaller than the bimolecular rate constant for malaoxon-AChE inhibition, the  $K_D$  will also be adjusted down by an order of magnitude to approximate the malaoxon-carboxylesterase dissociation constant. This approximation is necessary because the term for  $k_3$  has not been previously reported for malaoxon-carboxylesterase inhibition, and this term likely differs between enzymes.

## In Vitro to In Vivo Extrapolation

In order to use *in vitro* metabolism measurements in PBPK models, the values obtained *in vitro* must be scaled to describe an *in vivo* system. Scaling factors for IVIVE are based on knowledge about the concentrations of various compartments (such as microsomes and hepatocytes) of the intact liver. For microsomes, which was the *in vitro* system used in the metabolism studies for malathion, metabolic rates ( $V_{max}$ ) are expressed as metabolic product formed/time/mg microsomal protein (MSP). Thus, the appropriate scaling factor is the concentration of MSP per gram liver (mg MSP/g liver).

For malathion metabolism (both oxidation and hydrolysis), *in vitro*  $V_{max}$  values were extrapolated to *in vivo* values for use in the model by using the MSP scaling factor. The model requires units for  $V_{max}$  to be in  $\mu\text{mol/hr/kg tissue}$ . For the rat, 45 mg MSP/g liver was used; for the human, 33 mg MSP/g liver was used (Lipscomb and Poet 2008). The following equation is an example of how the extrapolations for  $V_{max}$  were performed.

$$V_{max_{in vivo}} \left( \frac{\frac{\mu\text{mol}}{\text{hr}}}{\text{kg tissue}} \right) = V_{max_{in vitro}} \left( \frac{\text{nmol/min}}{\text{mg MSP}} \right) * \frac{60 \text{ min}}{1 \text{ hr}} * \frac{1 \mu\text{mol}}{1000 \text{ nmol}} * \frac{45 \text{ mg MSP}}{\text{g liver}} * \frac{1000 \text{ g}}{1 \text{ kg}}$$

For  $K_m$ , no extrapolation was performed from the *in vitro* values, which is standard practice (Lipscomb and Poet 2008). Values are summarized in Table 14.

**Table 14. *In vitro* and extrapolated *in vivo* values for malathion metabolism**

<b>Malathion bioactivation to oxon in liver</b>				
	Rat <i>in vitro</i> (nmol/min/mg MSP) <sup>a</sup>	Rat <i>in vivo</i> (umol/hr/kg liver)	Human <i>in vitro</i> (nmol/min/mg MSP) <sup>b</sup>	Human <i>in vivo</i> (umol/hr/kg tissue)
$V_{max}$	5.22	16,628	23.39	46,314
$K_m$ (all in umol/L)	2730	2730	2320	2320
<b>Malathion hydrolysis to MCA+DCA in liver</b>				
	Rat <i>in vitro</i> (nmol/min/mg MSP) <sup>a</sup>	Rat <i>in vivo</i> (umol/hr/kg liver)	Human <i>in vitro</i> (nmol/min/mg MSP) <sup>b</sup>	Human <i>in vivo</i> (umol/hr/kg tissue)
$V_{max}$	8.66	23,382	17.6	34,848
$K_m$ (all in umol/L)	0.334	0.334	0.492	0.492

<sup>a</sup> For rat, a conversion factor of 45 mg MSP/g liver was used (Lipscomb and Poet 2008)

<sup>b</sup> For human, a conversion factor of 33 mg MSP/g liver was used (Lipscomb and Poet 2008)

## Rate Constants and Uptake Parameters

### Dermal uptake

Studies suggest that dermal absorption of malathion in humans is low; most report that less than 10% is recovered excreted in urine following dermal application (Rabovsky and Brown 1993; Dary et al. 1994; Dennis and Lee 1999). However, this is still a major exposure route during and following application to fields, aerial spraying, and residential use (ATSDR 2003). In the current model (dermal exposure is included only in the human model and not the rat models), dermal absorption is modeled as a one-compartment structure as was done in the CPF model (Poet et al. 2014; Smith et al. 2014). The volume of skin exposed to malathion is defined as the fraction of skin surface area exposed compared with total skin surface area, which assumes that diffusion in the skin is limited to the skin immediately below the application area. Thus, the dermal compartment consists of the volume of skin under the application site. The dose is assumed to be evenly spread over the application area, and absorption is based on the skin permeability coefficient ( $K_p$ ).

Bogen and Singhal (2017) obtained estimates of  $K_p$  by fitting a PBPK model they developed for malathion to observed data on excreted urinary metabolites in 29 human volunteers dermally exposed to malathion based on data from other literature studies (Bogen and Singhal 2017).

The mean value of  $K_p$  from the 29 volunteers was  $2.35 \times 10^{-6}$  cm/hr; this value is used in the model. A refined model is also available that models  $K_p$  as a function of surface loading.

### **Oral uptake**

Oral (gavage and dietary exposure) absorption of malathion or malaoxon from the stomach, transfer from the stomach to the intestine, and transfer from the intestine to the liver are all modeled as first-order processes. From rat and human PK data, malathion is well-absorbed orally; in the model, 100% of the dose is assumed to be absorbed. There are no existing data from which the oral absorption rate or transfer rates have been determined; thus, the rate constants for the rat and human are estimated by fitting the model to the available PK and PD data.

### **Inhalation exposure**

Due to low volatility, inhalation is not a significant route of exposure; however, inhalation of spray or mist is still anticipated to occur during occupational use (ATSDR 2003). Inhaled exposures to malathion are described in the same way as in the chlorpyrifos model, where a fraction of what is inhaled reaches the lung tissue and is absorbed while the remaining chemical is eliminated by mucociliary clearance and transferred to the GI tract and gets absorbed as an oral dose. Uptake by inhalation is based on alveolar ventilation rate, the concentration of chemical in the air, and the fraction available to the deep lung. The fraction available to the deep lung can be estimated using the Multiple Path Particle Dosimetry Model (MPPD) software (Price et al.) as was done with the chlorpyrifos model. The lung:air partition coefficient can be estimated using the Poulin and Krishnan (1995) methods. The rate constant for transfer from the lung to the GI tract will be fitted using rat data.

### **Elimination rate**

Urinary elimination of the malathion MCA+DCA metabolite is modeled as first-order processes. There are no existing data from which the urinary excretion rate constants could be determined; thus, the rate constants for the rat and human were estimated by fitting the model to the available data.



# Model Testing and Validation

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## Introduction

As described above, the PBPK/PD models attempt to describe the following processes:

- Activation of malathion to malaoxon via P450s.
- Metabolism of malathion via a catalytic reaction with carboxylesterase, including tracking the formation of MCA and DCA.
- Metabolism of malaoxon via a catalytic reaction with carboxylesterase.
- Metabolism of malaoxon via a stoichiometric reaction with carboxylesterase, including reactivation of carboxylesterase.
- Stoichiometric reactions of malaoxon with other esterases including BuChE and AChE, including esterase reactivation and aging.

The available data for each of these processes are summarized in Table 15.

**Table 15. Pathway and data source for malathion PBPK/PD modeling**

Pathway	Data Source
Activation of malathion to malaoxon	$V_{max}$ and $K_m$ from MSU experiments
Metabolism of malathion via carboxylesterase	$V_{max}$ and $K_m$ from limited MSU experiments
Metabolism of malaoxon via a catalytic reaction with carboxylesterase	Fitted with <i>in vivo</i> malaoxon data (see below)
Metabolism of malathion via a stoichiometric reaction with carboxylesterase, including reactivation with carboxylesterase	Estimated from Main and Dauterman (1966) and Hassan and Dauterman (1967)
Stoichiometric reactions of malaoxon with other esterases including BuChE and AChE, including esterase reactivation and aging	Herzsprung et al. (1992) and Mason et al. (2000)

The remaining part of this section shows some example runs of the PBPK/PD rat model in its current form, including the use of the oxon *in vivo* data to estimate oxon detoxication rates, fits to the urinary metabolite and blood data, and fits to malathion RBC AChE inhibition data.

## Oxon Detoxication

The oxon detoxication is the sum of the catalytic and non-catalytic processes. For non-catalytic detoxication, the bimolecular rate constant was measured by Main and Dauterman (1967) and Hassan and Dauterman (1968). The reactivation rate for carboxylesterase was estimated from Main and Dauterman (1967) and is consistent with other data. The model includes an estimate of the carboxylesterase active sites. Combined, these data are sufficient to estimate the contribution of the non-catalytic detoxication.

There are no available *in vitro* data for  $V_{max}$  and  $K_m$  for oxon detoxication. It would be challenging to conduct such an experiment because it would be difficult to separate the catalytic and non-catalytic processes which both occur via carboxylesterase. Originally, the catalytic detoxication was intended to be estimated via the urinary excretion of DMP. This was the technique used in the 2007 EPA PBPK model. DMP can be directly formed via the oxon detoxication and is the product of esterase reactivation following inhibition by the oxon. However, as described below, there is too much DMP formed than can be explained by oxon detoxication. Based on the *in vitro* data for malathion activation to the oxon, too little oxon forms to explain the amount of DMP that is formed. This may be explained by data from Chen et al. (2013). Chen et al. (2013) gavaged female Holtzman rats with 73  $\mu\text{moles/kg}$  of malathion, MDA, MCA, DMP, DMTP, and DMDTP. It showed that all of the dimethylphosphates are formed directly from MDA and MCA. From a modeling perspective, this makes the use of the acid and dimethylphosphate urinary metabolite data less clean in regards to separating the effects of the carboxylesterase and oxanase detoxication pathways. Furthermore, DMDTP is the only dimethylphosphate metabolite that can be directly formed from malathion as opposed to malaoxon (it has the P=S group, whereas DMTP and DMP have the P=O group). However, direct dosing of DMDTP resulted in small amounts of DMTP and DMP. Also, only about 50% of the dosed amounts of DMTP and DMDTP were recovered. Therefore, the urinary metabolite data are less useful for estimating oxon detoxication rates than originally thought.

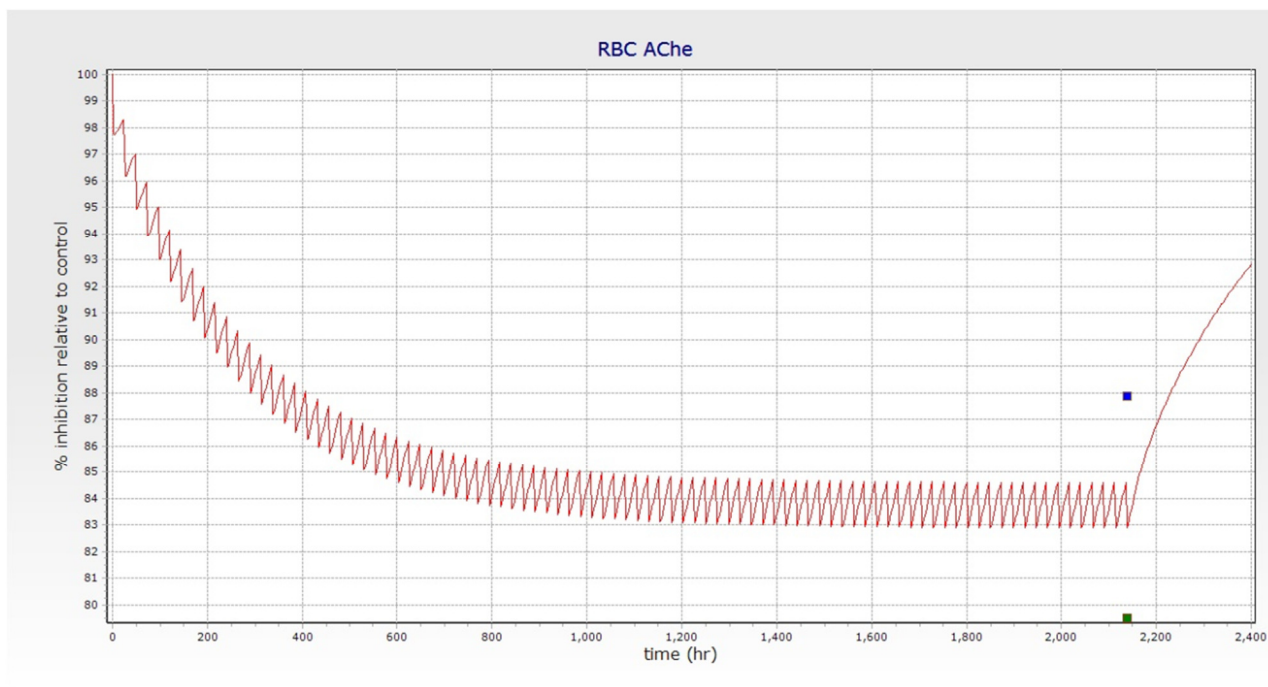
Given that the urinary data could not be used to fit the oxon detoxication data, it was decided to attempt to fit the detoxication rates using *in vivo* oxon dosing data. In this case, the PBPK/PD model can be run without any of the malathion-specific processes (activation to the oxon, malathion detoxication). The non-catalytic detoxication can be modeled as described above and the remaining required detoxication via the catalytic process was fit.

The starting point was a 2-year dietary study with malaoxon that measured RBC AChE inhibition at 90 days, 180 days, 1 year, and 2 years (Daly 1996). The focus of the analysis was dose groups with dietary concentrations of 20 and 1000 ppm. The average RBC AChE inhibition was 16% at 20 ppm and 57% at 1000 ppm. The corresponding doses are 1 mg/kg/day

for 20 ppm and 57 mg/kg/day for males and 58 mg/kg/day for females at 1000 ppm. The dietary study was modeled as a daily gavage dose, though the exact diurnal patterns of food consumption are unknown.

Figure 6 shows the RBC AChE inhibition time-course for a 90-day exposure scenario, including the male and female RBC AChE activity levels at the end of the 90 days. The oxon catalytic detoxication was fit to these data. A  $K_m=0.5$   $\mu\text{moles}$  was assumed similar to malathion detoxication and a value of  $V_{max}=2000$   $\mu\text{moles/hr/kg tissue}$  was fit for the oxon detoxication. This value is plausible given that  $V_{max}$  for malaoxon is expected to be lower than the  $V_{max}$  for malathion (which is 23,384  $\mu\text{moles/hr/kg tissue}$ ). Main and Dauterman (1967) suggested in a “semi-quantitative” analysis that the  $V_{max}$  for malathion was about an order of magnitude less than the  $V_{max}$  for malaoxon.

**Figure 6. RBC AChE activity for a 90-day malaoxon exposure to rats at 1 mg/kg/day. The oxon catalytic detoxication was fit with  $V_{max} = 2000$   $\mu\text{moles/hr/kg tissue}$  and  $K_m=0.5$   $\mu\text{moles}$ . Measured values at 90 days are shown as blue point (males) or green point (females).**



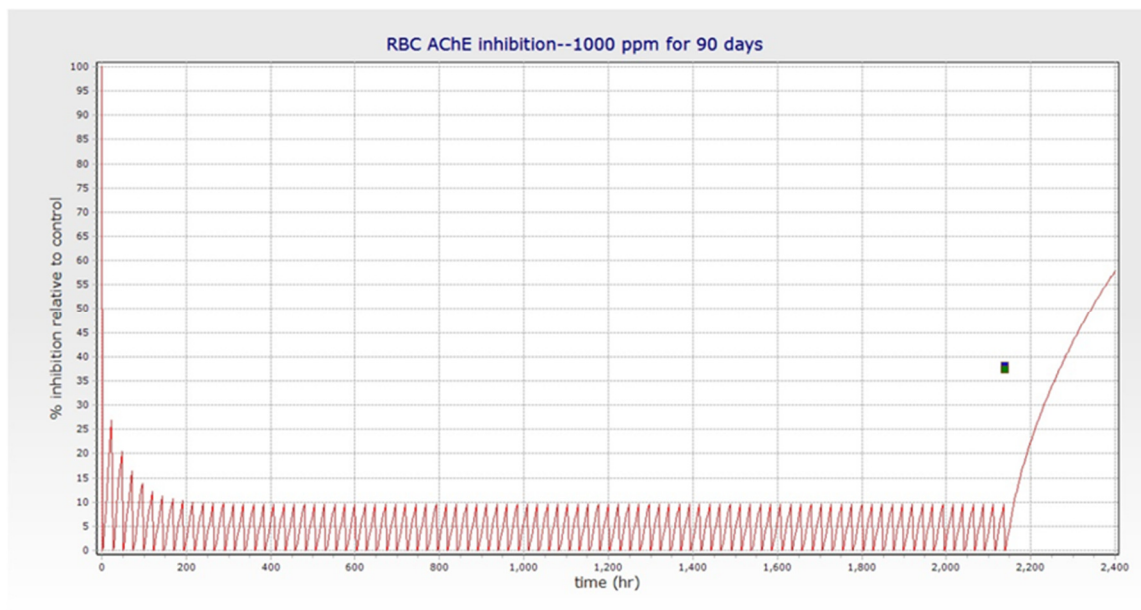
Of the total 214.2  $\mu\text{mole}$  dose, the following amounts were consumed by catalytic detoxication via carboxylesterase in the liver (207.1  $\mu\text{moles}$ , 96.7%) and non-catalytic detoxication via carboxylesterase in the liver (1.17  $\mu\text{moles}$ , 0.5%). Thus, detoxication via carboxylesterase in

the liver accounted for 97.2% of the total detoxication. Detoxication via the non-catalytic pathway was not a significant contributor; there is simply not enough carboxylesterase. It is worth noting that rat serum carboxylesterase is cited as a potential reason why rats may be less sensitive to malathion (Buratti et al., 2005). However, in this example, first pass metabolism in the liver is dominant. That is likely to be true for oral exposures, though this could be different for dermal or inhalation exposures.

Carboxylesterase inhibition peaked at about 10%, so there was not a large loss of carboxylesterase that would slow the malaoxon substrate reaction.

The fitted  $V_{max}$  and  $K_m$  were used for an additional model run with data for the 1000 ppm dose group (57-58 mg/kg/day). The simulated RBC AChE activity is shown in Figure 5 along with measured data for males and females. The model significantly overestimates the inhibition, indicating that there is insufficient detoxication. In this case, carboxylesterase inhibition was extreme with virtually complete inhibition at dosing time.

**Figure 7. RBC AChE activity for a 90-day malaoxon exposure to rats at 57 mg/kg/day. The oxon catalytic detoxication was fit with  $V_{max} = 2000 \mu\text{moles/hr/kg tissue}$  and  $K_m = 0.5 \mu\text{moles}$ . Measured values at 90 days are shown as blue point (males) or green point (females).**



There are a few possible explanations for the lack of fit for the 1000 ppm dose group data. First, our strategy of attempting to fit the oxon detoxication kinetics to the *in vivo* malaoxon data may be flawed. Second, it is possible that the minor oxanase detoxication pathway becomes important when carboxylesterase saturation occurs. Third, the situation where carboxylesterase

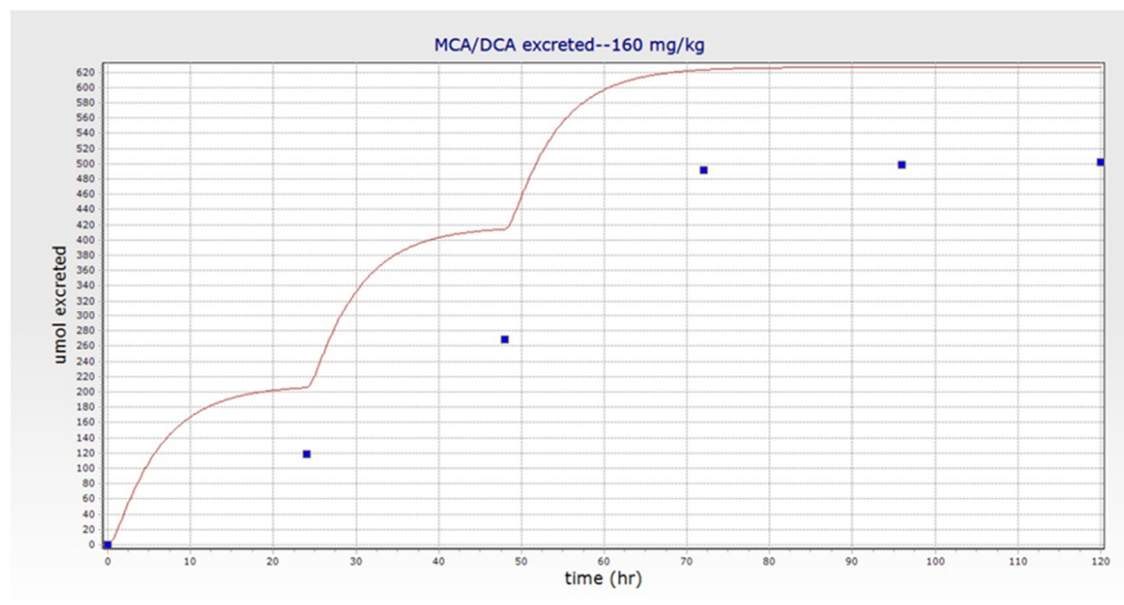
is completely inhibited may be too challenging to model. It is important to note that carboxylesterase saturation is likely to be irrelevant for any dose that is important for risk assessment.

Additional fitting for oxon detoxication can be done using the acute and repeat exposure data for post-natal rats.

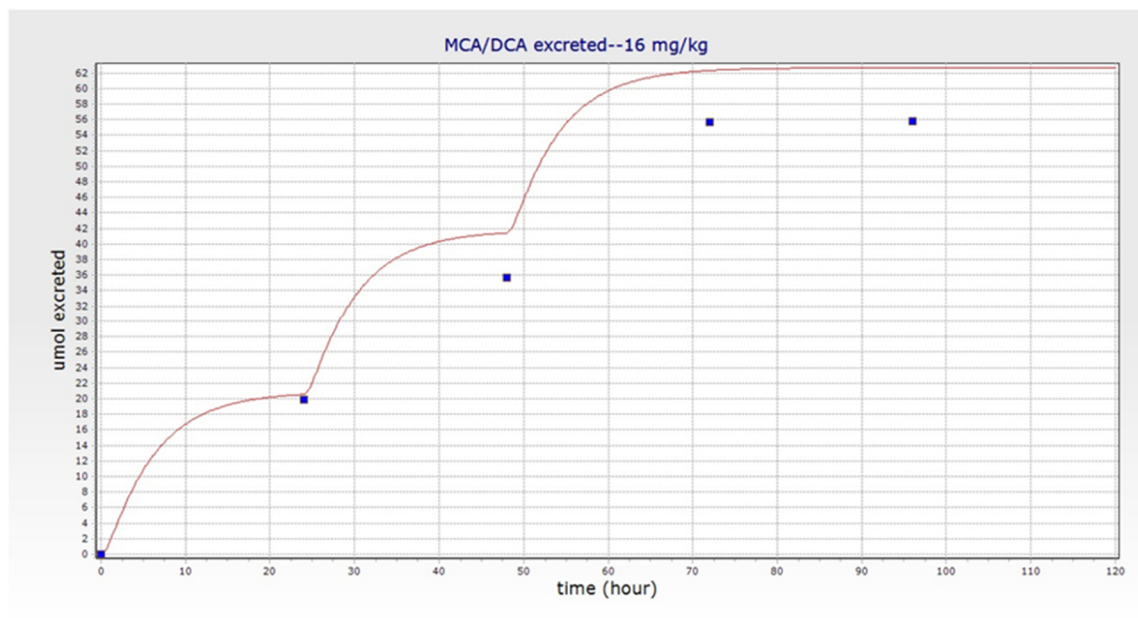
## Fit to Urinary Metabolite and Blood Data

The model was also run to compare its output with the urinary metabolite data in Bradway and Shafik (1977). Figures 8 and 9 show time course plots of the combined MCA+DCA metabolite masses in urine for gavage doses of 160 and 16 mg/kg delivered three times over three days. The fits to the data are quite good. The slight overestimate is plausible given that some MCA and DCA generated via carboxylesterase-mediated detoxication of malathion would be transformed to dimethylphosphates, which is unaccounted for in the model, and some MCA and DCA may be further metabolized to some unmeasured metabolites.

**Figure 8. Levels of MCA+DCA excreted in the Bradway and Shafik (1977) study versus model prediction for a dose of 160 mg/kg**



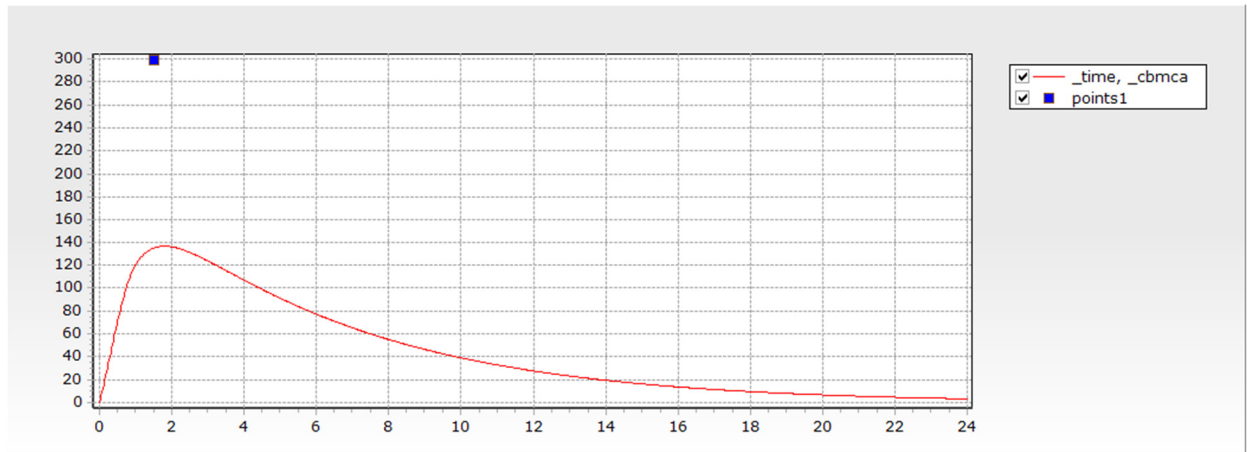
**Figure 9. Levels of MCA+DCA excreted in the Bradway and Shafik (1977) study versus model prediction for a dose of 16 mg/kg**



For the 160 mg/kg dose, there is a total of 626.6  $\mu$ moles of malathion. Of this amount, only 0.0823  $\mu$ moles were converted to malaoxon. For the 16 mg/kg dose, there is a total 62.7  $\mu$ moles of malathion of which 0.0056  $\mu$ moles is converted to malaoxon.

The fit to the blood data for Libberton (2017) was within about a factor of two. For a dose of 800 mg/kg, the predicted blood concentration at 1.5 hours was 289  $\mu$ molar, whereas the model predicts about 140  $\mu$ molar, as shown in Figure 10. It is possible as the malathion detoxication data is refined that this fit will improve.

**Figure 10. Fit to the MCA/DCA blood measurements at 1.5 hours in Libberton and Cheplin (2017)**



## Fits for Malathion Inhibition

As there is still uncertainty regarding the kinetic rates for malathion detoxication, fits for malathion inhibition have not yet been conducted.

## AcsIX Code

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The AcsIX code for the rat model, pre-natal rat model, and human model are delivered separately and text copies are in Appendix A (rat model), Appendix B (post-natal rat model), and Appendix C (human model). Tablx 16 summarizes the AcsIX m files provided with the model.

**Table 16. AcsIX m files**

Model	M file name	Description
Adult rat	Rat_parameters.m	Reads in parameter values for adult rat model
	Ryan_metabolites.m	Simulates MCA+DCA and malathion in blood following oral dose of 250 mg/kg
	Bradway_metabolites.m	Simulates MCA+DCA excreted following 3 different oral doses of malathion (doses each administered 3 times)
	Barnett_2012a.m	Simulates AChE inhibition in brain and RBCs following 90 days of dietary exposure to malathion (100, 500, 5000, or 10000 ppm)
	Barnett_2012b.m	Simulates AChE inhibition in brain and RBCs following 28 days of dietary exposure to malathion (100, 500, 5000, or 10000 ppm)
	Huntington_2001_repeat_dose.m	Simulates AChE inhibition in brain and RBCs and BuChE inhibition in plasma following 11 gavage doses of malathion (5, 50, or 150 mg/kg)



Model	M file name	Description
	Huntington_2001_single_dose.m	Simulates AChE inhibition in brain and RBCs and BuChE inhibition in plasma following a gavage dose of malathion (5, 50, 150, or 450 mg/kg)
	Oxon_90days.m	Simulates AChE inhibition in brain and RBCs following 90 days of dietary exposure to malaoxon (20, 1000, or 2000 ppm)
	FMC_metabolites.m	Simulates MCA+DCA in blood following single oral doses of malathion (800 or 1200 mg/kg)
Postnatal rat	Rat_parameters.m	Reads in parameters for postnatal rat model
	Barnett_2006.m	Simulates AChE inhibition in brain and RBCs following 11 doses (PND11-PND21) of malathion or oxon (malathion—5, 25, 50, or 150 mg/kg; oxon—0.1, 1.0, 2.5, or 4.0 mg/kg)
	Barnett_2008a.m	Simulates AChE inhibition in brain and RBCs from 0.5 to 2.5 hr postdose resulting from single oral dose of 150 mg/kg malathion (PND11)
	Barnett_2008b.m	Simulates AChE inhibition in brain and RBCs following a single oral dose (PND11) of malathion or oxon (malathion—10, 25, 50, 100, or 150 mg/kg; oxon—1.0, 3.5, 7.0, 10, or 12.5 mg/kg)
	Fulcher_2006.m	Simulates AChE inhibition in brain and RBCs from 0.5 to 6 hr postdose resulting from single oral dose of 150 mg/kg malathion (PND11)

Model	M file name	Description
	Huntington_2001_repeat_dose.m	Simulates AChE inhibition in brain and RBCs and BuChE inhibition in plasma following 11 gavage doses (PND11-PND21) of malathion (5, 50, or 150 mg/kg)
	Huntington_2001_single_dose.m	Simulates AChE inhibition in brain and RBCs and BuChE inhibition in plasma following a gavage dose (PND11) of malathion (5, 50, 150, or 450 mg/kg)
Human	Human_parameters.m	Reads in parameters for human life stage model
	Acute_dose.m	Simulates RBC AChE inhibition resulting from a single oral dose of malathion (adult)
	Subchronic_dose.m	Simulates RBC AChE inhibition resulting from subchronic (21 days) oral dosing with malathion (adult)
	Inhalation.m	Simulates RBC AChE inhibition resulting from subchronic (21 days) inhalation exposure to malathion (adult)
	Acute_oxon_dose.m	Simulates RBC AChE inhibition resulting from a single oral dose of malaoxon (adult)
	Subchronic_oxon_dose.m	Simulates RBC AChE inhibition resulting from subchronic (21 days) oral dosing with malaoxon (adult)

Model	M file name	Description
	Dermal_exposure_scenarios.m	Simulates RBC AChE inhibition resulting from dermal exposure to malathion. To simulate different scenarios, exposure duration, exposure frequency, fraction of skin in contact with malathion, and BW can all be adjusted within this file.
	Inhalation_scenarios.m	Simulates RBC AChE inhibition resulting from inhalation exposure to malathion. To simulate different scenarios, exposure duration, exposure frequency, breathing rate, and BW can all be adjusted within this file.
	Oral_exposure_subchronic.m	Simulates RBC AChE inhibition resulting from subchronic (21 days, once per day) oral dosing with malathion in 75 kg adult.
	Oral_exposure_child.m	Simulates RBC AChE inhibition resulting from subchronic (21 days, 1.5 hr/day) oral dosing with malathion in 11 kg child.
	Drinking_water.m	Simulates RBC AChE inhibition resulting from subchronic (21 days, 6 drinking events/day) drinking water exposure to malaoxon (BW and drinking rate can be adjusted for adult or child)

## References

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Aston, L. (2000). Determination of Residue of Malathion Dicarboxylic Acid (DCA), Malathion Monocarboxylic Acid (MCA), Dimethyl Phosphate (DMP), Dimethyl Thiophosphate (DMTP), and Dimethyl Dithiophosphate (DMDTP) in Human Urine. Project Number PTL119801. Unpublished study report prepared by Pacific Toxicology Laboratories. MRID #45244601.

ATSDR (2003). Toxicological Profile for Malathion. Atlanta, GA, Agency for Toxic Substances and Disease Registry, US Dept of Health and Human Services.

Barnett, J. (2006a). Oral (Gavage) Repeat Dose Comparative Cholinesterase Study of Malathion and Malaoxon in Juvenile Rats. Project number: TQC00013, MLXN00, TQC00013AA. Unpublished study prepared by Charles River Laboratories, Charles River Laboratories and QC Inc., Quality Control Lab. 233 p. MRID #46822201.

Barnett, J. (2006b). Oral (gavage) Repeat Dose Time of Peak Cholinesterase Depression Study of Malathion and Malaoxon in Juvenile Rats: Final Pilot Report. Project Number: TQC00012. Unpublished study prepared by Charles River Laboratories. 150 p. MRID #46825502.

Barnett, J. (2008a). Oral (gavage) Acute Dose Time of Peak Cholinesterase Depression Study of Malathion in Juvenile Rats: Final Study. Project Number: TQC00032. Unpublished study prepared by Charles River Laboratories. 123 p. MRID #47373702.

Barnett, J. (2008b). Oral (gavage) Acute Dose Comparative Cholinesterase Study of Malathion and Malaoxon in Juvenile Rats: Final Study. Project Number: TQC00017. Unpublished study prepared by Charles River Laboratories. 282 p. MRID # 47373704.

Barnett, J. (2012a). Oral (Diet) Repeated Dose 90-day Toxicity Study of Malathion Technical in Rats: Final Report. Project Number: TQC00066. Unpublished study prepared by Charles River Laboratories and Exponent. 786 p. MRID #49035701.

Barnett, J. (2012b). Oral (Diet) Repeated Dose 28-day Toxicity Study of Malathion Technical in Rats. Project Number: TQC00065. Unpublished study prepared by Charles River Laboratories and Exponent. 480 p. MRID #49035702.

Bogen, K. and A. Singhal (2017). "Malathion dermal permeability in relation to dermal load: Assessment by physiologically based pharmacokinetic modeling of in vivo human data." J Environ Sci Health B 52: 138-146.

Bouchard, M., N. Gosselin, R. Brunet, O. Samuel, M.-J. Dumoulin and G. Carrier (2003). "A Toxicokinetic Model of Malathion and Its Metabolites as a Tool to Assess Human Exposure and Risk through measurements of Urinary Biomarkers." Toxicol Sci 73: 182-194.

Bradway, D. and M. Shafik (1977). "Malathion Exposure Studies. Determination of Mono- and Dicarboxylic Acids and Alkyl Phosphates in Urine." J Agric Food Chem 25: 1342-1344.

Brown, R., M. Delp, S. Lindstedt, L. Rhomberg and R. Beliles (1997). "Physiological parameter values for physiologically based pharmacokinetic models." Toxicol Ind Health 13: 407-484.

Buratti, F., A. D'Aniello, M. Volpe, A. Meneguz and E. Testai (2005). "Malathion Bioactivation in the human liver: The contribution of different cytochrome P450 isoforms." Drug Metab Disp 33: 295-302.

Buratti, F. and E. Testai (2005). "Malathion Detoxification by Human Hepatic Carboxylesterases and its Inhibition by Isomalathion and Other Pesticides." J Biochem Mol Toxicol 19: 406-414.

Buratti, F. and E. Testai (2007). "Evidences for CYP3A4 autoactivation in the desulfuration of dimethoate by the human liver." Toxicology 241: 33-46.

Carr, R. and J. Chambers (1996). "Kinetic Analysis of the in Vitro Inhibition, Aging, and Reactivation of Brain Acetylcholinesterase from Rat and Channel Catfish by Paraoxon and Chlorpyrifos-oxon." Toxicol Appl Pharmacol 139: 365-373.

Chambers, J. and M. Meek (2017). Malathion in vitro testing, report pending, Mississippi State University.

Chen, L., T. Zhao, C. Pan, J. Ross, M. Ginevan, H. Vega and R. Krieger (2013). "Absorption and excretion of organophosphorous insecticide biomarkers of malathion in the rat: Implications for overestimateion bias and exposure misclassification from environmental biomonitoring." Regul Toxicol Pharmacol 65: 287-293.

Daly, I. (1996). 24-month oral toxicity/oncogenicity study of malaoxon in the rat via dietary administration: Final report. Prepared by Huntington Life Sciences. Lab Project Number:90-2234. US MRID 43975201.

Dary, C., J. Blancato, M. Castles, V. Reddy, M. Cannon, M. Saleh and G. Cash (1994). Dermal Absorption and Disposition of Formulation of Malathion in Sprague-Dawley Rats and Humans. Biomarkers of Human Exposure to Pesticides. Washington, DC, American Chemical Society: 231-263.

Dennis, G. and P. Lee (1999). "A Phase I Volunteer Study to Establish the Degree of Absorption and Effect on Cholinesterase Activity of Four Head Lice Preparations Containing Malathion." Clin Pharmacodynamics 18: 105-115.

Fenske, R. and J. Leffingwell (1989). "Method for Detemination of Dialkyl Phosphate Metabolites in Urine for Studies of Human Exposure to Malathion." J Agr Food Chem 37: 995-998.

Fulcher, S. (2001). Malathion: Effects on Cholinesterase in the CD Rat (Adult and Juvenile) by Oral Gavage Administration: Lab Project Number: CHV067/012452. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 339 p. Relates to L0000617 and L0000634. MRID #45566201.

Gillies, D. and J. Dickson (2000). A Randomised Double Blind Ascending Single Oral Dose Study with Malathion to Determine the No Effect Level on Plasma and RBC Cholinesterase

Activity: Lab Project Number: ICR 013177. Unpublished study prepared by Inveresk Research. 882 p. MRID #45125602.

Hassan, A. and W. Dauterman (1968). "Studies on the optically active isomers of O,O-diethyl malathion and O, O-diethyl malaoxon." Biochem Pharmacol 17: 1431-1439.

Hatfield, M., L. Tsurkan, M. Garrett, T. Shaver, J. Hyatt, C. Edwards, L. Hicks and P. Potter (2011). "Organ-specific carboxylesterase profiling identifies the small intestine and kidney as major contributors of activation of the anticancer prodrug CPT-11." Biochem Pharmacol 81: 24-31.

Hemmert, A., T. Otto, M. Wierdl, C. Edwards, C. Fleming, M. MacDonald, J. Cashman, P. Potter, D. Cerasoli and M. Redinbo (2010). "Human Carboxylesterase 1 Stereoselectively Binds the Nerve Agent Cyclosarin and Spontaneously Hydrolyzes the Nerve Agent Sarin." Molecular Pharmacol 77: 508-516.

Herzprung, P., L. Weil and R. Niessner (1992). "Measurement of Bimolecular Rate Constants  $k_i$  of the Cholinesterase Inactivation Reaction by 55 Insecticides and of the Influence of Various Pyridiniumoximes on  $k_i$ ." Int J Environ Anal Chem 47: 181-200.

Hines, R., P. Simpson and D. McCarver (2016). "Age-Dependent Human Hepatic Carboxylesterase 1 (CES1) and Carboxylesterase 2 (CES2) Postnatal Ontogeny." Drug Metab Disp Epub ahead of print.

Karanth, S. and C. Pope (2000). "Carboxylesterase and A-Esterase activities during maturation and aging: relationship to the toxicity of chlorpyrifos and parathion in rats." Toxicol Sci 58: 282-289.

Kitz, R. and I. Wilson (1962). "Esters of methanesulfonic acid as irreversible inhibitors of acetylcholinesterase." J Biol Chem 237: 3245-3249.

Krstic, D., M. Colovic, M. Kralj, M. Franko, K. Krinulovic, P. Trebse and V. Vasic (2008). "Inhibition of AChE by malathion and some structurally similar compounds." J Enzym Inhib Med Chem 23: 562-573.

Libberton, M. (2017). [14C]-Malathion: The Pharmacokinetics of [14C] Malathion in the Rat Following Single Oral and Intravenous Administration. Unpublished study prepared by Charles River Laboratories, MRID pending. .

Lipscomb, J. and T. Poet (2008). "In vitro measurements of metabolism for application in pharmacokinetic modeling." Pharmacol Therapeut 118: 82-103.

Luecke, R., B. Pearce, W. Wosilait, W. Slikker and J. Young (2007). "Postnatal Growth Considerations for PBPK Modeling." J Toxicol Environ Health Part A 70: 1027-1037.

Main, A. and P. Braid (1962). "Hydrolysis of Malathion by Ali-Esterases in vitro and in vivo." Biochem J 84: 255-263.

Main, A. and W. Dauterman (1966). "Kinetics for the inhibition of carboxylesterase by malaoxon." Can J Biochem 45: 757-771.

Main, A. and F. Iverson (1966). "Measurement of the affinity and phosphorylation constants governing irreversible inhibition of cholinesterases by di-isopropyl phosphorofluoridate." Biochem J 100: 525-531.

Mason, H., C. Sams, A. Stevenson and R. Rawbone (2000). "Rates of spontaneous reactivation and aging of acetylcholinesterase in human erythrocytes after inhibition by organophosphorus pesticides." Human Expt Toxicol 19: 511-516.

Mason, H., E. Waite, A. Stevenson and H. Wilson (1993). "Aging and Spontaneous Reactivation of Human Plasma Cholinesterase Activity after inhibition by organophosphorus insecticides." Human Expt Toxicol 12: 497-503.



Maxwell, D., D. Lenz, W. Groff, A. Kaminskis and H. Froehlich (1987). "The Effects of Blood Flow and Detoxification on In Vivo Cholinesterase Inhibition by Soman in Rats." Toxicol Appl Pharmacol 88: 66-76.

Maxwell, D., C. Lieske and K. Brecht (1994). "Oxime-Induced Reactivation of Carboxylesterase Inhibited by Organophosphorus Compounds." Chem Res Toxicol 7: 428-433.

Moser, V. and S. Padilla (2016). "Esterase detoxification of acetylcholinesterase inhibitors using human liver samples in vitro." Toxicology 353-354: 11-20.

Ohura, K., K. Tasaka, M. Hashimoto and T. Imai (2013). "Distinct Patterns of Aging Effects on the Expression and Activity of Carboxylesterases in Rat Liver and Intestine." Drug Metab Disp 42: 262-273.

Poet, T., A. Kousba, S. Dennison and C. Timchalk (2004). "Physiologically Based Pharmacokinetic/Pharmacodynamic Model for the Organophosphorus Pesticide Diazinon." Neurotoxicology 25: 1013-1030.

Poet, T., C. Timchalk, J. Hotchkiss and M. Bartels (2014). "Chlorpyrifos PBPK/PD model for multiple routes of exposure." Xenobiotica 44: 868-881.

Poulin, P. and F. Thiel (2000). "A priori prediction of tissue:plasma partition coefficients of drugs to facilitate the use of physiologically based pharmacokinetic models in drug discovery." J Pharmaceut Sci 89: 16-35.

Price, O., B. Asgharian, F. Miller, F. Cassee and R. de Winter-Sorkina Multiple Path Particle Dosimetry Model (MPPD v3.04).

Rabovsky, J. and J. Brown (1993). "Malathion metabolism and disposition in mammals." J Occup Med Toxicol 2: 131-168.

Reddy, V., T. Freeman and M. Cannon (1989). Disposition and Metabolism of <sup>14</sup>C-Labeled Malathion in Rats (Preliminary and Definitive Study): MRI Project no. 9354-B; JCSF Study No. 56. Unpublished study prepared by Midwest Research Institute. 117 p. MRID #41367701.

Ryan, D. and T. Fukuto (1985). "The Effect of Impurities on the Toxicokinetics of Malathion in Rats." Pesticide Biochem Physiol 23: 413-424.

Sams, C. and H. Mason (1999). "Detoxification of organophosphates by A-esterases in human serum." Human Expt Toxicol 18: 653-658.

Satoh, T. and M. Hosokawa (1998). "The mammalian carboxylesterases: from molecular to functions." Annu Rev Pharmacol Toxicol 38: 257-288.

Satoh, T. and M. Hosokawa (2006). "Structure, function, and regulation of carboxylesterases." Chem Biol Interact 162: 195-211.

Shafik, M. and H. Enos (1969). "Determination of metabolic and hydrolytic products of organophosphorus pesticide chemicals in human blood and urine." J Agr Food Chem 17: 1186-1189.

Shi, D., D. Yang, E. Prinssen, B. Davies and B. Yan (2011). "Surge in Expression of Carboxylesterase 1 During the Post-neonatal Stage Enables a Rapid Gain of the Capacity to Activate the Anti-influenza Prodrug Oseltamivir." 203: 937-942.

Skrinjaric-Spoljar, M., V. Simeon and E. Reiner (1973). "Spontaneous reactivation and aging of dimethylphosphorylated acetylcholinesterase and cholinesterase." Biochim Biophys Acta 315: 363-369.

Smith, J., P. Hinderliter, C. Timchalk, M. Bartels and T. Poet (2014). "A human life-stage physiologically based pharmacokinetic and pharmacodynamic model for chlorpyrifos: Development and validation." Regul Toxicol Pharmacol 69: 580-597.

Stannard, D. (2006). Malathion: Single dose time to peak effect cholinesterase study in juvenile rats. Project Number: CHV/0123, CHV/0123/050182. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 74 p. MRID #46756704.

Talcott, R. (1979). "Hepatic and Extrahepatic Malathion Carboxylesterases. Assay and Localization in the Rat." Toxicol Appl Pharmacol 47: 145-150.

Tarhoni, M., T. Lister, D. Ray and W. Carter (2008). "Albumin binding as a potential biomarker of exposure to moderately low levels of organophosphorus pesticides." Biomarkers 13: 343-363.

Timchalk, C., A. Kousba and T. Poet (2007). "An Age-Dependent Physiologically Based Pharmacokinetic/Pharmacodynamic Model for the Organophosphorus Insecticide Chlorpyrifos in the Preweanling Rat." Toxicol Sci 98: 348-365.

Timchalk, C., R. Nolan, A. Mendrala, D. Dittenber, K. Brzak and J. Mattsson (2002). "A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans." Toxicol Sci 66: 34-53.

USEPA (1988). Reference Physiological Parameters in Pharmacokinetic Modeling. Washington, DC, United States Environmental Protection Agency.

USEPA (2005). Benchmark dose analysis of brain and RBC data from the malathion comparative cholinesterase study in juvenile and adult rats (MRID no.45566201). Washington, DC, Office of Prevention, Pesticides, and Toxic Substances, United States Environmental Protection Agency.

USEPA (2007). Malathion Exposures During Lice Treatment: Use of Exposure Related Dose Estimating Model (ERDEM) and Factors Relating to the Evaluation of Risk, Office of Research and Development, USEPA.

USEPA (2014). Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review. Washington, DC, United States Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention.

USEPA (2015). Literature Review on Neurodevelopmental Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides. Washington, DC, Office of Chemical Safety and Pollution Prevention, United States Environmental Protection Agency.

USEPA (2016). Malathion: Human Health Draft Risk Assessment for Registration Review. Washington, DC, United States Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention.

Wallace, K. and U. Herzberg (1988). "Reactivation and Aging of Phosphorylated Brain Acetylcholinesterase from Fish and Rodents." Toxicol Appl Pharmacol 92: 307-314.

Worek, F., C. Diepold and P. Eyer (1999). "Dimethylphosphoryl-inhibited human cholinesterases: inhibition, reactivation, and aging kinetics." Arch Toxicol 73: 7-14.

Yang, D., R. Pearce, X. Wang, R. Gaedigk, Y. Wan and B. Yan (2009). "Human Carboxylesterases HCE1 and HCE2: Ontogenic Expression, Inter-Individual Variability and Differential Hydrolysis of Oseltamivir, Aspirin, Deltamethrin and Permethrin." Biochem Pharmacol 77: 238-247.

Young, J., R. Luecke, B. Pearce, T. Lee, H. Ahn, S. Baek, H. Moon, D. Dye, T. Davis and S. Taylor (2009). "Human Organ/Tissue Growth Algorithms that Include Obese Individuals and Black/White Population Organ Weight Similarities from Autopsy Data." J Toxicol Environ Health Part A 72: 527-540.

Zhu, H.-J., D. Appel, Y. Jiang and J. Markowitz (2009). "Age- and Sex-Related Expression and Activity of Carboxylesterase 1 and 2 in Mouse and Human Liver." Drug Metab Disp 37: 1819-1825.

# Appendix A – AcsxL Rat Model Code

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## Adult rat code

PROGRAM malathion\_adult\_rat

INITIAL

!Abbreviations:

!MAL--malathion

!MOX--malaoxon

!MCA--malathion monocarboxylic acid

!DCA--malathion dicarboxylic acid

!AChE--acetylcholinesterase

!BuChE--butyrylcholinesterase

!CaE--carboxylesterase

!compartments included for liver, kidney, brain, fat, and lumped for slow and rapidly perfused.

!exposure routes: oral only

!2-compartment model for oral exposure includes stomach and intestine (no blood flow)

!bioactivation (MAL -> MOX) occurs in liver

!inhibition of B-esterases (AChE, BuChE, CaE) described in liver, kidney, brain, plasma, RBCs.

!detoxification of MAL by CaE occurs in liver, kidney, brain, plasma, RBCs (to MCA/DCA; these are modeled as a single compartment).

!detoxification of oxon by B-esterases is stoichiometric; the oxon-enzyme complex serves as a "trap" for the oxon.

!CaE assumed to undergo reactivation; AChE and BuChE assumed to undergo reactivation/aging.

!catalytic detoxification of oxon in liver, plasma, brain, kidney

!Physiological parameters--rat

!blood flows (as fraction of cardiac output)

constant QCC = 0.0 !cardiac output (L/hr/kg BW<sup>0.75</sup>)

constant QFC = 0.0 !fraction of cardiac output to fat

constant QLC = 0.0 !fraction of cardiac output to liver

constant QKC = 0.0 !fraction of cardiac output to kidney

constant QBrC = 0.0 !fraction of cardiac output to brain

constant QRC = 0.0 !fraction of cardiac output to richly perfused tissues

!tissue volumes (as fraction of BW)

constant BW = 0.0 !rat body weight (kg)

constant VBLC = 0.0 !fractional volume of blood

constant VFC = 0.0 !fractional volume of fat

constant VLC = 0.0 !fractional volume of liver

constant VKC = 0.0 !fractional volume of kidney

constant VBrC = 0.0 !fractional volume of brain  
constant VSC = 0.0 !fractional volume of slowly perfused tissues  
constant HCT = 0.0 !hematocrit  
constant VRC = 0.0 !rapidly perfused  
constant VINTC = 0.0 !intestine

!partition coefficients (tissue:blood); from 2007 USEPA malathion lice assessment  
!malathion

constant PMaIF = 0.0 !fat:blood  
constant PMaIR = 0.0 !richly perfused:blood  
constant PMaIL = 0.0 !liver:blood  
constant PMaIK = 0.0 !kidney:blood  
constant PMaIBr = 0.0 !brain:blood  
constant PMaIS = 0.0 !slowly perfused:blood

!malaoxon

constant PMoXF = 0.0 !fat:blood  
constant PMoXR = 0.0 !richly perfused:blood  
constant PMoXL = 0.0 !liver:blood  
constant PMoXK = 0.0 !kidney:blood  
constant PMoXBr = 0.0 !brain:blood  
constant PMoXS = 0.0 !slowly perfused: blood

!volumes of distribution for metabolite compartments; from the USEPA malathion lice assessment (2009)

constant V1 = 0.0 !MCA + DCA (L)

!metabolism parameters

!maximum rates of metabolism (umol/hr/kg tissue)

!malathion detoxification

constant VMAXMALLC = 0.0 !liver--CaE; Chambers & Meek 2017  
constant VMAXMALKC = 0.0 !kidney--CaE; based on Talcott 1979  
constant VMAXMALBLC = 0.0 !plasma--CaE(umol/hr/L); based on Talcott 1979  
constant VMAXMALBRC = 0.0 !brain--CaE; 5% of liver

!malathion oxidation--malathion to oxon

constant VMAXMALMOXC = 0.0 !liver--CYPs; based on in vitro data (Chambers & Meek 2017)

!oxon detox (fit to oxon inhibition data)

constant VMOXDMPLC = 0.0 !liver  
constant VMOXDMPPC = 0.0 !plasma  
constant VMOXDMPBC = 0.0 !brain  
constant VMOXDMPKC = 0.0 !kidney

!affinity constants (umol/L)

!malathion detoxification

constant KMMALL = 0.0 !liver--CaE  
constant KMMALK = 0.0 !kidney--CaE

constant KMMALBL = 0.0 !plasma--CaE

constant KMMALBR = 0.0 !brain--CaE

!malathion oxidation--malathion to oxon

constant KMMALMOX = 0.0 !liver--CYPs; based on in vitro data (Chambers & Meek 2017)

!oxon detox

constant KMOXDMPL = 0.0 !liver

constant KMOXDMPP = 0.0 !plasma

constant KMOXDMPB = 0.0 !brain

constant KMOXDMPK = 0.0 !kidney

constant KI = 0.0 !dissociation constant (umol/L); from Krstic et al 2008 for AChE/malaoxon

!pharmacodynamics--esterase inhibition

!Enzyme turnover rates (umol hydrolysis/h/umol active sites)--not chemical specific

constant TRCE=0.0 !AChE (all tissues)

constant TRBE=0.0 !BuChE (all tissue)

constant TRCR=0.0 !CaE (all tissues)

!Enzyme activities (umol/hr/kg tissue)--not chemical-specific

!AChE

constant BACHE=0.0 !brain AChE

constant HACHE=0.0 !liver AChE

constant BLACHE=0.0 !Plasma AChE

constant RBCHE=0.0 !RBC AChE

constant KACHE=0.0 !kidney AChE

!BuChE

constant BBUCE=0.0 !brain BuChE

constant HBUCE=0.0 !liver BuChE

constant BLBUCE=0.0 !Plasma BuChE

constant KBUCE=0.0 !kidney BuChE

!CaE

constant BRCE=0.0 !Brain CaE

constant HECE=0.0 !liver CaE

constant PLOCE=0.0 !Plasma CaE

constant KECE=0.0 !kidney CaE

!chemical-specific parameters for inhibition

!liver AChE rate constants

constant KDHCE=0.0 ! degradation of esterase (/hr)

constant KIHCE=0.0 ! inhibition of esterase (per umol/L/hr)

constant KRHCE=0.0 ! reactivation of esterase (/hr)

constant KAHCE=0.0 ! aging of esterase (/hr)

!liver BuChE rate constants

constant KDHBE=0.0 ! degradation of esterase (/hr)

constant KIHBE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRHBE=0.0 ! reactivation of esterase (/hr)  
constant KAHBE=0.0 ! aging of esterase (/hr)

!liver CaE rate constants

constant KDHCR=0.0 ! degradation of esterase (/hr)  
constant KIHCR=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRHCR=0.0 ! reactivation of esterase (/hr)  
constant KAHCR=0.0 ! aging of esterase (/hr)

!brain AChE rate constants

constant KDBCE=0.0 ! degradation of esterase (/hr)  
constant KIBCE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRBCE=0.0 ! reactivation of esterase (/hr)  
constant KABCE=0.0 ! aging of esterase (/hr)

!brain BuChE rate constants

constant KDBBE=0.0 ! degradation of esterase (/hr)  
constant KIBBE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRBBE=0.0 ! reactivation of esterase (/hr)  
constant KABBE=0.0 ! aging of esterase (/hr)

!brain CaE rate constants

constant KDBCR=0.0 ! degradation of esterase (/hr)  
constant KIBCR=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRBCR=0.0 ! reactivation of esterase (/hr)  
constant KABCR=0.0 ! aging of esterase (/hr)

!kidney AChE rate constants

constant KDKCE=0.0 ! degradation of esterase (/hr)  
constant KIKCE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRKCE=0.0 ! HR-1; reactivation of esterase  
constant KAKCE=0.0 ! aging of esterase (/hr)

!kidney BuChE rate constants

constant KDKBE=0.0 ! degradation of esterase (/hr)  
constant KIKBE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRKBE=0.0 ! reactivation of esterase (/hr)  
constant KAKBE=0.0 ! aging of esterase (/hr)

!kidney CaE rate constants

constant KDKCR=0.0 ! degradation of esterase (/hr)  
constant KIKCR=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRKCR=0.0 ! reactivation of esterase (/hr)  
constant KAKCR=0.0 ! aging of esterase (/hr)

!plasma AChE rate constants

constant KDBLCE=0.0 ! degradation of esterase (/hr)



constant KIBLCE=0.0                   ! inhibition of esterase (per umol/L/hr)  
 constant KRBLCE=0.0               ! reactivation of esterase (/hr)  
 constant KABLCE=0.0               ! aging of esterase (/hr)

!plasma BuChE rate constants

constant KDBLBE=0.0               ! degradation of esterase (/hr)  
 constant KIBLBE=0.0               ! inhibition of esterase (per umol/L/hr)  
 constant KRBLBE=0.0    ! reactivation of esterase (/hr)  
 constant KABLBE=0.0    ! aging of esterase (/hr)

!plasma CaE rate constants

constant KDBLCR=0.0               ! degradation of esterase (/hr)  
 constant KIBLCR=0.0               ! inhibition of esterase (per umol/L/hr)  
 constant KRBLCR=0.0               ! reactivation of esterase (/hr)  
 constant KABLCR=0.0               ! aging of esterase (/hr)

!RBC AChE rate constants

constant KDRBCE=0.0               ! degradation of esterase (/hr)  
 constant KIRBCE=0.0               ! inhibition of esterase (per umol/L/hr)  
 constant KRRBCE=0.0               ! reactivation of esterase (/hr)  
 constant KARBCE=0.0               ! aging of esterase (/hr)

!uptake parameters

!malathion

constant KAS=0.0                   !transfer stomach to liver (/h)  
 constant KSI=0.0               !transfer stomach to intestine (/h)  
 constant KAI=0.0    !transfer intestine to liver (/h)  
 constant FA=0.0    !Fractional oral absorption

!oxon

constant KASO=0.0                   !transfer stomach to liver (/h)  
 constant KSIO=0.0               !transfer stomach to intestine (/h)  
 constant KAIO=0.0    !transfer intestine to liver (/h)  
 constant FAO=0.0    !fractional oral Absorption

!elimination rate constants for metabolite compartments (/h)

constant KE1=0.0    !MCA + DCA

!molecular weights

constant MWMAL=0.0 !molecular wt of malathion (g/mol)  
 constant MWMOX=0.0 !molecular wt of malaoxon (g/mol)

!oral dosing--single oral dose

constant ORALMAL=0.0    !MAL single oral bolus dose (ug/kg)  
 constant ORALMOX=0.0    !oxon oral bolus dose (ug/kg)

!oral dosing--multiple doses

!put times in the array DT (in hours) (absolute time, NOT time since last dose)  
 !Put the oral dose of malathion in the array DORAL (ug/kg) for malathion (DORALO for oxon)  
 !for dosing AMOUNT (ug rather than ug/kg,) put the oral dose of malathion (AMTMDOSE) or oxon (AMTODOSE)

!Add one additional dose of zero at a time greater than TSTOP to keep the model running

!BWT is in kg and is for simulating 90 day studies

DIMENSION DT(2000), DORAL(2000), DORALO(2000), AMTMDOSE(2000),  
 AMTODOSE(2000),BWT(2000)

INTEGER indx

indx=1 ! indx is a counter used to track multiple doses

SCHEDULE DOSEORAL .AT. DT(indx)

ODOSE=0.0 !oral dose (MAL; ug/kg BW)

ODOSEO=0.0 !oral dose (oxon; ug/kg BW)

ADOSEM=0.0 !oral dose (MAL; ug)

ADOSEO=0.0 !oral dose (oxon; ug)

! TIMING COMMANDS

constant POINTS=100.0 !OUTPUT POINTS

constant TSTOP=0.0 !STOP SIMULATION (HR)

CINT=TSTOP/POINTS !Communication interval

VARIABLE time = 0.0 !simulation time in hour

!scaling for blood flows

QC = QCC\*(BW\*\*0.75) !cardiac output (L/h)

QSC = 1.0-(QFC+QLC+QBrC+QRC+QKC) !% of blood flow not going to other tissues (i.e., % to slowly perfused)

QK = QKC\*QC !kidney (L/h)

QF = QFC\*QC !fat (L/h)

QL = QLC\*QC !liver (L/h)

QBr = QBrC\*QC !brain (L/h)

QR = QRC\*QC !rapidly perfused tissues (L/h)

QS = QSC\*QC !slowly perfused tissues (L/h)

QBal = QC-(QF+QL+QBr+QR+QS+QK) !balance check

!scaling for tissue volumes (L)

VBL = VBLC\*BW !blood

VPLAS = VBL\*(1-HCT) !plasma

VRBC = VBL\*HCT !RBCs

VF = VFC\*BW !fat

VL = VLC\*BW !liver

VBr = VBrC\*BW !brain

VK = VKC\*BW !kidney

VR = VRC\*BW !rapidly perfused tissues

VS = VSC\*BW !slowly perfused tissues

VINT = VINTC\*BW !intestine

!Tissue volume checks

VTOTCOMPARTMENTS=(VLC+VFC+VSC+VRC+VBRC+VBLC+VKC+VINTC)/0.9 !should be ~0.9-1.1

!scaling for Vmax for malathion detoxification (umol/hr/kg tissue to umol/hr)

VMAXMALL = VMAXMALLC \* VL !liver--CaE

VMAXMALK = VMAXMALKC \* VK !kidney--CaE

VMAXMALBL = VMAXMALBLC \* VBL \* (1-HCT) !plasma--CaE

VMAXMALBR = VMAXMALBRC \* VBR !brain--CaE

!oxidation--malathion to oxon

VMAXMALMOX = VMAXMALMOXC \* VL !liver--CYPs

!oxon detox

VMOXDMPL = VMOXDMPLC\*VL !liver oxon detox

VMOXDMPP = VMOXDMPPC\*VBL\*(1-HCT) !plasma oxon detox

VMOXDMPB = VMOXDMPBC\*VBr !brain oxon detox

VMOXDMPK = VMOXDMPKC\*VK !kidney oxon detox

!Enzyme activity (umol/h) in tissues

!AChE

SBACH=BACHE\*VBr !brain

SHACH=HACHE\*VL !liver

SBLACH=BLACHE\*VBL\*(1-HCT) !plasma

SRBACH=RBCHE\*VBL\*HCT !RBC

SKACH=KACHE\*VK !kidney

!BuChE

SBBUC=BBUCE\*VBr !brain

SHBUC=HBUCE\*VL !liver

SBLBUC=BLBUCE\*VBL\*(1-HCT) !plasma

SKBUC=KBUCE\*VK !kidney

!CaE

SBRCE=BRCE\*VBr !brain

SHECE=HECE\*VL !liver

SPLOCE=PLOCE\*VBL\*(1-HCT) !plasma

SKACE=KECE\*VK !kidney

!calculation of esterase binding sites (umol)

!AChE

IBCE=SBACH/TRCE !total brain AchE (umol)

IHCE=SHACH/TRCE !total liver AchE (umol)

IBLCE=SBLACH/TRCE !total plasma AchE (umol)

IRBCE=SRBACH/TRCE !total RBC AchE (umol)

IKCE=SKACH/TRCE !total kidney AchE (umol)

!BuChE

IBBE=SBBUC/TRBE                      !total brain BuChE (umol)  
 IHBE=SHBUC/TRBE                      !total liver BuChE (umol)  
 IBLBE=SBLBUC/TRBE                  !total plasma BuChE (umol)  
 IKBE=SKBUC/TRBE                      !total kidney BuChE (umol)

!CaE  
 IBCR=SBRCR/TRCR                      !total brain CaE (umol)  
 IHCR=SHRCR/TRCR                      !total liver CaE (umol)  
 IBLCR=SPLOC/TRCR                      !total plasma CaE (umol)  
 IKCR=SKACR/TRCR                      !total kidney CaE (umol)

!calculation of enzyme synthesis rates in tissues (umol/hr)

!liver

KSHCE=IHCE\*KDHCE                  !AChE  
 KSHBE=IHBE\*KDHBE                  !BuChE  
 KSHCR=IHCR\*KDHCR                  !CaE

!brain

KSBCE=IBCE\*KDBCE                  !AChE  
 KSBBE=IBBE\*KDBBE                  !BuChE  
 KSBCE=IBCE\*KDBCE                  !CaE

!kidney

KSKCE=IKCE\*KDKCE                  !AChE  
 KSKBE=IKBE\*KDKBE                  !BuChE  
 KSKCR=IKCR\*KDKCR                  !CaE

!plasma

KSBLC=IBLC\*KDBLC                  !AChE  
 KSBLC=IBLC\*KDBLC                  !BuChE  
 KSBLC=IBLC\*KDBLC                  !CaE

!RBC

KSRBCE=KDRBCE\*IRBCE !AChE

ODOSEMAL=ORALMAL\*(BW/MWMAL)\*FA                  ! MAL dose conversion (ug/kg to umol)  
 ODOSEMOX=ORALMOX\*(BW/MWMOX)\*FAO                  ! malaoxon dose conversion (ug/kg to umol)

END ! INITIAL

DYNAMIC

ALGORITHM IALG = 2                  !Gear algorithm  
 TERMT(TIME .GE. TSTOP)                  !stop execution

!scaling for blood flows

QC = QCC\*(BW\*\*0.75)                  !cardiac output (L/h)

QSC = 1.0-(QFC+QLC+QBrC+QRC+QKC) !% of blood flow not going to other tissues (i.e., % to slowly perfused)

$QK = QKC * QC$  !kidney (L/h)  
 $QF = QFC * QC$  !fat (L/h)  
 $QL = QLC * QC$  !liver (L/h)  
 $QBr = QBrC * QC$  !brain (L/h)  
 $QR = QRC * QC$  !rapidly perfused tissues (L/h)  
 $QS = QSC * QC$  !slowly perfused tissues (L/h)

$QBal = QC - (QF + QL + QBr + QR + QS + QK)$  !balance check

!scaling for tissue volumes (L)

$VBL = VBLC * BW$  !blood  
 $VPLAS = VBL * (1 - HCT)$  !plasma  
 $VRBC = VBL * HCT$  !RBCs  
 $VF = VFC * BW$  !fat  
 $VL = VLC * BW$  !liver  
 $VBr = VBrC * BW$  !brain  
 $VK = VKC * BW$  !kidney  
 $VR = VRC * BW$  !rapidly perfused tissues  
 $VS = VSC * BW$  !slowly perfused tissues  
 $VINT = VINTC * BW$  !intestine

!Tissue volume checks

$VTOTCOMPARTMENTS = (VLC + VFC + VSC + VRC + VBrC + VBLC + VKC + VINTC) / 0.9$  !should be ~0.9-1.1

!scaling for Vmax for malathion detoxification (umol/hr/kg tissue to umol/hr)

$VMAXMALL = VMAXMALLC * VL$  !liver--CaE  
 $VMAXMALK = VMAXMALKC * VK$  !kidney--CaE  
 $VMAXMALBL = VMAXMALBLC * VBL * (1 - HCT)$  !plasma--CaE  
 $VMAXMALBR = VMAXMALBRC * VBr$  !brain--CaE

!oxidation--malathion to oxon

$VMAXMALMOX = VMAXMALMOXC * VL$  !liver--CYPs

!oxon detox

$VMOXDMPL = VMOXDMPLC * VL$  !liver oxon detox  
 $VMOXDMPP = VMOXDMPPC * VBL * (1 - HCT)$  !plasma oxon detox  
 $VMOXDMPB = VMOXDMPBC * VBr$  !brain oxon detox  
 $VMOXDMPK = VMOXDMPKC * VK$  !kidney oxon detox

!Enzyme activity (umol/h) in tissues--these are currently scaled to BW (as with the adult rat); may need to update based on data on ontogeny of enzyme activity

!AChE

$SBACH = BACHE * VBr$  !brain  
 $SHACH = HACHE * VL$  !liver  
 $SBLACH = BLACHE * VBL * (1 - HCT)$  !plasma  
 $SRBACH = RBACHE * VBL * HCT$  !RBC  
 $SKACH = KACHE * VK$  !kidney

!BuChE  
 SBBUC=BBUCE\*VBr !brain  
 SHBUC=HBUCE\*VL !liver  
 SBLBUC=BLBUCE\*VBL\*(1-HCT) !plasma  
 SKBUC=KBUCE\*VK !kidney

!CaE  
 SBRCE=BRCE\*VBr !brain  
 SHECE=HECE\*VL !liver  
 SPLOCE=PLOCE\*VBL\*(1-HCT) !plasma  
 SKACE=KECE\*VK !kidney

!calculation of esterase binding sites (umol)

!AChE  
 IBCE=SBACH/TRCE !total brain AChE (umol)  
 IHCE=SHACH/TRCE !total liver AChE (umol)  
 IBLCE=SBLACH/TRCE !total plasma AChE (umol)  
 IRBCE=SRBACH/TRCE !total RBC AChE (umol)  
 IKCE=SKACH/TRCE !total kidney AChE (umol)

!BuChE  
 IBBE=SBBUC/TRBE !total brain BuChE (umol)  
 IHBE=SHBUC/TRBE !total liver BuChE (umol)  
 IBLBE=SBLBUC/TRBE !total plasma BuChE (umol)  
 IKBE=SKBUC/TRBE !total kidney BuChE (umol)

!CaE  
 IBCR=SBRCE/TRCR !total brain CaE (umol)  
 IHCR=SHECE/TRCR !total liver CaE (umol)  
 IBLCR=SPLOCE/TRCR !total plasma CaE (umol)  
 IKCR=SKACE/TRCR !total kidney CaE (umol)

!calculation of enzyme synthesis rates in tissues (umol/hr)

!liver  
 KSHCE=IHCE\*KDHCE !AChE  
 KSHBE=IHBE\*KDHBE !BuChE  
 KSHCR=IHCR\*KDHCR !CaE  
 !brain  
 KSBCE=IBCE\*KDBCE !AChE  
 KSBBE=IBBE\*KDBBE !BuChE  
 KSBCE=IBCE\*KDBCE !CaE  
 !kidney  
 KSKCE=IKCE\*KDKCE !AChE  
 KSKBE=IKBE\*KDKBE !BuChE  
 KSKCR=IKCR\*KDKCR !CaE  
 !plasma  
 KSKLCE=IBLCE\*KDBLCE !AChE

KSBLBE=IBLBE\*KDBLBE !BuChE  
 KSBLCR=IBLCR\*KDBLCR !CaE  
 !RBC  
 KSRBCE=KDRBCE\*IRBCE !AChE

!-----

!oral dose molar conversion with fractional absorption

ODOSEMAL=ORALMAL\*(BW/MWMAL)\*FA !MAL dose conversion (ug/kg to umol)  
 ODOSEMOX=ORALMOX\*(BW/MWMOX)\*FAO !malaoxon dose conversion (ug/kg to umol)

!oral dose

DISCRETE DOSEORAL

!BW=BWT(indx)

ODOSE=ODOSE+DORAL(indx)\*(BW/MWMAL)\*FA !malathion dose ug/kg to umol

ODOSEO=ODOSEO+DORALO(indx)\*(BW/MWMOX)\*FAO !oxon dose ug/kg to umol

ADOSEO=ADOSEO+AMTODOSE(indx)\*FA/MWMAL !malathion dose ug to umol--check that

ADOSEM=ADOSEM+AMTMDOSE(indx)\*FAO/MWMOX !oxon dose ug to umol

indx=indx+1

SCHEDULE DOSEORAL .AT. DT(indx)

END

DERIVATIVE

!-----

!malathion (parent compound)-----

!-----

!stomach

RSTOM = -kas\*Astom-ksi\*Astom !rate of change in stomach (umol/hr)

ASTOM = ODOSE+ADOSEM+ODOSEMAL + integ(RSTOM,0.0) !amount in stomach (umol)

!intestine

RINST = ksi\*ASTOM - kai\*AlntM !rate of change in intestine (umol/hr)

AlntM=integ(RINST,0.0) !amount in intestine (umol)

CINTM = AlntM/VINT !concentration in intestine (umol/L)

!liver

RALM=QL\*CBL-QL\*CVLM+kai\*AlntM+kas\*ASTOM-RALM1-RALM2 !rate of change in liver (umol/hr)

ALM = integ(RALM,0.0) !amt in liver (umol)

CLM = ALM/VL !concentration in liver (umol/L)

CVLM = CLM/PMaLL !concn in venous blood leaving liver (umol/L)

!Liver metabolism of Mal by CYPs--oxidation

RALM1 = (VmaxMalMox\*CVLM)/(KmMalMox+CVLM) !rate of metabolite formation (umol/hr)

ALM1 = integ(RALM1, 0.0)                      !amt oxon formed (umol)  
  
 !Liver--metabolism of MAL to MCA/DCA by CaE  
 RALM2 = VMAXMALL\*CVLM/(KMMALL\*(1+CVLO/KI)+CVLM) !rate of metabolism (umol/hr)  
 ALM2 = integ(RALM2,0.0)                      !amount of metabolites formed (umol)  
  
 !kidney  
 RAKM = QK\*CBL-QK\*CVKM-RAKM2                      !rate of change in kidney (umol/hr)  
 AKM = integ(RAKM,0.0)                      !amt in kidney (umol)  
 CKM = AKM/VK                      !concn in kidney (umol/L)  
 CVKM = CKM/PMaIK                      !concn in venous blood leaving kidney (umol/L)  
  
 !kidney--metabolism of MAL to MCA/DCA by CaE  
 RAKM2 = VMAXMALK\*CVKM/(KMMALK\*(1+CVKO/KI)+CVKM) !rate of metabolism (umol/hr)  
 AKM2 = integ(RAKM2,0.0)                      !amount of metabolites formed (umol)  
  
 !fat  
 RAFM = QF\*CBL-QF\*CVFM                      !rate of change in fat (umol/hr)  
 AFM = integ(RAFM, 0.0)                      !amt in fat (umol)  
 CFM = AFM/VF                      !concn. in fat (umol/L)  
 CVFM = CFM/PMaIF                      !concn. in venous blood leaving fat (umol/L)  
  
 !slowly perfused  
 RASM = QS\*CBL-QS\*CVSM                      !rate of change in slowly perfused (umol/hr)  
 ASMaI = integ(RASM, 0.0)                      !amt in slowly perfused (umol)  
 CSM = ASMaI/VS                      !concn. in slowly perfused (umol/L)  
 CVSM = CSM/PMaIS                      !concn. in venous blood leaving slowly perfused (umol/L)  
  
 !brain  
 RABrM = QBr\*CBL-QBr\*CVBrM-RABRM2                      !rate of change in brain (umol/hr)  
 ABrM = integ(RABrM,0.0)                      !amt. in brain (umol)  
 CBrM = ABrM/VBr                      !concn in brain (umol/L)  
 CVBrM = CBrM/PMaIBr                      !concn. in venous blood leaving brain (umol/L)  
  
 !brain--metabolism of MAL to MCA/DCA by CaE  
 RABRM2 = VMAXMALBR\*CVBrM/(KMMALBr\*(1+CVBrO/KI)+CVBrM)!rate of metabolism (umol/hr)  
 ABRM2 = integ(RABRM2,0.0)                      !amount of metabolites formed (umol)  
  
 !rapidly perfused tissues  
 RARM = QR\*CBL-QR\*CVRM                      !rate of change in rapidly perfused (umol/hr)  
 ARM = integ(RARM,0.0)                      !amt in rapidly perfused (umol)  
 CRM = ARM/VR                      !concn in rapidly perfused (umol/L)  
 CVRM = CRM/PMaIR                      !concn in venous blood leaving rapidly perfused (umol/L)  
  
 !blood  
 RABL=QC\*(CV-CBL)-RABLM2                      !rate of change in mixed blood (arterial & venous; umol/hr)  
 ABL=INTEG(RABL,0.0)                      !amount in mixed blood (umol)  
 CBL=ABL/VBL                      !concn in mixed blood (umol/L)



!blood--metabolism of MAL to MCA/DCA by CaE

RABLM2 = VMAXMALBL\*CBL/(KMMALBL\*(1+CBLO/KI)+CBL) !rate of metabolism (umol/hr)

ABLM2 = integ(RABLM2,0.0) !amt metabolite formed (umol)

CV=(QL\*CVLM+QF\*CVFM+QK\*CVKM+QS\*CVSM+QBr\*CVBrM+QR\*CVRM)/QC !concentration free in venous blood (umol/L)

!-----  
!oxon  
!-----

!stomach

RAStomO = -ksiO\*ASTomO-kasO\*ASTomO !rate of change in stomach (umol/hr)

ASTomO = integ(RASTomO,0.0)+ODOSEO+ADOSEO+ODOSEMOX !amount in stomach (umol)

!intestine

RAIntO = -kaiO\*AINTO+ksiO\*ASTomO !rate of change in intestine (umol/hr)

AINTO = integ(RAIntO,0.0) !amount in intestine (umol)

CINTO = AINTO/VINT !concn in intestine (umol/L)

!liver

RALO = QL\*(CBLO-CVLO)+RALM1-(RHPCE+RHPBE+RHPCR)+kaiO\*AINTO+kasO\*ASTomO-RALOD

!rate of change in liver (umol/hr)

ALO = integ(RALO,0.0) !amt in liver (umol)

CLO = ALO/VL !concentration in liver (umol/L)

CVLO = CLO/PMOXL !concn in venous blood leaving liver (umol/L)

!oxon detox--catalytic

RALOD = (VMOXDMPL\*CVLO)/(KMOXDMPL\*(1+CVLO/KI)+CVLO)

AALOD = integ(RALOD,0.0)

!AChE activity--liver

RHCE=KSHCE-AHCE\*(KDHCE+KIHCE\*CLO)+HOCE\*KRHCE ! rate of AChE activity (umol/hr)

AHCE=INTEG(RHCE,IHCE) ! liver AChE activity (umol)

RNHCE=KSHCE-ANHCE\*KDHCE ! rate of baseline AChE activity (umol/hr)

ANHCE=INTEG(RNHCE,IHCE) ! Amt of baseline AChE (umol)

RHOCE=AHCE\*KIHCE\*CLO-HOCE\*(KAHCE+KRHCE) ! rate of AChE inhibition (umol/hr)

HOCE=INTEG(RHOCE,0.0) ! amt of liver AChE inhibited (umol)

RHPCE=AHCE\*KIHCE\*CLO ! rate of oxon consumption (umol/hr)

AHPCE=integ(RHPCE,0.0) ! amt of oxon consumed (umol)

!BuChE activity--liver

RHBE=KSHBE-AHBE\*(KDHBE+KIHBE\*CLO)+HOBE\*KRHBE ! rate of BuChE activity (umol/hr)

AHBE=INTEG(RHBE,IHBE)	! liver BuChE activity (umol)
RNHBE=KSHBE-ANHBE*KDHBE	! rate of baseline BuChE act
(umol/hr)	
ANHBE=INTEG(RNHBE,IHBE)	! Amt of baseline BuChE (umol)
RHOBE=AHBE*KIHBE*CLO-HOBE*(KAHBE+KRHBE)	! rate of BuChE inhibition
(umol/hr)	
HOBE=INTEG(RHOBE,0.0)	! amount of liver BuChE inhibited
(umol)	
RHPBE=AHBE*KIHBE*CLO	! rate of oxon consumption
(umol/hr)	
AHPBE=integ(RHPBE,0.0)	! amt of oxon consumed (umol)
!CaE activity--liver	
RHCR=KSHCR-AHCR*(KDHCR+KIHCR*CLO)+HOCCR*KRHCR	! rate of CaE activity (umol/hr)
AHCR=INTEG(RHCR,IHCR)	! liver CaE activity (umol)
RNHCR=KSHCR-ANHCR*KDHCR	! rate of baseline CaE act (umol/hr)
ANHCR=INTEG(RNHCR,IHCR)	! Amt of baseline CaE (umol)
RHOCCR=AHCR*KIHCR*CLO-HOCCR*(KAHCR+KRHCR)	! rate of CaE inhibition (umol/hr)
HOCCR=INTEG(RHOCCR,0.0)	! amount of liver CaE inhibited
(umol)	
RHPCR=AHCR*KIHCR*CLO	! rate of oxon consumption
(umol/hr)	
AHPCR=integ(RHPCR,0.0)	! amt of oxon consumed (umol)
!total amount detoxified--non-catalytic in liver	
AHTOTNC=AHPCE+AHPBE+AHPCR	!umol
!liver B-esterase (AChE, BuChE, and CaE) activity	
HBE=AHCE+AHBE+AHCR	! total B-esterase=AChE+BuChE+CaE
(umol)	
IHE=100*(HBE/(ANHCE+ANHBE+ANHCR))	! % total B-esterase inhibition
HCE=100*(AHCE/ANHCE)	! % AChE inhibition
HBES=100*(AHBE/ANHBE)	! % BuChE inhibition
HCR=100*(AHCR/ANHCR)	! % CaE inhibition
!fat	
RAFO = QF*CBLO-QF*CVFO	!rate of change in fat (umol/hr)
AFO = integ(RAFO, 0.0)	!amt in fat (umol)
CFO = AFO/VF	!concn. in fat (umol/L)
CVFO = CFO/PMOXF	!concn. in venous blood leaving fat (umol/L)
!slowly perfused	
RASO = QS*CBLO-QS*CVSO	!rate of change in slowly perfused (umol/hr)
ASO = integ(RASO, 0.0)	!amt in slowly perfused (umol)
CSO = ASO/VS	!concn. in slowly perfused (umol/L)
CVSO = CSO/PMOXS	!concn. in venous blood leaving slowly perfused (umol/L)

!rapidly perfused tissues  
RARO = QR\*CBLO-QR\*CVRO !rate of change in rapidly perfused (umol/hr)  
ARO = integ(RARO,0.0) !amt in rapidly perfused (umol)  
CRO = ARO/VR !concn in rapidly perfused (umol/L)  
CVRO = CRO/PMOXR !concn in venous blood leaving rapidly perfused (umol/L)

!brain  
RABrO = QBr\*CBLO-QBr\*CVBrO-(RBPCE+RBPBE+RBPCR)-RABROD !rate of change in brain (umol/hr)  
ABrO = integ(RABrO,0.0) !amt. in brain (umol)  
CBrO = ABrO/VBr !concn in brain (umol/L)  
CVBrO = CBrO/PMOXR !concn. in venous blood leaving brain (umol/L)

!oxon detox--catalytic  
RABROD = (VMOXDMPB\*CVBrO)/(KMOXDMPB\*(1+CVBrO/KI)+CVBrO)  
AABRO = integ(RABROD,0.0)

!AChE activity--brain  
RBCE=KSBCE-ABCE\*(KDBCE+KIBCE\*CBrO)+BOCE\*KRBCE ! rate of AChE activity (umol/hr)  
ABCE=INTEG(RBCE,IBCE) ! brain AChE activity (umol)  
RNBCE=KSBCE-ANBCE\*KDBCE ! rate of baseline AChE act  
(umol/hr)  
ANBCE=INTEG(RNBCE,IBCE) ! Amt of baseline AChE (umol)  
RBOCE=ABCE\*KIBCE\*CBrO-BOCE\*(KABCE+KRBCE) ! rate of AChE inhibition  
(umol/hr)  
BOCE=INTEG(RBOCE,0.0) ! amount of brain AChE inhibited  
(umol)  
RBPCE=ABCE\*KIBCE\*CBrO ! rate of oxon consumption  
(umol/hr)  
ABPCE=integ(RBPCE,0.0) ! amt of oxon consumed (umol)

!BuChE activity--brain  
RBBE=KSBBE-ABBE\*(KDBBE+KIBBE\*CBrO)+BOBE\*KRBBE ! rate of BuChE activity (umol/hr)  
ABBE=INTEG(RBBE,IBBE) ! brain BuChE activity (umol)  
RNBBE=KSBBE-ANBBE\*KDBBE ! rate of baseline BuChE act  
(umol/hr)  
ANBBE=INTEG(RNBBE,IBBE) ! Amt of baseline BuChE (umol)  
RBOBE=ABBE\*KIBBE\*CBrO-BOBE\*(KABBE+KRBBE) ! rate of BuChE inhibition  
(umol/hr)  
BOBE=INTEG(RBOBE,0.0) ! amount of brain BuChE  
inhibited (umol)  
RBPBE=ABBE\*KIBBE\*CBrO ! rate of oxon consumption  
(umol/hr)  
ABPBE=integ(RBPBE,0.0) ! amt of oxon consumed (umol)

!CaE activity--brain  
RBCR=KSBCE-ABCE\*(KDBCE+KIBCE\*CBrO)+BOCE\*KRBCE ! rate of CaE activity (umol/hr)  
ABCE=INTEG(RBCE,IBCE) ! brain CaE activity (umol)

$RNBCR = KSBCE - ANBCR * KDBCE$  ! rate of baseline CaE act  
 (umol/hr)  
 $ANBCR = INTEG(RNBCR, IBCR)$  ! Amt of baseline CaE (umol)  
 $RBOCR = ABCR * KIBCE * CBrO - BOCR * (KABCE + KRBCR)$  ! rate of CaE inhibition (umol/hr)  
 $BOCR = INTEG(RBOCR, 0.0)$  ! amount of brain CaE inhibited  
 (umol)  
 $RBPCR = ABCR * KIBCE * CBrO$  ! rate of oxon consumption  
 (umol/hr)  
 $ABPCR = integ(RBPCR, 0.0)$  ! amt of oxon consumed (umol)

!brain B-esterase (AChE, BuChE, and CaE) activity  
 $BBE = ABCE + ABBE + ABCR$  ! total B-esterase = AChE + BuChE + CaE (umol)  
 $IBE = 100 * (BBE / (ANBCE + ANBBE + ANBCR))$  ! total B-esterase inhibition  
 $BCE = 100 * (ABCE / ANBCE)$  ! % AChE inhibition  
 $BBES = 100 * (ABBE / ANBBE)$  ! % BuChE inhibition  
 $BCR = 100 * (ABCR / ANBCR)$  ! % CaE inhibition

!kidney  
 $RAKO = QK * CBLO - QK * CVKO - (RKPCE + RKPBE + RKPCE) - RAKOD$  !rate of change in kidney (umol/hr)  
 $AKO = integ(RAKO, 0.0)$  !amt in kidney (umol)  
 $CKO = AKO / VK$  !concn in kidney (umol/L)  
 $CVKO = CKO / PMOXX$  !concn in venous blood leaving kidney (umol/L)

!oxon detox--catalytic  
 $RAKOD = (VMOXDMPK * CVKO) / (KMOXDMPK * (1 + CVKO / KI) + CVKO)$   
 $AAKOD = integ(RAKOD, 0.0)$

! AChE activity--kidney  
 $RKCE = KSKCE - AKCE * (KDKCE + KIKCE * CKO) + KOCE * KRKCE$  ! rate of AChE activity (umol/hr)  
 $AKCE = INTEG(RKCE, IKCE)$  ! kidney AChE activity (umol)  
 $RNKCE = KSKCE - ANKCE * KDKCE$  ! rate of baseline AChE act  
 (umol/hr)  
 $ANKCE = INTEG(RNKCE, IKCE)$  ! Amt of baseline AChE (umol)  
 $RKOCE = AKCE * KIKCE * CKO - KOCE * (KAKCE + KRKCE)$  ! rate of AChE inhibition  
 (umol/hr)  
 $KOCE = INTEG(RKOCE, 0.0)$  ! amount of kidney AChE  
 inhibited (umol)  
 $RKPCE = AKCE * KIKCE * CKO$  ! rate of oxon consumption  
 (umol/hr)  
 $AKPCE = integ(RKPCE, 0.0)$  ! amt of oxon consumed (umol)

! BuChE activity--kidney  
 $RKBE = KSKBE - AKBE * (KDKBE + KIKBE * CKO) + KOBE * KRKBE$  ! rate of BuChE activity (umol/hr)  
 $AKBE = INTEG(RKBE, IKBE)$  ! kidney BuChE activity (umol)  
 $RNKBE = KSKBE - ANKBE * KDKBE$  ! rate of baseline BuChE act  
 (umol/hr)  
 $ANKBE = INTEG(RNKBE, IKBE)$  ! Amt of baseline BuChE (umol)

RKOB<sub>E</sub>=AKBE\*KIKBE\*CKO-KOB<sub>E</sub>\*(KAKBE+KRKB<sub>E</sub>) ! rate of BuChE inhibition  
 (umol/hr)  
 KOB<sub>E</sub>=INTEG(RKOB<sub>E</sub>,0.0) ! amount of kidney BuChE  
 inhibited (umol)  
 RKPBE=AKBE\*KIKBE\*CKO ! rate of oxon consumption  
 (umol/hr)  
 AKPBE=integ(RKPBE,0.0) ! amt of oxon consumed (umol)

! CaE activity--kidney  
 RKCR=KSKCR-AKCR\*(KDKCR+KIKCR\*CKO)+KOCR\*KRKCR ! rate of CaE activity (umol/hr)  
 AKCR=INTEG(RKCR,IKCR) ! kidney CaE activity (umol)  
 RNKCR=KSKCR-ANKCR\*KDKCR ! rate of baseline CaE act  
 (umol/hr)  
 ANKCR=INTEG(RNKCR,IKCR) ! Amt of baseline CaE (umol)  
 RKOCR=AKCR\*KIKCR\*CKO-KOCR\*(KAKCR+KRKCR) ! rate of CaE inhibition  
 (umol/hr)  
 KOCR=INTEG(RKOCR,0.0) ! amount of kidney CaE  
 inhibited (umol)  
 RKPCR=AKCR\*KIKCR\*CKO ! rate of oxon consumption  
 (umol/hr)  
 AKPCR=integ(RKPCR,0.0) ! amt of oxon consumed (umol)

!kidney B-esterase (AChE, BuChE, and CaE) activity  
 KBE=AKCE+AKBE+AKCR ! total B-esterase=AChE+BuChE+CaE (umol)  
 IKE=100\*(KBE/(ANKCE+ANKBE+ANKCR)) ! % total B-esterase inhibition  
 KCE=100\*(AKCE/ANKCE) ! % AChE inhibition  
 KBES=100\*(AKBE/ANKBE) ! % BuChE inhibition  
 KCR=100\*(AKCR/ANKCR) ! % CaE inhibition

!blood  
 RABLO=QC\*(CVO-CBLO)-(RBLPBE+RBLPCR+RBLPCE+RRBPCE)-RAPLD !rate of change in blood (umol/hr)  
 ABLO=integ(RABLO,0.0) !amount in blood (umol)  
 CBLO=ABLO/VBL !concn in blood (umol/L)

CVO=(QL\*CVLO+QF\*CVFO+QS\*CVSO+QR\*CVRO+QBr\*CVBrO+QK\*CVKO)/QC !concn in venous blood  
 (umol/L)

!oxon detox--catalytic  
 RAPLD = (VMOXDMPP\*CBLO)/(KMOXDMPP\*(1+CBLO/KI)+CBLO)  
 AAPLD = integ(RAPLD,0.0)

! AChE activity--plasma  
 RBLCE=KSBLC<sub>E</sub>-ABLCE\*(KDBLC<sub>E</sub>+KIBLC<sub>E</sub>\*CBLO)+BLOC<sub>E</sub>\*KRBLCE ! rate of AChE activity (umol/hr)  
 ABLCE=INTEG(RBLCE,IBLC<sub>E</sub>) ! plasma AChE activity (umol)  
 RNBLCE=KSBLC<sub>E</sub>-ANBLCE\*KDBLC<sub>E</sub> ! rate of baseline AChE act  
 (umol/hr)

ANBLCE=INTEG(RNBLCE,IBLCE) ! Amt of baseline AChE (umol)  
 RBLOCE=ABLCE\*KIBLCE\*CBLO-BLOCE\*(KABLCE+KRBLCE) ! rate of AChE inhibition (umol/hr)  
 BLOCE=INTEG(RBLOCE,0.0) ! amount of plasma AChE inhibited (umol)  
 RBLPCE=ABLCE\*KIBLCE\*CBLO ! rate of oxon consumption (umol/hr)  
 ABLPCE=integ(RBLPCE,0.0) ! amt of oxon consumed (umol)

! BuChE activity--plasma  
 RBLBE=KSBLBE-ABLBE\*(KDBLBE+KIBLBE\*CBLO)+BLOBE\*KRBLBE ! rate of BuChE activity (umol/hr)  
 ABLBE=INTEG(RBLBE,IBLBE) ! plasma BuChE activity (umol)  
 RNBLBE=KSBLBE-ANBLBE\*KDBLBE ! rate of baseline BuChE act (umol/hr)  
 ANBLBE=INTEG(RNBLBE,IBLBE) ! Amt of baseline BuChE (umol)  
 RBLOBE=ABLBE\*KIBLBE\*CBLO-BLOBE\*(KABLBE+KRBLBE) ! rate of BuChE inhibition (umol/hr)  
 BLOBE=INTEG(RBLOBE,0.0) ! amount of plasma BuChE inhibited (umol)  
 RBLPBE=ABLBE\*KIBLBE\*CBLO ! rate of oxon consumption (umol/hr)  
 ABLPBE=integ(RBLPBE,0.0) ! Amt of oxon consumed (umol)

! CaE activity--plasma  
 RBLCR=KSBLCR-ABLCR\*(KDBLCR+KIBLCR\*CBLO)+BLOCR\*KRBLCR ! rate of CaE activity (umol/hr)  
 ABLCR=INTEG(RBLCR,IBLCR) ! plasma CaE activity (umol)  
 RNBLCR=KSBLCR-ANBLCR\*KDBLCR ! rate of baseline CaE act (umol/hr)  
 ANBLCR=INTEG(RNBLCR,IBLCR) ! Amt of baseline CaE (umol)  
 RBLOCR=ABLCR\*KIBLCR\*CBLO-BLOCR\*(KABLCR+KRBLCR) ! rate of CaE inhibition (umol/hr)  
 BLOCR=INTEG(RBLOCR,0.0) ! amount of plasma CaE inhibited (umol)  
 RBLPCR=ABLCR\*KIBLCR\*CBLO ! rate of oxon consumption (umol/hr)  
 ABLPCR=integ(RBLPCR,0.0) ! amt of oxon consumed (umol)

!plasma B-esterase (AChE, BuChE, and CaE) activity  
 BLBE=ABLCE+ABLBE+ABLCR !total B-esterase=AChE+BuChE+CaE (umol)  
 IBLE=100\*(BLBE/(ANBLCE+ANBLBE+ANBLCR)) ! % Total B-esterase inhibition in plasma  
 BLCE=100\*(ABLCE/ANBLCE) ! % AChE inhibition in plasma  
 BLBES=100\*(ABLBE/ANBLBE) ! % BuChE inhibition in plasma  
 BLCR=100\*(ABLCR/ANBLCR) ! % CaE inhibition in plasma

! AChE activity--RBCs  
 RRBCE=KSRBCE-ARBCE\*(KDRBCE+KIRBCE\*CBLO)+RBCOCE\*KRRBCE ! rate of AChE activity (umol/hr)

```

ARBCE=INTEG(RRBCCE,IRBCE)                                ! RBC AChE activity (umol)
RNRBCCE=KSRBCE-ANRBCE*KDRBCE                                ! rate of baseline AChE act
(umol/hr)
ANRBCE=INTEG(RNRBCCE,IRBCE)                                ! Amt of baseline AChE
(umol)
RRBOCE=ARBCE*KIRBCE*CBLO-RBCOCE*(KARBCE+KRRBCE)           ! rate of AChE inhibition
(umol/hr)
RBCOCE=INTEG(RRBOCE,0.0)                                    ! amount of RBC AChE inhibited
(umol)
RRBPCE=ARBCE*KIRBCE*CBLO                                    ! rate of oxon consumption
(umol/hr)
ARBPCCE=integ(RRBPCE,0.0)                                    ! amt of oxon consumed (umol)

!RBC B-esterase (AChE only)activity
RBCCE=100*(ARBCE/ANRBCE)                                    ! % AChE inhibition in RBCs
RBCPERC=100-RBCCE                                           ! % inhibited
versus inhibition

```

```

!-----
!metabolite excretion
!MCA + DCA metabolites
RAMCA = RAKM2+RABRM2+RABLM2+RALM2                        !total rate of metabolite formation (umol/hr)
AMCA = integ(RAMCA, 0.0)                                  !amount of MCA+DCA (umol)
RA1 = RAMCA-RAMCAex                                       !rate of change of MCA+DCA in compartment 1 (umol/hr)
A1 = integ(RA1,0.0)                                       !amount of MCA+DCA in compartment 1 (umol)
CBMCA = A1/V1                                             !concentration of MCA+DCA in compartment 1 (umol/L)
RAMCAex = A1*KE1                                           !rate of MCA+DCA excretion (umol/hr)
AMCAex = integ(RAMCAex,0.0)                               !amount of MCA+DCA excreted (umol)

```

end !dynamic

```

!-----
!mass balance
TMASSMAL=ASTOM+AINTM+ALM+AKM+AFM+ASMaI+ABRM+ARM+ABL      !umol
TMASSMOX=ASTOMO+AINTO+ALO+AFO+ASO+AKO+ARO+ABRO+ABLO      !umol
TMASSMCA=A1+AMCAEX                                         !umol
TMASSODT=AAPLD+AALOD+AAKOD+AABRO                          !catalytic oxon detox (umol)
TMASSOXONNC=AHPCE+AHPBE+AHPCR+ABPCE+ABPBE+ABPCR+AKPCE+AKPBE+AKPCR+ABLPCE+ABLPB
E+ABLPCE+ARBPCCE    !non-catalytic oxon detox (umol)
TMASSTOTAL=TMASSMAL+TMASSMOX+TMASSMCA+TMASSODT+TMASSOXONNC !umol--total
better add up to what went in

```

```

TDOSE=ODOSE+ODOSEMAL+ADOSEM+ADOSEO+ODOSEO+ODOSEMOX      !total dose (umol)
MASSBAL=TDOSE/(TMASSTOTAL+1E-99) !should equal 1 (or really close to it)

```

```
end !derivative  
end !program
```



## Appendix B – AcsxL Post-Natal Model

---

### Postnatal rat code

PROGRAM malathion\_postnatal\_rat

INITIAL

!Abbreviations:

!MAL--malathion

!MOX--malaoxon

!DMP--dimethyl phosphate metabolites

!MCA--malathion monocarboxylic acid

!DCA--malathion dicarboxylic acid

!AChE--acetylcholinesterase

!BuChE--butyrylcholinesterase

!CaE--carboxylesterase

!no skin compartment/dermal exposure or lung compartment/inhalation exposure in postnatal rat

!compartments included for liver, kidney, brain, fat, and lumped for slow and rapidly perfused.

!exposure routes: oral

!bioactivation (MAL -> MOX) occurs in liver

!inhibition of B-esterases (AChE, BuChE, CaE) described in liver, kidney, brain, plasma, RBCs.

!detoxification of MAL by CaE occurs in liver, kidney, brain, plasma, RBCs (to MCA/DCA; these are modeled as a single compartment).

!detoxification of oxon by B-esterases is stoichiometric; the oxon-enzyme complex serves as a "trap" for the oxon.

!CaE assumed to undergo reactivation; AChE and BuChE assumed to undergo reactivation/aging.

!Physiological parameters--rat

!blood flows (as fraction of cardiac output)

constant MVC = 0.0 !minute ventilation rate (L/kg/BW)

constant QCC = 0.0 !cardiac output (L/hr/kg BW<sup>0.75</sup>)

constant QFC = 0.0 !fraction of cardiac output to fat

constant QLC = 0.0 !fraction of cardiac output to liver

constant QKC = 0.0 !fraction of cardiac output to kidney

constant QBrC = 0.0 !fraction of cardiac output to brain

constant QRC = 0.0 !fraction of cardiac output to richly perfused tissues

variable time = 0.0 !hr

constant AGE0 = 1.0 !age

!tissue volumes (as fraction of BW)

constant BW0 = 0.0 !rat body weight (kg)

constant VBLC = 0.0 !fractional volume of blood  
 constant VFC = 0.0 !fractional volume of fat  
 constant VKC = 0.0 !fractional volume of kidney  
 constant VSC = 0.0 !fractional volume of slowly perfused tissues  
 constant HCT = 0.0 !hematocrit  
 constant VRC = 0.0 !rapidly perfused  
 constant VINTC = 0.0 !intestine

!partition coefficients (tissue:blood); from 2007 USEPA malathion lice assessment  
 !malathion

constant PMaIF = 0.0 !fat:blood  
 constant PMaIR = 0.0 !richly perfused:blood  
 constant PMaIL = 0.0 !liver:blood  
 constant PMaIK = 0.0 !kidney:blood  
 constant PMaIBr = 0.0 !brain:blood  
 constant PMaIS = 0.0 !slowly perfused:blood

!malaoxon

constant PMoIF = 0.0 !fat:blood  
 constant PMoIR = 0.0 !richly perfused:blood  
 constant PMoIL = 0.0 !liver:blood  
 constant PMoIK = 0.0 !kidney:blood  
 constant PMoIBr = 0.0 !brain:blood  
 constant PMoIS = 0.0 !slowly perfused: blood

!volumes of distribution for metabolite compartment  
 constant V1 = 0.0 !MCA + DCA (L)

!metabolism parameters

!maximum rates of metabolism (umol/hr/kg tissue)

!malathion detoxification

constant VMAXMALLC = 0.0 !liver--CaE; based on in vitro data (Chambers & Meek 2017)  
 constant VMAXMALKC = 0.0 !kidney--CaE; 60% of liver, based on Talcott 1979  
 constant VMAXMALBLC = 0.0 !plasma--CaE(umol/hr/L serum); CaE--74% of liver, based on Talcott 1979  
 constant VMAXMALBRC = 0.0 !brain--CaE; 5% of liver, based on Talcott 1979

!malathion oxidation--malathion to oxon

constant VMAXMALMOXC = 0.0 !liver--CYPs; based on in vitro data (Chambers & Meek 2017)

!oxon detox--fit to oxon oral dose data

constant VMOXDMPLC = 0.0 !liver  
 constant VMOXDMPPC = 0.0 !plasma  
 constant VMOXDMPBC = 0.0 !brain  
 constant VMOXDMPKC = 0.0 !kidney

!affinity constants (umol/L)

!malathion detoxification

constant KMMALL = 0.0 !liver--CaE (Chambers & Meek 2017)

constant KMMALK = 0.0 !kidney--CaE

constant KMMALBL = 0.0 !plasma--CaE

constant KMMALBR = 0.0 !brain--CaE

!malathion oxidation--malathion to oxon

constant KMMALMOX = 0.0 !liver--CYPs; based on in vitro data (Chambers & Meek 2017)

constant KMOXDMPL = 0.0 !liver

constant KMOXDMPP = 0.0 !plasma

constant KMOXDMPB = 0.0 !brain

constant KMOXDMPK = 0.0 !kidney

constant KI = 0.0 !dissociation constant

!pharmacodynamics--esterase inhibition

!Enzyme turnover rates (umol hydrolysis/h/umol active sites)--not chemical specific

constant TRCE=0.0 !AChE (all tissues)

constant TRBE=0.0 !BuChE (all tissue)

constant TRCR=0.0 !CaE (all tissues)

!Enzyme activities (umol/hr/kg tissue)--not chemical-specific

!AChE

constant BACHE=0.0 !brain AChE

constant HACHE=0.0 !liver AChE

constant BLACHE=0.0 !Plasma AChE

constant RBCHE=0.0 !RBC AChE

constant KACHE=0.0 !kidney AChE

!BuChE

constant BBUCE=0.0 !brain BuChE

constant HBUCE=0.0 !liver BuChE

constant BLBUCE=0.0 !Plasma BuChE

constant KBUCE=0.0 !kidney BuChE

!CaE

constant BRCE=0.0 !Brain CaE

constant HECE=0.0 !liver CaE

constant PLOCE=0.0 !Plasma CaE

constant KECE=0.0 !kidney CaE

!chemical-specific parameters for inhibition

!liver AChE rate constants

constant KDHCE=0.0 ! degradation of esterase (/hr)

constant KIHCE=0.0 ! inhibition of esterase (per umol/L/hr)

constant KRHCE=0.0 ! reactivation of esterase (/hr)

constant KAHCE=0.0 ! aging of esterase (/hr)

!liver BuChE rate constants

constant KDHBE=0.0 ! degradation of esterase (/hr)  
constant KIHBE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRHBE=0.0 ! reactivation of esterase (/hr)  
constant KAHBE=0.0 ! aging of esterase (/hr)

!liver CaE rate constants

constant KDHCR=0.0 ! degradation of esterase (/hr)  
constant KIHCR=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRHCR=0.0 ! reactivation of esterase (/hr)  
constant KAHCR=0.0 ! aging of esterase (/hr)

!brain AChE rate constants

constant KDBCE=0.0 ! degradation of esterase (/hr)  
constant KIBCE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRBCE=0.0 ! reactivation of esterase (/hr)  
constant KABCE=0.0 ! aging of esterase (/hr)

!brain BuChE rate constants

constant KDBBE=0.0 ! degradation of esterase (/hr)  
constant KIBBE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRBBE=0.0 ! reactivation of esterase (/hr)  
constant KABBE=0.0 ! aging of esterase (/hr)

!brain CaE rate constants

constant KDBCR=0.0 ! degradation of esterase (/hr)  
constant KIBCR=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRBCR=0.0 ! reactivation of esterase (/hr)  
constant KABCR=0.0 ! aging of esterase (/hr)

!kidney AChE rate constants

constant KDKCE=0.0 ! degradation of esterase (/hr)  
constant KIKCE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRKCE=0.0 ! HR-1; reactivation of esterase  
constant KAKCE=0.0 ! aging of esterase (/hr)

!kidney BuChE rate constants

constant KDKBE=0.0 ! degradation of esterase (/hr)  
constant KIKBE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KKRBE=0.0 ! reactivation of esterase (/hr)  
constant KAKBE=0.0 ! aging of esterase (/hr)

!kidney CaE rate constants

constant KDKCR=0.0 ! degradation of esterase (/hr)  
constant KIKCR=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KKRCR=0.0 ! reactivation of esterase (/hr)  
constant KAKCR=0.0 ! aging of esterase (/hr)

!plasma AChE rate constants

constant KDBLCE=0.0           ! degradation of esterase (/hr)  
constant KIBLCE=0.0           ! inhibition of esterase (per umol/L/hr)  
constant KRBLCE=0.0           ! reactivation of esterase (/hr)  
constant KABLCE=0.0           ! aging of esterase (/hr)

!plasma BuChE rate constants

constant KDBLBE=0.0           ! degradation of esterase (/hr)  
constant KIBLBE=0.0           ! inhibition of esterase (per umol/L/hr)  
constant KRBLBE=0.0           ! reactivation of esterase (/hr)  
constant KABLBE=0.0           ! aging of esterase (/hr)

!plasma CaE rate constants

constant KDBLCR=0.0           ! degradation of esterase (/hr)  
constant KIBLCR=0.0           ! inhibition of esterase (per umol/L/hr)  
constant KRBLCR=0.0           ! reactivation of esterase (/hr)  
constant KABLCR=0.0           ! aging of esterase (/hr)

!RBC AChE rate constants

constant KDRBCE=0.0           ! degradation of esterase (/hr)  
constant KIRBCE=0.0           ! inhibition of esterase (per umol/L/hr)  
constant KRRBCE=0.0           ! reactivation of esterase (/hr)  
constant KARBCE=0.0           ! aging of esterase (/hr)

!uptake parameters

!malathion

constant KAS=0.0               !transfer stomach to liver (/h)  
constant KSI=0.0               !transfer stomach to intestine (/h)  
constant KAI=0.0               !transfer intestine to liver (/h)  
constant FA=0.0                !Fractional oral Absorption

!oxon

constant KASO=0.0               !transfer stomach to liver (/h)  
constant KSIO=0.0               !transfer stomach to intestine (/h)  
constant KAIO=0.0               !transfer intestine to liver (/h)  
constant FAO=0.0                !fractional oral Absorption

!elimination rate constants for metabolite compartments (/h)

constant KE1=0.0   !MCA + DCA

!molecular weights

constant MWMAL=0.0 !molecular wt of malathion (g/mol)  
constant MWMOX=0.0 !molecular wt of malaoxon (g/mol)

!oral dosing--single oral dose

constant ORALMAL=0.0   !MAL single oral bolus dose (ug/kg)

constant ORALMOX=0.0    !oxon oral bolus dose (ug/kg)

!oral dosing--multiple doses

!put times in the array DT (in hours) (absolute time, NOT time since last dose)

!Put the oral dose of malathion in the array DORAL (UG/KG)(DORALO FOR OXON)

!for dosing AMOUNT instead of /kg (ug) put the oral dose of malathion (AMTMDOSE) OR OXON (AMTODOSE)

!Add one additional dose of zero at a time greater than TSTOP to keep the model running  
DIMENSION DT(2000), DORAL(2000), DORALO(2000), AMTMDOSE(2000), AMTODOSE(2000)

INTEGER indx

indx=1                            ! indx is a counter used to track multiple doses

SCHEDULE DOSEX .AT. DT(indx)

ODOSE=0.0    !oral dose (MAL; ug/kg BW)

ODOSEO=0.0    !oral dose (oxon; ug/kg BW)

ADOSEM=0.0    !oral dose (MAL; ug)

ADOSEO=0.0    !oral dose (oxon; ug)

! TIMING COMMANDS

constant POINTS=100.0            !OUTPUT POINTS

constant TSTOP=0.0                !STOP SIMULATION (HR)

CINT=TSTOP/POINTS    !Communication interval

VARIABLE time = 0.0    !simulation time in hour

END ! INITIAL

DYNAMIC

ALGORITHM IALG = 2    !Gear algorithm

TERMT(TIME .GE. TSTOP)            !stop execution

PND = time/24+AGE0

DERIVATIVE

AGE = AGE0+(TIME/24)    !age in days

!growth of postnatal rat

BW= BW0+ (-1E-8 \* AGE\*\*4) + (5E-7 \* AGE\*\*3) + (8E-5 \* AGE\*\*2)- (0.0001 \* AGE) + 0.0101

!body weight (kg)

VBrC= 0.02956 + (0.003697 \* AGE) + (-2.989E-4 \* AGE\*\*2)+ (6.84E-6 \* AGE\*\*3) + (-4.99E-8 \* AGE\*\*4)

!brain, % of BW

VLC= 0.04738 + (-0.004162 \* AGE) + (2.796E-4 \* AGE\*\*2)+ (-6.054E-6 \* AGE\*\*3) + (4.187E-8 \* AGE\*\*4)

!liver, % of BW

!scaling for blood flows

QC = QCC\*(BW\*\*0.75)            !cardiac output (L/h)

QSC = 1.0-(QFC+QLC+QBrC+QRC+QKC) !% of blood flow not going to other tissues (i.e., % to slowly perfused)

$QK = QKC * QC$  !kidney (L/h)  
 $QF = QFC * QC$  !fat (L/h)  
 $QL = QLC * QC$  !liver (L/h)  
 $QBr = QBrC * QC$  !brain (L/h)  
 $QR = QRC * QC$  !rapidly perfused tissues (L/h)  
 $QS = QSC * QC$  !slowly perfused tissues (L/h)

$QBal = QC - (QF + QL + QBr + QR + QS + QK)$  !balance check

!scaling for tissue volumes (L)

$VL = VBL * BW$  !blood  
 $VF = VFC * BW$  !fat  
 $VL = VLC * BW$  !liver  
 $VBr = VBrC * BW$  !brain  
 $VK = VKC * BW$  !kidney  
 $VR = VRC * BW$  !rapidly perfused tissues  
 $VS = VSC * BW$  !slowly perfused tissues  
 $VINT = VINTC * BW$  !intestine  
 $VPLAS = VBL * (1 - HCT)$  !plasma  
 $VRBC = VBL * HCT$  !RBC

!Tissue volume checks

$VTOTCOMPARTMENTS = (VLC + VFC + VSC + VRC + VBrC + VBL + VK + VINTC) / 0.9$  !should be ~0.9-1.1

!scaling for Vmax for malathion detoxification (umol/hr/kg tissue to umol/hr)

$VMAXMALL = VMAXMALLC * VL$  !liver--CaE  
 $VMAXMALK = VMAXMALKC * VK$  !kidney--CaE  
 $VMAXMALBL = VMAXMALBLC * VBL * (1 - HCT)$  !plasma--CaE  
 $VMAXMALBR = VMAXMALBRC * VBr$  !brain--CaE

!oxidation--malathion to oxon

$VMAXMALMOX = VMAXMALMOXC * VL$  !liver--CYPs

!oxon detox

$VMOXDMPL = VMOXDMPLC * VL$  !liver oxon detox  
 $VMOXDMPP = VMOXDMPPC * VBL * (1 - HCT)$  !plasma oxon detox  
 $VMOXDMPB = VMOXDMPBC * VBr$  !brain oxon detox  
 $VMOXDMPK = VMOXDMPKC * VK$  !kidney oxon detox

!Enzyme activity (umol/h) in tissues--these are currently scaled to BW

!AChE

$SBACH = BACHE * VBr$  !brain  
 $SHACH = HACHE * VL$  !liver  
 $SBLACH = BLACHE * VBL * (1 - HCT)$  !plasma  
 $SRBACH = RBACHE * VBL * HCT$  !RBC  
 $SKACH = KACHE * VK$  !kidney

!BuChE

$SBBUC = BBUCE * VBr$                       !brain  
 $SHBUC = HBUCE * VL$                               !liver  
 $SBLBUC = BLBUC * VBL * (1 - HCT)$    !plasma  
 $SKBUC = KBUC * VK$                               !kidney

!CaE  
 $SBRCE = BRCE * VBr$                               !brain  
 $SHECE = HECE * VL$                                       !liver  
 $SPLOCE = PLOCE * VBL * (1 - HCT)$    !plasma  
 $SKACE = KECE * VK$                               !kidney

!calculation of esterase binding sites (umol)

!AChE  
 $IBCE = SBACH / TRCE$                               !total brain AchE (umol)  
 $IHCE = SHACH / TRCE$                               !total liver AchE (umol)  
 $IBLCE = SBLACH / TRCE$    !total plasma AchE (umol)  
 $IRBCE = SRBACH / TRCE$    !total RBC AchE (umol)  
 $IKCE = SKACH / TRCE$                               !total kidney AchE (umol)

!BuChE  
 $IBBE = SBBUC / TRBE$                               !total brain BuChE (umol)  
 $IHBE = SHBUC / TRBE$                               !total liver BuChE (umol)  
 $IBLBE = SBLBUC / TRBE$    !total plasma BuChE (umol)  
 $IKBE = SKBUC / TRBE$                               !total kidney BuChE (umol)

!CaE  
 $IBCR = SBRCE / TRCR$                               !total brain CaE (umol)  
 $IHCR = SHECE / TRCR$                               !total liver CaE (umol)  
 $IBLCR = SPLOCE / TRCR$    !total plasma CaE (umol)  
 $IKCR = SKACE / TRCR$                               !total kidney CaE (umol)

!calculation of enzyme synthesis rates in tissues (umol/hr)

!liver  
 $KSHCE = IHCE * KDHCE$    !AChE  
 $KSHBE = IHBE * KDHBE$    !BuChE  
 $KSHCR = IHCR * KDHCR$    !CaE  
 !brain  
 $KSBCE = IBCE * KDBCE$    !AChE  
 $KSBBE = IBBE * KDBBE$    !BuChE  
 $KSBCR = IBCR * KDBCR$    !CaE  
 !kidney  
 $KSKCE = IKCE * KDKCE$    !AChE  
 $KSKBE = IKBE * KDKBE$    !BuChE  
 $KSKCR = IKCR * KDKCR$    !CaE  
 !plasma  
 $KSBLCE = IBLCE * KDBLCE$  !AChE  
 $KSBLBE = IBLBE * KDBLBE$  !BuChE



KSBLCR=IBLCR\*KDBLCR !CaE  
 !RBC  
 KSRBCE=KDRBCE\*IRBCE !AChE

!oral dose molar conversion with fractional absorption--single oral dose  
 ODOSEMAL=ORALMAL\*(BW/MWMAL)\*FA !MAL dose conversion (ug/kg to umol)  
 ODOSEMOX=ORALMOX\*(BW/MWMOX)\*FAO !malaoxon dose conversion (ug/kg to umol)

!oral dose--multiple dose  
 DISCRETE DOSEX  
 ODOSE=ODOSE+DORAL(indx)\*(BW/MWMAL)\*FA !malathion dose ug/kg to umol  
 ODOSEO=ODOSEO+DORALO(indx)\*(BW/MWMOX)\*FAO !oxon dose ug/kg to umol  
 ADOSEO=ADOSEO+AMTODOSE(indx)\*FA/MWMAL !malathion dose ug to umol  
 ADOSEM=ADOSEM+AMTMDOSE(indx)\*FAO/MWMOX !oxon dose ug to umol  
 indx=indx+1  
 SCHEDULE DOSEX .AT. DT(indx)  
 END

!-----  
 !malathion (parent compound)-----  
 !-----

!stomach  
 RSTOM = -kas\*Astom-ksi\*Astom !rate of change in stomach (umol/hr)  
 Astom = integ(RStom,0.0)+ODOSE+ADOSEM+ODOSEMAL !amount in stomach (umol)

!intestine  
 RInst = ksi\*Astom - kai\*AlntM !rate of change in intestine (umol/hr)  
 AlntM=integ(RInst,0.0) !amount in intestine (umol)  
 CINTM = AINTM/VINT !concentration in intestine (umol/L)

!liver  
 RALM=QL\*CBL-QL\*CVLM+kai\*AlntM+kas\*Astom-RALM1-RALM2 !rate of change in liver (umol/hr)  
 ALM = integ(RALM,0.0) !amt in liver (umol)  
 CLM = ALM/VL !concentration in liver (umol/L)  
 CVLM = CLM/PMaLL !concn in venous blood leaving liver (umol/L)

!Liver metabolism of Mal by CYPs--oxidation  
 RALM1 = (VmaxMalMox\*CVLM)/(KmMalMox+CVLM) !rate of metabolite formation (umol/hr)  
 ALM1 = integ(RALM1, 0.0) !amt oxon formed (umol)

!Liver--metabolism of MAL to MCA/DCA by CaE  
 RALM2 = VMAXMALL\*CVLM/(KMMALL\*(1+CVLO/KI)+CVLM) !rate of metabolites formed (umol/hr)  
 ALM2 = integ(RALM2,0.0) !amount of metabolites formed (umol)

!kidney

$RAKM = QK \cdot CBL - QK \cdot CVKM - RAKM2$  !rate of change in kidney (umol/hr)  
 $AKM = \text{integ}(RAKM, 0.0)$  !amt in kidney (umol)  
 $CKM = AKM / VK$  !concn in kidney (umol/L)  
 $CVKM = CKM / PMaIK$  !concn in venous blood leaving kidney (umol/L)

!kidney--metabolism of MAL to MCA/DCA by CaE  
 $RAKM2 = VMAXMALK \cdot CVKM / (KMMALK \cdot (1 + CVKO/KI) + CVKM)$  !rate of metabolites formed (umol/hr)  
 $AKM2 = \text{integ}(RAKM2, 0.0)$  !amount of metabolites formed (umol)

!fat  
 $RAFM = QF \cdot CBL - QF \cdot CVFM$  !rate of change in fat (umol/hr)  
 $AFM = \text{integ}(RAFM, 0.0)$  !amt in fat (umol)  
 $CFM = AFM / VF$  !concn. in fat (umol/L)  
 $CVFM = CFM / PMaIF$  !concn. in venous blood leaving fat (umol/L)

!slowly perfused  
 $RASM = QS \cdot CBL - QS \cdot CVSM$  !rate of change in slowly perfused (umol/hr)  
 $ASMaI = \text{integ}(RASM, 0.0)$  !amt in slowly perfused (umol)  
 $CSM = ASMaI / VS$  !concn. in slowly perfused (umol/L)  
 $CVSM = CSM / PMaIS$  !concn. in venous blood leaving slowly perfused (umol/L)

!brain  
 $RABrM = QBr \cdot CBL - QBr \cdot CVBrM - RABRM2$  !rate of change in brain (umol/hr)  
 $ABrM = \text{integ}(RABrM, 0.0)$  !amt. in brain (umol)  
 $CBrM = ABrM / VBr$  !concn in brain (umol/L)  
 $CVBrM = CBrM / PMaIBr$  !concn. in venous blood leaving brain (umol/L)

!brain--metabolism of MAL to MCA/DCA by CaE  
 $RABRM2 = VMAXMALBR \cdot CVBrM / (KMMALBr \cdot (1 + CVBrO/KI) + CVBrM)$  !rate of metabolites formed (umol/hr)  
 $ABRM2 = \text{integ}(RABRM2, 0.0)$  !amount of metabolites formed (umol)

!rapidly perfused tissues  
 $RARM = QR \cdot CBL - QR \cdot CVRM$  !rate of change in rapidly perfused (umol/hr)  
 $ARM = \text{integ}(RARM, 0.0)$  !amt in rapidly perfused (umol)  
 $CRM = ARM / VR$  !concn in rapidly perfused (umol/L)  
 $CVRM = CRM / PMaIR$  !concn in venous blood leaving rapidly perfused (umol/L)

!blood  
 $RABL = QC \cdot (CV - CBL) - RABLM2$  !rate of change in mixed blood (arterial & venous; umol/hr)  
 $ABL = \text{INTEG}(RABL, 0.0)$  !amount in mixed blood (umol)  
 $CBL = ABL / VBL$  !concn in mixed blood (umol/L)

!blood--metabolism of MAL to MCA/DCA by CaE  
 $RABLM2 = VMAXMALBL \cdot CBL / (KMMALBL \cdot (1 + CBLO/KI) + CBL)$  !rate of metabolism (umol/hr)  
 $ABLM2 = \text{integ}(RABLM2, 0.0)$  !amt metabolite formed (umol)

CV=(QL\*CVLM+QF\*CVFM+QK\*CVKM+QS\*CVSM+QBr\*CVBrM+QR\*CVRM)/QC !concentration free in venous blood (umol/L)

!-----  
!oxon  
!-----

!stomach  
RAStomO = -ksiO\*ASTomO-kasO\*ASTomO !rate of change in stomach (umol/hr)  
ASTomO = integ(RASTomO,0.0)+ODOSEO+ADOSEO+ODOSEMOX !amount in stomach (umol)

!intestine  
RAIntO = -kaiO\*AINTO+ksiO\*ASTomO !rate of change in intestine (umol/hr)  
AintO = integ(RAIntO,0.0) !amount in intestine (umol)  
CINTO = AINTO/VINTC !conc in intestine (umol/L)

!liver  
RALO = QL\*CBLO-QL\*CVLO+kaiO\*AINTO+kasO\*ASTomO+RALM1-(RHPCE+RHPBE+RHPCR)-RALOD  
!rate of change in liver (umol/hr)  
ALO = integ(RALO,0.0) !amt in liver (umol)  
CLO = ALO/VL !concentration in liver (umol/L)  
CVLO = CLO/PMOXL !conc in venous blood leaving liver (umol/L)

!oxon detox  
RALOD = (VMOXDMPL\*CVLO)/(KMOXDMPL\*(1+CVLO/KI)+CVLO)  
AALOD = integ(RALOD,0.0)

!AChE activity--liver  
RHCE=KSHCE-AHCE\*(KDHCE+KIHCE\*CLO)+HOCE\*KRHCE ! rate of AChE activity (umol/hr)  
AHCE=INTEG(RHCE,IHCE) ! liver AChE activity (umol)  
RNHCE=KSHCE-ANHCE\*KDHCE ! rate of baseline AChE act (umol/hr)  
ANHCE=INTEG(RNHCE,IHCE) ! Amt of baseline AChE (umol)  
RHOCE=AHCE\*KIHCE\*CLO-HOCE\*(KAHCE+KRHCE) ! rate of AChE inhibition (umol/hr)  
HOCE=INTEG(RHOCE,0.0) ! amount of liver AChE inhibited (umol)  
RHPCE=AHCE\*KIHCE\*CLO ! rate of oxon consumption (umol/hr)  
AHPCE=integ(RHPCE,0.0) ! amt of oxon consumed (umol)

!BuChE activity--liver  
RHBE=KSHBE-AHBE\*(KDHBE+KIHBE\*CLO)+HOBE\*KRHBE ! rate of BuChE activity (umol/hr)  
AHBE=INTEG(RHBE,IHBE) ! liver BuChE activity (umol)  
RNHBE=KSHBE-ANHBE\*KDHBE ! rate of baseline BuChE act (umol/hr)  
ANHBE=INTEG(RNHBE,IHBE) ! Amt of baseline BuChE (umol)

$RHOBE = AHBE * KIHBE * CLO - HOBE * (KAHBE + KRHBE)$  ! rate of BuChE inhibition  
 (umol/hr)  
 $HOBE = INTEG(RHOBE, 0.0)$  ! amount of liver BuChE inhibited  
 (umol)  
 $RHPBE = AHBE * KIHBE * CLO$  ! rate of oxon consumption  
 (umol/hr)  
 $AHPBE = integ(RHPBE, 0.0)$  ! amt of oxon consumed (umol)

!CaE activity--liver  
 $RHCR = KSHCR - AHCR * (KDHCR + KIHCR * CLO) + HOOCR * KRHCR$  ! rate of CaE activity (umol/hr)  
 $AHCR = INTEG(RHCR, IHCR)$  ! liver CaE activity (umol)  
 $RNHCR = KSHCR - ANHCR * KDHCR$  ! rate of baseline CaE act (umol/hr)  
 $ANHCR = INTEG(RNHCR, IHCR)$  ! Amt of baseline CaE (umol)  
 $RHOOCR = AHCR * KIHCR * CLO - HOOCR * (KAHCR + KRHCR)$  ! rate of CaE inhibition (umol/hr)  
 $HOOCR = INTEG(RHOOCR, 0.0)$  ! amount of liver CaE inhibited  
 (umol)  
 $RHPCR = AHCR * KIHCR * CLO$  ! rate of oxon consumption  
 (umol/hr)  
 $AHPCR = integ(RHPCR, 0.0)$  ! amt of oxon consumed (umol)

!liver B-esterase (AChE, BuChE, and CaE) activity  
 $HBE = AHCE + AHBE + AHCR$  ! total B-esterase = AChE + BuChE + CaE  
 (umol)  
 $IHE = 100 * (HBE / (ANHCE + ANHBE + ANHCR))$  ! % total B-esterase inhibition  
 $HCE = 100 * (AHCE / ANHCE)$  ! % AChE inhibition  
 $HBES = 100 * (AHBE / ANHBE)$  ! % BuChE inhibition  
 $HCR = 100 * (AHCR / ANHCR)$  ! % CaE inhibition

!fat  
 $RAFO = QF * CBLO - QF * CVFO$  !rate of change in fat (umol/hr)  
 $AFO = integ(RAFO, 0.0)$  !amt in fat (umol)  
 $CFO = AFO / VF$  !concn. in fat (umol/L)  
 $CVFO = CFO / PMOXF$  !concn. in venous blood leaving fat (umol/L)

!slowly perfused  
 $RASO = QS * CBLO - QS * CVSO$  !rate of change in slowly perfused (umol/hr)  
 $ASO = integ(RASO, 0.0)$  !amt in slowly perfused (umol)  
 $CSO = ASO / VS$  !concn. in slowly perfused (umol/L)  
 $CVSO = CSO / PMOXS$  !concn. in venous blood leaving slowly perfused (umol/L)

!rapidly perfused tissues  
 $RARO = QR * CBLO - QR * CVRO$  !rate of change in rapidly perfused (umol/hr)  
 $ARO = integ(RARO, 0.0)$  !amt in rapidly perfused (umol)  
 $CRO = ARO / VR$  !concn in rapidly perfused (umol/L)  
 $CVRO = CRO / PMOXR$  !concn in venous blood leaving rapidly perfused (umol/L)

!brain  
 $RABrO = QBr * CBLO - QBr * CVBrO - (RBPCE + RBPBE + RBPCR) - RABROD$  !rate of change in brain (umol/hr)

ABrO = integ(RABrO,0.0) !amt. in brain (umol)  
 CBrO = ABrO/VBr !concn in brain (umol/L)  
 CVBrO = CBrO/PMOXBBr !concn. in venous blood leaving brain (umol/L)

!oxon detox  
 RABROD = (VMOXDMPB\*CVBrO)/(KMOXDMPB\*(1+CVBrO/KI)+CVBrO)  
 AABRO = integ(RABROD,0.0)

!AChE activity--brain  
 RBCE=KSBCE-ABCE\*(KDBCE+KIBCE\*CBrO)+BOCE\*KRBCE ! rate of AChE activity (umol/hr)  
 ABCE=INTEG(RBCE,IBCE) ! brain AChE activity (umol)  
 RNBCE=KSBCE-ANBCE\*KDBCE ! rate of baseline AChE act  
 (umol/hr)  
 ANBCE=INTEG(RNBCE,IBCE) ! Amt of baseline AChE (umol)  
 RBOCE=ABCE\*KIBCE\*CBrO-BOCE\*(KABCE+KRBCE) ! rate of AChE inhibition  
 (umol/hr)  
 BOCE=INTEG(RBOCE,0.0) ! amount of brain AChE inhibited  
 (umol)  
 RBPCE=ABCE\*KIBCE\*CBrO ! rate of oxon consumption  
 (umol/hr)  
 ABPCE=integ(RBPCE,0.0) ! amt of oxon consumed (umol)

!BuChE activity--brain  
 RBBE=KSBBE-ABBE\*(KDBBE+KIBBE\*CBrO)+BOBE\*KRBBE ! rate of BuChE activity (umol/hr)  
 ABBE=INTEG(RBBE,IBBE) ! brain BuChE activity (umol)  
 RNBBE=KSBBE-ANBBE\*KDBBE ! rate of baseline BuChE act  
 (umol/hr)  
 ANBBE=INTEG(RNBBE,IBBE) ! Amt of baseline BuChE (umol)  
 RBOBE=ABBE\*KIBBE\*CBrO-BOBE\*(KABBE+KRBBE) ! rate of BuChE inhibition  
 (umol/hr)  
 BOBE=INTEG(RBOBE,0.0) ! amount of brain BuChE  
 inhibited (umol)  
 RBPBE=ABBE\*KIBBE\*CBrO ! rate of oxon consumption  
 (umol/hr)  
 ABPBE=integ(RBPBE,0.0) ! amt of oxon consumed (umol)

!CaE activity--brain  
 RBCR=KSBCE-ABCE\*(KDBCE+KIBCE\*CBrO)+BOCE\*KRBCE ! rate of CaE activity (umol/hr)  
 ABCR=INTEG(RBCR,IBCR) ! brain CaE activity (umol)  
 RNBCR=KSBCE-ANBCE\*KDBCE ! rate of baseline CaE act  
 (umol/hr)  
 ANBCR=INTEG(RNBCR,IBCR) ! Amt of baseline CaE (umol)  
 RBOCR=ABCR\*KIBCE\*CBrO-BOCE\*(KABCE+KRBCE) ! rate of CaE inhibition (umol/hr)  
 BOCR=INTEG(RBOCR,0.0) ! amount of brain CaE inhibited  
 (umol)  
 RBPCR=ABCR\*KIBCE\*CBrO ! rate of oxon consumption  
 (umol/hr)

ABPCR=integ(RBPCR,0.0) ! amt of oxon consumed (umol)

!brain B-esterase (AChE, BuChE, and CaE) activity  
 BBE=ABCE+ABBE+ABCR ! total B-esterase=AChE+BuChE+CaE (umol)  
 IBE=100\*(BBE/(ANBCE+ANBBE+ANBCR)) ! total B-esterase inhibition  
 BCE=100\*(ABCE/ANBCE) ! % AChE inhibition  
 BBES=100\*(ABBE/ANBBE) ! % BuChE inhibition  
 BCR=100\*(ABCR/ANBCR) ! % CaE inhibition

!kidney  
 RAKO = QK\*CBLO-QK\*CVKO-(RKPCE+RKPBE+RKPCE)-RAKOD !rate of change in kidney (umol/hr)  
 AKO = integ(RAKO,0.0) !amt in kidney (umol)  
 CKO = AKO/VK !concn in kidney (umol/L)  
 CVKO = CKO/PMOXX !concn in venous blood leaving kidney (umol/L)

!oxon detox  
 RAKOD = (VMOXDMPK\*CVKO)/(KMOXDMPK\*(1+CVKO/KI)+CVKO)  
 AAKOD = integ(RAKOD,0.0)

! AChE activity--kidney  
 RKCE=KSKCE-AKCE\*(KDKCE+KIKCE\*CKO)+KOCE\*KRKCE ! rate of AChE activity (umol/hr)  
 AKCE=INTEG(RKCE,IKCE) ! kidney AChE activity (umol)  
 RNKCE=KSKCE-ANKCE\*KDKCE ! rate of baseline AChE act  
 (umol/hr)  
 ANKCE=INTEG(RNKCE,IKCE) ! Amt of baseline AChE (umol)  
 RKOCE=AKCE\*KIKCE\*CKO-KOCE\*(KAKCE+KRKCE) ! rate of AChE inhibition  
 (umol/hr)  
 KOCE=INTEG(RKOCE,0.0) ! amount of kidney AChE  
 inhibited (umol)  
 RKPCE=AKCE\*KIKCE\*CKO ! rate of oxon consumption  
 (umol/hr)  
 AKPCE=integ(RKPCE,0.0) ! amt of oxon consumed (umol)

! BuChE activity--kidney  
 RKBE=KSKBE-AKBE\*(KDKBE+KIKBE\*CKO)+KOBCE\*KRKBE ! rate of BuChE activity (umol/hr)  
 AKBE=INTEG(RKBE,IKBE) ! kidney BuChE activity (umol)  
 RNKBE=KSKBE-ANKBE\*KDKBE ! rate of baseline BuChE act  
 (umol/hr)  
 ANKBE=INTEG(RNKBE,IKBE) ! Amt of baseline BuChE (umol)  
 RKOBCE=AKBE\*KIKBE\*CKO-KOBCE\*(KAKBE+KRKBE) ! rate of BuChE inhibition  
 (umol/hr)  
 KOBCE=INTEG(RKOBCE,0.0) ! amount of kidney BuChE  
 inhibited (umol)  
 RKPBE=AKBE\*KIKBE\*CKO ! rate of oxon consumption  
 (umol/hr)  
 AKPBE=integ(RKPBE,0.0) ! amt of oxon consumed (umol)

! CaE activity--kidney  
 $RKCR = KSKCR - AKCR * (KDKCR + KIKCR * CKO) + KOCCR * KRKCR$  ! rate of CaE activity (umol/hr)  
 $AKCR = INTEG(RKCR, IKCR)$  ! kidney CaE activity (umol)  
 $RNKCR = KSKCR - ANKCR * KDKCR$  ! rate of baseline CaE act  
(umol/hr)  
 $ANKCR = INTEG(RNKCR, IKCR)$  ! Amt of baseline CaE (umol)  
 $RKOCCR = AKCR * KIKCR * CKO - KOCCR * (KAKCR + KRKCR)$  ! rate of CaE inhibition  
(umol/hr)  
 $KOCCR = INTEG(RKOCCR, 0.0)$  ! amount of kidney CaE  
inhibited (umol)  
 $RKPCR = AKCR * KIKCR * CKO$  ! rate of oxon consumption  
(umol/hr)  
 $AKPCR = integ(RKPCR, 0.0)$  ! amt of oxon consumed (umol)

!kidney B-esterase (AChE, BuChE, and CaE) activity  
 $KBE = AKCE + AKBE + AKCR$  ! total B-esterase = AChE + BuChE + CaE (umol)  
 $IKE = 100 * (KBE / (ANKCE + ANKBE + ANKCR))$  ! % total B-esterase inhibition  
 $KCE = 100 * (AKCE / ANKCE)$  ! % AChE inhibition  
 $KBES = 100 * (AKBE / ANKBE)$  ! % BuChE inhibition  
 $KCR = 100 * (AKCR / ANKCR)$  ! % CaE inhibition

!blood  
 $RABLO = QC * (CVO - CBLO) - (RBLPBE + RBLPCR + RBLPCE + RRBPC) - RAPLD$  !rate of change in blood (umol/hr)  
 $ABLO = integ(RABLO, 0.0)$  !amount in blood (umol)  
 $CBLO = ABLO / VBL$  !concn in blood (umol/L)

$CVO = (QL * CVLO + QF * CVFO + QS * CVSO + QR * CVRO + QBr * CVBrO + QK * CVKO) / QC$  !concn in venous blood (umol/L)

!oxon detox--catalytic  
 $RAPLD = (VMOXDMPP * CBLO) / (KMOXDMPP * (1 + CBLO / KI) + CBLO)$   
 $AAPLD = integ(RAPLD, 0.0)$

! AChE activity--plasma  
 $RBLCE = KSBLC - ABLCE * (KDBLCE + KIBLCE * CBLO) + BLOCE * KRBLCE$  ! rate of AChE activity (umol/hr)  
 $ABLCE = INTEG(RBLCE, IBLCE)$  ! plasma AChE activity (umol)  
 $RNBLCE = KSBLC - ANBLCE * KDBLCE$  ! rate of baseline AChE act  
(umol/hr)  
 $ANBLCE = INTEG(RNBLCE, IBLCE)$  ! Amt of baseline AChE (umol)  
 $RBLOCE = ABLCE * KIBLCE * CBLO - BLOCE * (KABLCE + KRBLCE)$  ! rate of AChE inhibition (umol/hr)  
 $BLOCE = INTEG(RBLOCE, 0.0)$  ! amount of plasma AChE  
inhibited (umol)  
 $RBLPCE = ABLCE * KIBLCE * CBLO$  ! rate of oxon consumption  
(umol/hr)  
 $ABLPCE = integ(RBLPCE, 0.0)$  ! amt of oxon consumed (umol)

! BuChE activity--plasma

$RBLBE = KSBLBE - ABLBE * (KDBLBE + KIBLBE * CBLO) + BLOBE * KRBLBE$  ! rate of BuChE activity (umol/hr)  
 $ABLBE = INTEG(RBLBE, IBLBE)$  ! plasma BuChE activity (umol)  
 $RNBLBE = KSBLBE - ANBLBE * KDBLBE$  ! rate of baseline BuChE act (umol/hr)  
 $ANBLBE = INTEG(RNBLBE, IBLBE)$  ! Amt of baseline BuChE (umol)  
 $RBLOBE = ABLBE * KIBLBE * CBLO - BLOBE * (KABLBE + KRBLBE)$  ! rate of BuChE inhibition (umol/hr)  
 $BLOBE = INTEG(RBLOBE, 0.0)$  ! amount of plasma BuChE inhibited (umol)  
 $RBLPBE = ABLBE * KIBLBE * CBLO$  ! rate of oxon consumption (umol/hr)  
 $ABLPBE = integ(RBLPBE, 0.0)$  ! amt of oxon consumed (umol)

! CaE activity--plasma  
 $RBLCR = KSBLCR - ABLCR * (KDBLCR + KIBLCR * CBLO) + BLOCRCR * KRBLCR$  ! rate of CaE activity (umol/hr)  
 $ABLCR = INTEG(RBLCR, IBLCR)$  ! plasma CaE activity (umol)  
 $RNBLCR = KSBLCR - ANBLCR * KDBLCR$  ! rate of baseline CaE act (umol/hr)  
 $ANBLCR = INTEG(RNBLCR, IBLCR)$  ! Amt of baseline CaE (umol)  
 $RBLOCRCR = ABLCR * KIBLCR * CBLO - BLOCRCR * (KABLCR + KRBLCR)$  ! rate of CaE inhibition (umol/hr)  
 $BLOCRCR = INTEG(RBLOCRCR, 0.0)$  ! amount of plasma CaE inhibited (umol)  
 $RBLPCR = ABLCR * KIBLCR * CBLO$  ! rate of oxon consumption (umol/hr)  
 $ABLPCR = integ(RBLPCR, 0.0)$  ! amt of oxon consumed (umol)

!plasma B-esterase (AChE, BuChE, and CaE) activity  
 $BLBE = ABLCE + ABLBE + ABLCR$  !total B-esterase=AChE+BuChE+CaE (umol)  
 $IBLE = 100 * (BLBE / (ANBLCE + ANBLBE + ANBLCR))$  ! % Total B-esterase inhibition in plasma  
 $BLCE = 100 * (ABLCE / ANBLCE)$  ! % AChE inhibition in plasma  
 $BLBES = 100 * (ABLBE / ANBLBE)$  ! % BuChE inhibition in plasma  
 $BLCR = 100 * (ABLCR / ANBLCR)$  ! % CaE inhibition in plasma

! AChE activity--RBCs  
 $RRBCCE = KSRBCE - ARBCE * (KDRBCE + KIRBCE * CBLO) + RBCOCE * KRRBCE$  ! rate of AChE activity (umol/hr)  
 $ARBCE = INTEG(RRBCCE, IRBCE)$  ! RBC AChE activity (umol)  
 $RNRBCCE = KSRBCE - ANRBCE * KDRBCE$  ! rate of baseline AChE act (umol/hr)  
 $ANRBCE = INTEG(RNRBCCE, IRBCE)$  ! Amt of baseline AChE (umol)  
 $RRBOCE = ARBCE * KIRBCE * CBLO - RBCOCE * (KARBCE + KRRBCE)$  ! rate of AChE inhibition (umol/hr)  
 $RBCOCE = INTEG(RRBOCE, 0.0)$  ! amount of RBC AChE inhibited (umol)



```

RRBPCE=ARBCE*KIRBCE*CBLO                                ! rate of oxon consumption
(umol/hr)
ARBPCCE=integ(RRBPCE,0.0)                                ! amt of oxon consumed (umol)

!RBC B-esterase (AChE only)activity
RBCCE=100*(ARBCE/ANRBCE)                                ! % AChE inhibition in RBCs
RBCPERC=100-RBCCE                                        ! % inhibited
versus inhibition

!-----
!metabolite excretion
!MCA + DCA metabolites--compartment 1
RAMCA = RAKM2+RABRM2+RABLM2+RALM2 !          !total rate of metabolite formation (umol/hr)
AMCA = integ(RAMCA, 0.0)                !amount of MCA+DCA (umol)
RA1 = RAMCA-RAMCAex                    !rate of change of MCA+DCA in compartment 1 (umol/hr)
A1 = integ(RA1,0.0)                    !amount of MCA+DCA in compartment 1 (umol)
CBMCA = A1/V1                          !concentration of MCA+DCA in compartment 1 (umol/L)
RAMCAex = A1*KE1                       !rate of MCA+DCA excretion (umol/hr)
AMCAex = integ(RAMCAex,0.0)            !amount of MCA+DCA excreted (umol)

end !dynamic

!-----
!mass balance
TMASSMAL=ASTOM+AINTM+ALM+AKM+AFM+ASMaI+ABRM+ARM+ABL      !umol
TMASSMOX=ASTOMO+AINTO+ALO+AFO+ASO+AKO+ARO+ABRO+ABLO      !umol
TMASSMCA=A1+AMCAEX                                         !umol
TMASSODT=AAPLD+AALOD+AAKOD+AABRO                         !catalytic oxon detox (umol)
TMASSOXONNC=AHPCE+AHPBE+AHPCE+ABPCE+ABPBE+ABPCR+AKPCE+AKPBE+AKPCR+ABLPCE+ABLPB
E+ABLPCE+ARBPCCE !non-catalytic oxon detox (umol)
TMASSTOTAL=TMASSMAL+TMASSMOX+TMASSMCA+TMASSODT+TMASSOXONNC !umol--total
better add up to what went in

TDOSE=ODOSE+ODOSEMAL+ADOSEM+ADOSEO+ODOSEO+ODOSEMOX !total dose (umol)
MASSBAL=TDOSE/(TMASSTOTAL+1E-99) !should equal 1 (or really close to it)

end !derivative
end !program

```

## Appendix C – Human Model Code

---

### Human model code

PROGRAM malathion\_lifestage

!Abbreviations: --remember to put the variability terms back in

!MAL--malathion  
!MOX--malaoxon  
!DMP--dimethyl phosphate metabolites  
!MCA--malathion monocarboxylic acid  
!DCA--malathion dicarboxylic acid  
!AChE--acetylcholinesterase  
!BuChE--butyrylcholinesterase  
!CaE--carboxylesterase

!skin compartment/dermal exposure for malathion only  
!lung compartment/inhalation exposure for malathion only

!compartments included for liver, kidney, brain, fat, and lumped for slow and rapidly perfused.  
!exposure routes: oral, dermal, inhalation (malathion); oral (oxon)  
!bioactivation (MAL -> MOX) occurs in liver  
!inhibition of B-esterases (AChE, BuChE, CaE) described in liver, kidney, brain, plasma, RBCs.

!detoxification of MAL by CaE occurs in liver, kidney, brain, plasma, RBCs (to MCA/DCA; these are modeled as a single compartment).

!detoxification of oxon by B-esterases is stoichiometric; the oxon-enzyme complex serves as a "trap" for the oxon.

!CaE assumed to undergo reactivation; AChE and BuChE assumed to undergo reactivation/aging.

!catalytic detoxification of oxon in liver, brain, kidney (plasma for rat only)

INITIAL

!constants should be in human parameters m file

VARIABLE TIME=0.0 !hr

CONSTANT AGE0=0.0 !Initial age (D for rats; Y for humans)

CONSTANT SPECIES=0 !Species dummy variable: 0=male rat, 1=male human

CONSTANT BWSW=0.0 !Switch for age (set =0) or set (set =1) determination of BW

CONSTANT BWST=0.0 !Set value of BW (kg)

! Gompertz equation parameters

CONSTANT B1=0.0

CONSTANT B2=0.0

```

CONSTANT B3=0.0
CONSTANT B4=0.0
CONSTANT B5=0.0
CONSTANT B6=0.0
CONSTANT B7=0.0
CONSTANT B8=0.0
CONSTANT AGE1=0.0
CONSTANT AGE2=0.0
CONSTANT AGE3=0.0
CONSTANT SEX=0

! Age dependent B4 & B8 for humans (Luecke 2007)
IF ((AGE0 .GE. AGE1) .AND. (AGE0 .LT. AGE2) .AND. (SPECIES .EQ. 1)) THEN
  B4x=B4;B8x=0
ELSE IF ((AGE0 .GE. AGE3) .AND. (SPECIES .EQ. 1)) THEN
  B4x=0;B8x=B8
ELSE
  B4x=0;B8x=0
END IF

! Initial BWT (kg) (Luecke 2007)
IF (AGE0 .GE. AGE2) THEN
  BWT=B5*EXP((B6/B7)*(1-EXP(-B7*(AGE0-AGE2))))+B8x*(AGE0-AGE3)
ELSE
  BWT=B1*EXP((B2/B3)*(1-EXP(-B3*AGE0)))+B4x*(AGE0-AGE1)
END IF
BWT=BWT*(1.-BWSW)+BWST*BWSW
! BWT kg to grams for compartment volume calculations
BWTG=BWT*1000

! Log of BWT for rat brain calculation
IF (SPECIES .EQ. 0) THEN
  LBWT=LOG(BWT)
ELSE
  LBWT=BWT*1000
END IF

! Initial Height (HT: fit to CDC growth chart      (human))
HT=19.994+(5.5408*BWT)-(0.0671*BWT**2)+0.0003*BWT**3

! Initial compartment volumes (frac of BWT)(developed by Luecke 2007 & Young 2009; Described in
Smith et al., 2013)
!Brain
VBrC=VB0+(VB1*LBWT)+(VB2*LBWT**2)+(VB3*LBWT**3)+(VB4*LBWT**4)+(VB5*LBWT**5)+(VB6*LB
WT**6)
!Liver
VHC=VH0+(VH1*BWTG)+(VH2*BWTG**2)+(VH3*BWTG**3)+(VH4*BWTG**4)

```

!Blood  
 $V_{BLC} = V_{BL0} + (V_{BL1} * BWTG) + (V_{BL2} * BWTG^{**2})$   
 !Fat  
 $V_{FC} = V_{F0} + (V_{F1} * BWTG) + (V_{F2} * BWTG^{**2}) + (V_{F3} * BWTG^{**3}) + (V_{F4} * BWTG^{**4}) + (V_{F5} * BWTG^{**5}) + (V_{F6} * BWTG^{**6})$   
 !Adipose  
 $V_{AC} = V_{A0} + (V_{A1} * BWTG) + (V_{A2} * BWTG^{**2}) + (V_{A3} * BWTG^{**3}) + (V_{A4} * BWTG^{**4}) + (V_{A5} * BWTG^{**5}) + (V_{A6} * BWTG^{**6})$   
 !Kidney (in rapidly perfused tissues)  
 $V_{KC} = V_{K0} + (V_{K1} * BWTG) + (V_{K2} * BWTG^{**2})$   
 !Spleen (in rapidly perfused tissues)  
 $V_{SPC} = V_{SP0} + (V_{SP1} * BWTG) + (V_{SP2} * BWTG^{**2})$   
 !Lung (in rapidly perfused tissues)  
 $V_{LC} = V_{L0} + (V_{L1} * BWTG) + (V_{L2} * BWTG^{**2})$   
 !GI, both stomach and intestine (in rapidly perfused tissues)  
 $V_{GIC} = V_{GI0} + (V_{GI1} * BWTG) + (V_{GI2} * BWTG^{**2}) + (V_{GI3} * BWTG^{**3})$   
 !Muscle (in slowly perfused tissues)  
 $V_{MC} = V_{M0} + (V_{M1} * BWTG) + (V_{M2} * BWTG^{**2}) + (V_{M3} * BWTG^{**3}) + (V_{M4} * BWTG^{**4})$   
 !Skin  
 $V_{SKC} = V_{SK0} + (V_{SK1} * BWTG) + (V_{SK2} * BWTG^{**2}) + (V_{SK3} * BWTG^{**3}) + (V_{SK4} * BWTG^{**4}) + (V_{SK5} * BWTG^{**5})$

CONSTANT FATVAR = 0.0  
 CONSTANT VOLUNCERT = 0.0  
 CONSTANT VHCv=0.0  
 !CONSTANT VBLC=0.0  
 $V_{FCv} = GAUSS(0., FATVAR * V_{FC})$   
 $V_{FCv} = RSW(V_{FC} + V_{FCv} .LT. 0.1 * V_{FC}, 0.1 * V_{FC}, V_{FCv})$  ! check to ensure that vol is > 0  
 $V_{FC} = V_{FC} + V_{FCv}$   
 CONSTANT VBMC=0.0 ! Bone marrow (in slow)  
 $V_{RC} = V_{KC} + V_{SPC} + V_{LC} + V_{GIC} + V_{PC}$  ! Rapid  
 $V_{SC} = V_{MC} + V_{SKC} + VBMC + (V_{AC} - V_{FC})$  ! Slow  
 !CONSTANT VDC=0.0 ! Diaphragm  
 CONSTANT VPC=0.0 ! Pancreas (in rapid)  
 !CONSTANT VINTC=0.0 ! Small intestine

! Volume variability, set VOLUNCERT=0 to turn off  
 $V_{SCu} = GAUSS(0., VOLUNCERT * V_{SC})$   
 $V_{RCu} = GAUSS(0., VOLUNCERT * V_{RC})$   
 $V_{BCu} = GAUSS(0., VOLUNCERT * V_{SC})$   
 $V_{HCU} = GAUSS(0., VOLUNCERT * V_{HC})$  !This can be used if total uncertainty is desired, or the next line to vary just hepatic volume based on P3M  
 $V_{HCU} = GAUSS(0., V_{HCv} * V_{HC})$   
 $V_{FCu} = GAUSS(0., VOLUNCERT * V_{FC})$   
 $V_{BLCu} = GAUSS(0., VOLUNCERT * V_{BLC})$   
 $V_{BLCu} = GAUSS(0., V_{BLCv} * V_{BLC})$   
 $V_{DCu} = GAUSS(0., VOLUNCERT * V_{DC})$   
 $V_{INTCu} = GAUSS(0., VOLUNCERT * V_{INTC})$

$VSCu = RSW(VSC + VSCu \cdot LT. 0.1 \cdot VSC, 0.1 \cdot VSC, VSCu)$  ! check to ensure that vol is > 0  
 $VRCu = RSW(VRC + VRCu \cdot LT. 0.1 \cdot VRC, 0.1 \cdot VRC, VRCu)$  ! check to ensure that vol is > 0  
 $VBCu = RSW(VBC + VBCu \cdot LT. 0.1 \cdot VBC, 0.1 \cdot VBC, VBCu)$  ! check to ensure that vol is > 0  
 $VHCu = RSW(VHC + VHCu \cdot LT. 0.1 \cdot VHC, 0.1 \cdot VHC, VHCu)$  ! check to ensure that vol is > 0  
 $VFCu = RSW(VFC + VFCu \cdot LT. 0.1 \cdot VFC, 0.1 \cdot VFC, VFCu)$  ! check to ensure that vol is > 0  
 $VBLCu = RSW(VBLC + VBLCu \cdot LT. 0.1 \cdot VBLC, 0.1 \cdot VBLC, VBLCu)$  ! check to ensure that vol is > 0  
 $!VDCu = RSW(VDC + VDCu \cdot LT. 0.1 \cdot VDC, 0.1 \cdot VDC, VDCu)$  ! check to ensure that vol is > 0  
 $VINTCu = RSW(VINTC + VINTCu \cdot LT. 0.1 \cdot VINTC, 0.1 \cdot VINTC, VINTCu)$  ! check to ensure that vol is > 0

$VSC = VSC + VSCu$   
 $VRC = VRC + VRCu$   
 $VBC = VBC + VBCu$   
 $VHC = VHC + VHCu$   
 $VFC = VFC + VFCu$   
 $VBLC = VBLC + VBLCu$   
 $!VDC = VDC + VDCu$   
 $VINTC = VINTC + VINTCu$

! Compartment volume polynomial parameters

$CONSTANT VB0=0.0; CONSTANT VB1=0.0; CONSTANT VB2=0.0; CONSTANT VB3=0.0; CONSTANT VB4=0.0$   
 $CONSTANT VB5=0.0; CONSTANT VB6=0.0$   
 $CONSTANT VH0=0.0; CONSTANT VH1=0.0; CONSTANT VH2=0.0; CONSTANT VH3=0.0; CONSTANT VH4=0.0$   
 $CONSTANT VBL0=0.0; CONSTANT VBL1=0.0; CONSTANT VBL2=0.0$   
 $CONSTANT VF0=0.0; CONSTANT VF1=0.0; CONSTANT VF2=0.0; CONSTANT VF3=0.0; CONSTANT VF4=0.0$   
 $CONSTANT VF5=0.0; CONSTANT VF6=0.0$   
 $CONSTANT VA0=0.0; CONSTANT VA1=0.0; CONSTANT VA2=0.0; CONSTANT VA3=0.0; CONSTANT VA4=0.0$   
 $CONSTANT VA5=0.0; CONSTANT VA6=0.0$   
 $CONSTANT VK0=0.0; CONSTANT VK1=0.0; CONSTANT VK2=0.0$   
 $CONSTANT VSP0=0.0; CONSTANT VSP1=0.0; CONSTANT VSP2=0.0$   
 $CONSTANT VL0=0.0; CONSTANT VL1=0.0; CONSTANT VL2=0.0$   
 $CONSTANT VGI0=0.0; CONSTANT VGI1=0.0; CONSTANT VGI2=0.0; CONSTANT VGI3=0.0$   
 $CONSTANT VM0=0.0; CONSTANT VM1=0.0; CONSTANT VM2=0.0; CONSTANT VM3=0.0; CONSTANT VM4=0.0$   
 $CONSTANT VSK0=0.0; CONSTANT VSK1=0.0; CONSTANT VSK2=0.0; CONSTANT VSK3=0.0;$   
 $CONSTANT VSK4=0.0; CONSTANT VSK5=0.0$

! Compartment volumes (L)

$VBr = VBrC \cdot BWT$  !brain  
 $VH = VHC \cdot BWT$  !liver  
 $VBL = VBLC \cdot BWT$  !blood  
 $VAB = 0.46 \cdot VBL$  !arterial blood  
 $VF = VFC \cdot BWT$  !fat  
 $VR = VRC \cdot BWT$  !rapidly perfused  
 $VS = VSC \cdot BWT$  !slowly perfused  
 $VINT = VINTC \cdot BWT$  !intestine  
 $VL = VL \cdot BWT$  !lung  
 $VPLAS = VBL \cdot (1 - HCT)$  !plasma

VRBC=VBL\*HCT !RBC  
VK=VKC\*BWT !kidney

! Blood flow to each compartment (L/h/kg)

CONSTANT QFC=0.0 !fat  
CONSTANT QHC=0.0 !liver  
CONSTANT QSC=0.0 !slowly perfused  
CONSTANT QBrC=0.0 !brain  
constant QKC=0.0 !kidney  
CONSTANT QRC=0.0 !rapidly perfused

!Variability to activity level

CONSTANT WORK= 0.0 !Work in Watts

DVO2=WORK\*10.1/1000.;

! Blood Flow adjustments

QFCv = 0.017\*DVO2\*60.

!QHCv = -0.19\*DVO2\*60. !FOR WORK, OR FOR VARIABILITY COMMENT OUT and use following line

QHCv=GAUSS(0.,QHCv\*QHC)

QSCv = 7.38\*DVO2\*60.

QRCv = -0.08\*DVO2\*60.

!Partition coefficients (tissue:blood); from 2009 USEPA malathion lice assessment

!malathion

constant PMaIF = 0.0 !fat:blood

constant PMaIR = 0.0 !richly perfused:blood

constant PMaIL = 0.0 !liver:blood

constant PMaIK = 0.0 !kidney:blood

constant PMaIBr = 0.0 !brain:blood

constant PMaIS = 0.0 !slowly perfused:blood

constant PSKL = 0.0 !skin:blood

constant PLAir = 0.0 !tissue:air PC

!malaoxon

constant PMoXF = 0.0 !fat:blood

constant PMoXR = 0.0 !richly perfused:blood

constant PMoXL = 0.0 !liver:blood

constant PMoXK = 0.0 !kidney:blood

constant PMoXBr = 0.0 !brain:blood

constant PMoXS = 0.0 !slowly perfused: blood

!volumes of distribution for metabolite compartments (L)

constant V1 = 0.0 !MCA + DCA

!constant HCT=0.0 !hematocrit

!metabolism parameters

!maximum rates of metabolism (umol/hr/kg tissue)

!malathion detoxification

constant VMAXMALLC = 0.0 !liver--CaE; based on in vitro data (Chambers & Meek 2017)

constant VMAXMALKC = 0.0 !kidney--CaE; 60% of liver based on Talcott 1979

constant VMAXMALBLC = 0.0 !plasma--CaE(umol/hr/L); 74% of liver based on Talcott 1979

constant VMAXMALBRC = 0.0 !brain--CaE; 5% of liver

!malathion oxidation--malathion to oxon

constant VMAXMALMOXC = 0.0 !liver--CYPs; based on in vitro data (Chambers & Meek 2017)

!oxon detox

constant VMOXDMPLC = 0.0 !liver

constant VMOXDMPPC = 0.0 !plasma

constant VMOXDMPBC = 0.0 !brain

constant VMOXDMPKC = 0.0 !kidney

!affinity constants (umol/L)

!malathion detoxification

constant KMMALL = 0.0 !liver--CaE--Chambers & Meek 2017

constant KMMALK = 0.0 !kidney--CaE

constant KMMALBL = 0.0 !plasma--CaE

constant KMMALBR = 0.0 !brain--CaE

!malathion oxidation--malathion to oxon

constant KMMALMOX = 0.0 !liver--CYPs; based on in vitro data (Chambers & Meek 2017)

!oxon detox

constant KMOXDMPL = 0.0 !liver

constant KMOXDMPP = 0.0 !plasma

constant KMOXDMPB = 0.0 !brain

constant KMOXDMPK = 0.0 !kidney

constant KI = 0.0 !dissociation constant

!pharmacodynamics--esterase inhibition

!Enzyme turnover rates (umol hydrolysis/h/umol active sites)--not chemical specific

constant TRCE=0.0 !AChE (all tissues)

constant TRBE=0.0 !BuChE (all tissue)

!constant TRCR=0.0 !CaE (all tissues)

!Enzyme activities (umol/hr/kg tissue)--not chemical-specific

!AChE

constant BACHE=0.0 !brain AChE

constant HACHE=0.0 !liver AChE

constant BLACHE=0.0 !Plasma AChE

constant RBCHE=0.0 !RBC AChE

constant KACHE=0.0 !kidney AChE

!BuChE

constant BBUCE=0.0           !brain BuChE  
constant HBUCE=0.0           !liver BuChE  
constant BLBUCE=0.0   !Plasma BuChE  
constant KBUCE=0.0   !kidney BuChE

!CaE

constant BRCE=0.0   !Brain CaE  
!constant HECE=0.0   !liver CaE  
constant PLOCE=0.0           !Plasma CaE  
constant KECE=0.0   !kidney CaE

!chemical-specific parameters for inhibition

!liver AChE rate constants

constant KDHCE=0.0   ! degradation of esterase (/hr)  
constant KIHCE=0.0   ! inhibition of esterase (per umol/L/hr)  
constant KRHCE=0.0   ! reactivation of esterase (/hr)  
constant KAHCE=0.0   ! aging of esterase (/hr)

!liver BuChE rate constants

constant KDHBE=0.0   ! degradation of esterase (/hr)  
constant KIHBE=0.0   ! inhibition of esterase (per umol/L/hr)  
constant KRHBE=0.0   ! reactivation of esterase (/hr)  
constant KAHBE=0.0   ! aging of esterase (/hr)

!liver CaE rate constants

constant KDHCR=0.0   ! degradation of esterase (/hr)  
constant KIHCR=0.0   ! inhibition of esterase (per umol/L/hr)  
!constant KRHCR=0.0   ! reactivation of esterase (/hr)  
constant KAHCR=0.0   ! aging of esterase (/hr)

!brain AChE rate constants

constant KDBCE=0.0   ! degradation of esterase (/hr)  
constant KIBCE=0.0   ! inhibition of esterase (per umol/L/hr)  
constant KRBCE=0.0   ! reactivation of esterase (/hr)  
constant KABCE=0.0   ! aging of esterase (/hr)

!brain BuChE rate constants

constant KDBBE=0.0   ! degradation of esterase (/hr)  
constant KIBBE=0.0   ! inhibition of esterase (per umol/L/hr)  
constant KRBBE=0.0   ! reactivation of esterase (/hr)  
constant KABBE=0.0   ! aging of esterase (/hr)

!brain CaE rate constants

constant KDBCR=0.0   !degradation of esterase (/hr)  
constant KIBCR=0.0   !inhibition of esterase (per umol/L/hr)



constant KRBCR=0.0 !reactivation of esterase (/hr)  
constant KABCR=0.0 !aging of esterase (/hr)

!kidney AChE rate constants

constant KDKCE=0.0 ! degradation of esterase (/hr)  
constant KIKCE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRKCE=0.0 ! HR-1;reactivation of esterase  
constant KAKCE=0.0 ! aging of esterase (/hr)

!kidney BuChE rate constants

constant KDKBE=0.0 ! degradation of esterase (/hr)  
constant KIKBE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRKBE=0.0 ! reactivation of esterase (/hr)  
constant KAKBE=0.0 ! aging of esterase (/hr)

!kidney CaE rate constants

constant KDKCR=0.0 ! degradation of esterase (/hr)  
constant KIKCR=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRKCR=0.0 ! reactivation of esterase (/hr)  
constant KAKCR=0.0 ! aging of esterase (/hr)

!plasma AChE rate constants

constant KDBLCE=0.0 ! degradation of esterase (/hr)  
constant KIBLCE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRBLCE=0.0 ! reactivation of esterase (/hr)  
constant KABLCE=0.0 ! aging of esterase (/hr)

!plasma BuChE rate constants

constant KDBLBE=0.0 ! degradation of esterase (/hr)  
constant KIBLBE=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRBLBE=0.0 ! reactivation of esterase (/hr)  
constant KABLBE=0.0 ! aging of esterase (/hr)

!plasma CaE rate constants

constant KDBLCR=0.0 ! degradation of esterase (/hr)  
constant KIBLCR=0.0 ! inhibition of esterase (per umol/L/hr)  
constant KRBLCR=0.0 ! reactivation of esterase (/hr)  
constant KABLCR=0.0 ! aging of esterase (/hr)

!RBC AChE rate constants

!constant KDRBCE=0.0 ! degradation of esterase (/hr)  
!constant KIRBCE=0.0 ! inhibition of esterase (per umol/L/hr)  
!constant KRRBCE=0.0 ! reactivation of esterase (/hr)  
constant KARBCE=0.0 ! aging of esterase (/hr)

!uptake parameters

!malathion  
constant KAS=0.0 !transfer stomach to liver (/h)

!constant KSI=0.0 !transfer stomach to intestine (/h)  
 constant KAI=0.0 !transfer intestine to liver (/h)  
 constant FA=0.0 !Fractional oral Absorption  
 constant KMUC=0.0 !rate constant for swallowing part of an inhaled dose (/h); mucocillary clearance  
 constant FRACIN=0.0 !fraction to deep lung

FRLUGI=1-FRACIN !FRACTIONAL transfer of MAL to GI tract

!oxon  
 constant KASO=0.0 !transfer stomach to liver (/h)  
 constant KSIO=0.0 !transfer stomach to intestine (/h)  
 constant KAIO=0.0 !transfer intestine to liver (/h)  
 constant FAO=0.0 !fractional oral Absorption

!elimination rate constants for metabolite compartment (/h)  
 constant KE1=0.0 !MCA + DCA

!molecular weights  
 constant MWMAL=0.0 !molecular wt of malathion (g/mol)  
 constant MWMOX=0.0 !molecular wt of malaoxon (g/mol)

!oral dosing--single oral dose  
 constant ORALMAL=0.0 !MAL single oral bolus dose (ug/kg)  
 constant ORALMOX=0.0 !oxon oral bolus dose (ug/kg)

!oral dosing--multiple doses  
 !put times in the array DT (in hours) (absolute time, NOT time since last dose)  
 !Put the oral dose of malathion in the array DORAL (ug/kg)(DORALO for oxon dosing)  
 !for dosing amount in ug (instead of ug/kg), use AMTMDOSE (malathion) or AMTODOSE (oxon)  
 !Add one additional dose of zero at a time greater than TSTOP to keep the model running  
 DIMENSION DT(2000), DORAL(2000), DORALO(2000), AMTMDOSE(2000), AMTODOSE(2000)  
 INTEGER indx  
 indx=1 ! indx is a counter used to track multiple doses  
 SCHEDULE DOSEX .AT. DT(indx)  
 ODOSE=0.0 !oral dose (MAL; ug/kg BW)  
 ODOSEO=0.0 !oral dose (oxon; ug/kg BW)  
 ADOSEM=0.0 !oral dose (MAL; ug)  
 ADOSEO=0.0 !oral dose (oxon; ug)

!dermal dose  
 constant DERM=0.0 !dermal dose, ug/kg or ug; see lines below for DDOSE  
 constant SAF=0.0 !fraction of total skin surface area exposed  
 constant KDIS=0.0 !dissipative loss rate (/hr)  
 TSA=71.81\*(BWT\*\*0.425)\*(HT\*\*0.725) !total human skin surface area (cm2)  
 SA=SAF\*TSA !surface area exposed (cm2)

!constant SA = 0.0  
 VSKCC=VSKC\*(SA/TSA)                      !volume of skin tissue exposed

constant VLIQ=0.0                      !volume of liquid applied to skin (L)  
 constant KPL=0.0                      !skin permeability coefficient (cm/hr)  
 constant FAD=0.0                      !fraction of dermal dose absorbed; assume 100%  
 DDOSE=((DERM\*BWT)/MWMAL)\*FAD !total dermal dose (umol); use when dose is ug/kg  
 !DDOSE=DERM/MWMAL\*FAD\*VLIQ !use this when dose is in ug  
 constant TCHNG=12                      !time that chemical is in contact with skin (hr)

!inhalation  
 constant CONCPPM = 0.0    !Inhaled conc of malathion (ppm)  
 constant CONCMGM = 0.0                      !Inhaled conc of malathion (mg/m3)  
 constant CONCPPMO=0.0    !Inhaled conc of oxon (ppm)  
 constant CONCMGMO=0.0    !Inhaled conc of oxon (mg/m3)  
 constant TCHNG1=2                      !total exposure time (hr)

!oral dose--child exposure  
 constant ODOSECHILD = 0.0 !ug/kg  
 constant TCHNG4=504;                      !total exposure time (21 days)  
 ODMALCHILD=ODOSECHILD\*(BWT/MWMAL)\*FA !MAL dose conversion (ug/kg to umol)

!oral dose molar conversion with fractional absorption  
 ODOSEMAL=ORALMAL\*(BWT/MWMAL)\*FA                      ! MAL dose conversion (ug/kg to umol)  
 ODOSEMOX=ORALMOX\*(BWT/MWMOX)\*FAO ! malaoxon dose conversion (ug/kg to umol)

! Blood flow to each compartment (L/h)  
 QF=QFC\*VF    !fat  
 QH=QHC\*VH    !liver  
 QR=QRC\*VR    !rapidly perfused  
 QS=QSC\*VS    !slowly perfused  
 QBr=QBrC\*VBr    !brain  
 QK=QKC\*VK                      !kidney

QC=QF+QH+QR+QS+QBr+QK                      !Total cardiac output (L/h)

!scaling for Vmax for malathion detoxification (umol/hr/kg tissue to umol/hr)  
 VMAXMALL = VMAXMALLC \* VH                      !liver--CaE  
 VMAXMALK = VMAXMALKC \* VK                      !kidney--CaE  
 VMAXMALBL = VMAXMALBLC \* VBL \* (1-HCT)                      !plasma--CaE  
 VMAXMALBR = VMAXMALBRC \* VBR                      !brain--CaE

!oxidation--malathion to oxon  
 VMAXMALMOX = VMAXMALMOXC \* VH                      !liver--CYPs

!oxon detox  
 VMOXDMPL = VMOXDMPLC\*VH !liver oxon detox  
 VMOXDMPP = VMOXDMPPC\*VBL\*(1-HCT) !plasma oxon detox  
 VMOXDMPB = VMOXDMPBC\*VBr !brain oxon detox  
 VMOXDMPK = VMOXDMPKC\*VK !kidney oxon detox

!Enzyme activity (umol/h) in tissues

!AChE  
 SBACH=BACHE\*VBr !brain  
 SHACH=HACHE\*VH !liver  
 SBLACH=BLACHE\*VBL\*(1-HCT) !plasma  
 SRBACH=RBCHE\*VBL\*HCT !RBC  
 SKACH=KACHE\*VK !kidney

!BuChE  
 SBBUC=BBUCE\*VBr !brain  
 SHBUC=HBUCE\*VH !liver  
 SBLBUC=BLBUCE\*VBL\*(1-HCT) !plasma  
 SKBUC=KBUCE\*VK !kidney

!CaE  
 SBRCE=BRCE\*VBr !brain  
 SHECE=HECE\*VH !liver  
 SPLOCE=PLOCE\*VBL\*(1-HCT) !plasma  
 SKACE=KECE\*VK !kidney

!calculation of esterase binding sites (umol)

!AChE  
 IBCE=SBACH/TRCE !total brain AchE (umol)  
 IHCE=SHACH/TRCE !total liver AchE (umol)  
 IBLCE=SBLACH/TRCE !total plasma AchE (umol)  
 IRBCE=SRBACH/TRCE !total RBC AchE (umol)  
 IKCE=SKACH/TRCE !total kidney AchE (umol)

!BuChE  
 IBBE=SBBUC/TRBE !total brain BuChE (umol)  
 IHBE=SHBUC/TRBE !total liver BuChE (umol)  
 IBLBE=SBLBUC/TRBE !total plasma BuChE (umol)  
 IKBE=SKBUC/TRBE !total kidney BuChE (umol)

!CaE  
 IBCR=SBRCE/TRCR !total brain CaE (umol)  
 IHCR=SHECE/TRCR !total liver CaE (umol)  
 IBLCR=SPLOCE/TRCR !total plasma CaE (umol)  
 IKCR=SKACE/TRCR !total kidney CaE (umol)

!calculation of enzyme synthesis rates in tissues (umol/hr)

```

!liver
KSHCE=IHCE*KDHCE      !AChE
KSHBE=IHBE*KDHBE      !BuChE
KSHCR=IHCR*KDHCR      !CaE
!brain
KSBCE=IBCE*KDBCE      !AChE
KSBBE=IBBE*KDBBE      !BuChE
KSBCE=IBCE*KDBCE      !CaE
!kidney
KSKCE=IKCE*KDKCE      !AChE
KSKBE=IKBE*KDKBE      !BuChE
KSKCR=IKCR*KDKCR      !CaE
!plasma
KSLCE=IBLCE*KDBLCE    !AChE
KSLBE=IBLBE*KDBLBE    !BuChE
KSLCR=IBLCR*KDBLCR    !CaE
!RBC
KSRBCE=KDRBCE*IRBCE !AChE

! Enzymatic variability
CONSTANT VMLv=0.0
CONSTANT VMBLv=0.0
CONSTANT VMHCPv=0.0
CONSTANT VMHHCov=0.0
CONSTANT VMCBCPv=0.0
CONSTANT VMCBCov=0.0
CONSTANT VINTTv=0.0
CONSTANT VINTOv=0.0

! lognormal distributions
VMLvv=exp(GAUSS(LOG(VMAXMALL)-(SQRT(LOG((VMLv)**2.+1))**2.)/2.,SQRT(LOG((VMLv)**2.+1))))-
VMAXMALL      !malathion detox--liver
VMHCPvv=exp(GAUSS(LOG(VMAXMALK)-
(SQRT(LOG((VMHCPv)**2.+1))**2.)/2.,SQRT(LOG((VMHCPv)**2.+1))))-VMAXMALK      !malathion
detox--kidney
VMHHCovv=exp(GAUSS(LOG(VMAXMALBR)-
(SQRT(LOG((VMHHCov)**2.+1))**2.)/2.,SQRT(LOG((VMHHCov)**2.+1))))-VMAXMALBR !malathion
detox--brain
VMCBCPvv=exp(GAUSS(LOG(VMAXMALMOX)-
(SQRT(LOG((VMCBCPv)**2.+1))**2.)/2.,SQRT(LOG((VMCBCPv)**2.+1))))-VMAXMALMOX !malathion
activation
VMCBCOvv=exp(GAUSS(LOG(VMOXDMPL)-
(SQRT(LOG((VMCBCov)**2.+1))**2.)/2.,SQRT(LOG((VMCBCov)**2.+1))))-VMOXDMPL !oxon detox--
liver
VINTOvv=exp(GAUSS(LOG(VMOXDMPB)-
(SQRT(LOG((VINTOv)**2.+1))**2.)/2.,SQRT(LOG((VINTOv)**2.+1))))-VMOXDMPB      !oxon detox--
brain

```

VINTTv=exp(GAUSS(LOG(VMOXDMPK)-  
(SQRT(LOG((VINTTv)\*\*2.+1))\*\*2.)/2.,SQRT(LOG((VINTTv)\*\*2.+1))))-VMOXDMPK !oxon detox--  
kidney

!If the Vmax is less than 1% of the nominal, set equal to 1% of nominal (by setting error to 99% of nominal)

VMLvv = RSW(VMLvv+VMAXMALL .LT. .01\*VMAXMALL, -0.99\*VMAXMALL, VMLvv) !malathion  
detox--liver

VMHCPvv = RSW(VMHCPvv+VMAXMALK .LT. .01\*VMAXMALK, -0.99\*VMAXMALK, VMHCPvv)

!malathion detox--kidney

VMHHCOvv = RSW(VMHHCOvv+VMAXMALBR .LT. .01\*VMAXMALBR, -0.99\*VMAXMALBR, VMHHCOvv)

!malathion detox--brain

VMCBCPvv = RSW(VMCBCPvv+VMAXMALMOX .LT. .01\*VMAXMALMOX, -0.99\*VMAXMALMOX, VMCBCPvv) !malathion activation

VMCBCOvv = RSW(VMCBCOvv+VMOXDMPL .LT. .01\*VMOXDMPL, -0.99\*VMOXDMPL, VMCBCOvv)

!oxon detox--liver

VINTTv = RSW(VINTTv+VMOXDMPB .LT. .01\*VMOXDMPB, -0.99\*VMOXDMPB, VINTTv) !oxon  
detox--brain

VINTOvv = RSW(VINTOvv+VMOXDMPK .LT. .01\*VMOXDMPK, -0.99\*VMOXDMPK, VINTOvv) !oxon  
detox--kidney

! set variable Vmax's

VMAXMALL=VMLvv+VMAXMALL

VMAXMALK=VMHCPvv+VMAXMALK

VMAXMALBR=VMHHCOvv+VMAXMALBR

VMAXMALMOX=VMCBCPvv+VMAXMALMOX

VMOXDMPL=VMCBCOvv+VMOXDMPL

VMOXDMPB=VINTTv+VMOXDMPB

VMOXDMPK=VINTOvv+VMOXDMPK

!other variability based on point (LOCAL) sensitivity analyses

CONSTANT KDRBCEv=0.0

CONSTANT KIRBCEv=0.0

CONSTANT HCTv=0.0

CONSTANT TRCRv=0.0

CONSTANT HECEv=0.0

CONSTANT KRHCRv=0.0

CONSTANT KSIv=0.0

CONSTANT KRRBCEv=0.0

! lognormal distributions

KDRBCEvv=exp(GAUSS(LOG(KDRBCE)-

(SQRT(LOG((KDRBCEv)\*\*2.+1))\*\*2.)/2.,SQRT(LOG((KDRBCEv)\*\*2.+1))))-KDRBCE

KIRBCEvv=exp(GAUSS(LOG(KIRBCE)-

(SQRT(LOG((KIRBCEv)\*\*2.+1))\*\*2.)/2.,SQRT(LOG((KIRBCEv)\*\*2.+1))))-KIRBCE

KRRBCEvv=exp(GAUSS(LOG(KRRBCE)-

(SQRT(LOG((KRRBCEv)\*\*2.+1))\*\*2.)/2.,SQRT(LOG((KRRBCEv)\*\*2.+1))))-KRRBCE

HCTvv=exp(GAUSS(LOG(HCT)-(SQRT(LOG((HCTv)\*\*2.+1))\*\*2.)/2.,SQRT(LOG((HCTv)\*\*2.+1))))-HCT

```

TRCRvv=exp(GAUSS(LOG(TRCR)-(SQRT(LOG((TRCRv)**2.+1))**2.)/2.,SQRT(LOG((TRCRv)**2.+1))))-TRCR
KRHCRvv=exp(GAUSS(LOG(KRHCR)-
(SQRT(LOG((KRHCRv)**2.+1))**2.)/2.,SQRT(LOG((KRHCRv)**2.+1))))-KRHCR
HECEvv=exp(GAUSS(LOG(HECE)-(SQRT(LOG((HECEv)**2.+1))**2.)/2.,SQRT(LOG((HECEv)**2.+1))))-HECE
KSIvv=exp(GAUSS(LOG(KSI)-(SQRT(LOG((KSIv)**2.+1))**2.)/2.,SQRT(LOG((KSIv)**2.+1))))-KSI
!IF value goes below 1%, set to 1% of nominal mean
KDRBCEvv = RSW(KDRBCEvv+KDRBCE .LT. .01*KDRBCE, -0.99*KDRBCE, KDRBCEvv)
KIRBCEvv = RSW(KIRBCEvv+KIRBCE .LT. .01*KIRBCE, -0.99*KIRBCE, KIRBCEvv)
KRRBCEvv = RSW(KRRBCEvv+KRRBCE .LT. .01*KRRBCE, -0.99*KRRBCE, KRRBCEvv)
HCTvv=RSW(HCTvv+HCT .LT. .01*HCT, -0.99*HCT, HCTvv)
TRCRvv = RSW(TRCRvv+TRCR .LT. .01*TRCR, -0.99*TRCR, TRCRvv)
KRHCRvv = RSW(KRHCRvv+KRHCR .LT. .01*KRHCR, -0.99*KRHCR, KRHCRvv)
HECEvv = RSW(HECEvv+HECE .LT. .01*HECE, -0.99*HECE, HECEvv)
KSIvv = RSW(KSIvv+KSI .LT. .01*KSI, -0.99*KSI, KSIvv)
! set variable params
KDRBCE=KDRBCEvv+KDRBCE
KIRBCE=KIRBCEvv+KIRBCE
KRRBCE=KRRBCEvv+KRRBCE
HCT=HCTvv+HCT
TRCR=TRCRvv+TRCR
HECE=HECEvv+HECE
KSI=KSIvv+KSI
KOSI=KSIvv+KOSI
KRHCR=KRHCRvv+KRHCR

```

!INITIALIZING MODEL VARIABLES BEFORE EACH SIMULATION

```

DZONE=1.0          !turn dermal exposure on
INZONE=1.0         !turn inhalation exposure on
OZONE=1.0          !turn child oral exposure on

```

! TIMING COMMANDS

```

constant POINTS=100.0      !OUTPUT POINTS
constant TSTOP=0.0         !STOP SIMULATION (HR)
CINT=TSTOP/POINTS         !Communication interval
VARIABLE time = 0.0        !simulation time in hour

```

END ! INITIAL

DYNAMIC

```

ALGORITHM IALG = 2        !Gear algorithm
TERMT(TIME .GE. TSTOP)    !stop execution

```

! Age

IF (SPECIES .EQ. 0) THEN

```

AGE=AGE0+TIME/24.0        ! Rat age (D)

```

```

ELSE
AGE=AGE0+TIME/8766          ! Human age (Y)
END IF

! Age dependent B4 & B8 for humans (Luecke 2007)
IF ((AGE .GE. AGE1) .AND. (AGE .LT. AGE2) .AND. (SPECIES .EQ. 1)) THEN
B4x=B4;B8x=0
ELSE IF ((AGE .GE. AGE3) .AND. (SPECIES .EQ. 1)) THEN
B4x=0;B8x=B8
ELSE
B4x=0;B8x=0
END IF

! BWT (kg) Gompertz (Luecke 2007)
IF (AGE .GE. AGE2) THEN
BWT=B5*EXP((B6/B7)*(1-EXP(-B7*(AGE-AGE2))))+B8x*(AGE-AGE3)
ELSE
BWT=B1*EXP((B2/B3)*(1-EXP(-B3*AGE)))+B4x*(AGE-AGE1)
END IF
BWT=BWT*(1.-BWSW)+BWST*BWSW

BWTG=BWT*1000          ! BWT kg to grams for compartment volume calculations

IF (SPECIES .EQ. 0) THEN
LBWT=LOG(BWT)          ! Log of BWT for rat brain calc
ELSE
LBWT=BWT*1000
END IF

! Compartment volumes (frac of BWT)
!brain
VBrC=VB0+(VB1*LBWT)+(VB2*LBWT**2)+(VB3*LBWT**3)+(VB4*LBWT**4)+(VB5*LBWT**5)+(VB6*LB
WT**6)
!liver
VHC=VH0+(VH1*BWTG)+(VH2*BWTG**2)+(VH3*BWTG**3)+(VH4*BWTG**4)
!blood
VBLC=VBL0+(VBL1*BWTG)+(VBL2*BWTG**2)
!fat--this is lipid content only
VFC=VF0+(VF1*BWTG)+(VF2*BWTG**2)+(VF3*BWTG**3)+(VF4*BWTG**4)+(VF5*LBWT**5)+(VF6*LB
WT**6)
!adipose--this is all tissue associated with adipose tissue (connective and lipid)
VAC=VA0+(VA1*BWTG)+(VA2*BWTG**2)+(VA3*BWTG**3)+(VA4*BWTG**4)+(VA5*BWTG**5)+(VA6*
BWTG**6)
!kidney (in rapidly perfused)
VKC=VK0+(VK1*BWTG)+(VK2*BWTG**2)
!spleen (in rapidly perfused)
VSPC=VSP0+(VSP1*BWTG)+(VSP2*BWTG**2)
!lung

```



$$VLC=VL0+(VL1*BWTG)+(VL2*BWTG**2)$$

!intestines (in rapidly perfused)

$$VGIC=VGIO+(VGI1*BWTG)+(VGI2*BWTG**2)+(VGI3*BWTG**3)$$

!muscle (in slowly perfused)

$$VMC=VM0+(VM1*BWTG)+(VM2*BWTG**2)+(VM3*BWTG**3)+(VM4*BWTG**4)$$

!skin (in slowly perfused)

$$VSKC=VSK0+(VSK1*BWTG)+(VSK2*BWTG**2)+(VSK3*BWTG**3)+(VSK4*BWTG**4)+(VSK5*BWTG**5)$$

$$VFC=VFC$$

$$VRC=VKC+VSPC+VGIC+VPC$$

! Rapid +VLC

$$VSC=VMC+VSKC+VBMC+(VAC-VFC)$$

! Slow

$$VSC=VSC+VSCu$$

$$VRC=VRC+VRCu$$

$$VBC=VBC+VBCu$$

$$VHC=VHC+VHCu$$

$$VFC=VFC+VFCu$$

$$VBLC=VBLC+VBLCu$$

$$!VDC=VDC+VDCu$$

$$VINTC=VINTC+VINTCu$$

! Compartment volumes (L)

$$VBr=VBrC*BWT \quad !\text{brain}$$

$$VH=VHC*BWT \quad !\text{liver}$$

$$VBL=VBLC*BWT \quad !\text{blood}$$

$$VPLAS=VBL*(1-HCT) \quad !\text{plasma}$$

$$VRBC=VBL*HCT \quad !\text{RBC}$$

$$VF=VFC*BWT \quad !\text{fat}$$

$$VR=VRC*BWT \quad !\text{rapidly perfused}$$

$$VS=VSC*BWT \quad !\text{slow perfused}$$

$$VK=VKC*BWT \quad !\text{kidney}$$

$$VINT=VINTC*BWT \quad !\text{intestine}$$

$$VL=VLC*BWT \quad !\text{lung}$$

$$VTOTCOMPARTMENTS=(VLC+VFC+VSC+VRC+VBRC+VBLC+VKC+VINTC+VHC)/0.9 \quad !\text{should be } \sim 0.9-1.1$$

$$BWT\text{Total}=(0.9*BWT)-(VL+VF+VS+VR+VBr+VBL+VK+VH) \quad !\text{should } < 10 \text{ for a human}$$

! Blood flow to each compartment (L/h)

$$QF=QFC*VF$$

$$QH=QHC*VH$$

$$QR=QRC*VR$$

$$QS=QSC*VS$$

$$QBr=QBrC*VBr$$

$$QK=QKC*VK$$

QC=QF+QH+QR+QS+QBr+QK !total cardiac output (L/h)

constant BreatheRATE = 29 !L/min; HED Pesticide Exposure Database

QALV = BreatheRATE\*60\*0.7 !adult

!QALV = 330\*0.7 ! Kids

!scaling for Vmax for malathion detoxification (umol/hr/kg tissue to umol/hr)

VMAXMALL = VMAXMALLC \* VH !liver--CaE

VMAXMALK = VMAXMALKC \* VK !kidney--CaE

VMAXMALBL = VMAXMALBLC \* VBL \* (1-HCT) !plasma--CaE

VMAXMALBR = VMAXMALBRC \* VBR !brain--CaE

!oxidation--malathion to oxon

VMAXMALMOX = VMAXMALMOXC \* VH !liver--CYPs

!oxon detox

VMOXDMPL = VMOXDMPLC\*VH !liver oxon detox

VMOXDMPP = VMOXDMPPC\*VBL\*(1-HCT) !plasma oxon detox

VMOXDMPB = VMOXDMPBC\*VBr !brain oxon detox

VMOXDMPK = VMOXDMPKC\*VK !kidney oxon detox

!Enzyme activity (umol/h) in tissues

!AChE

SBACH=BACHE\*VBr !brain

SHACH=HACHE\*VH !liver

SBLACH=BLACHE\*VBL\*(1-HCT) !plasma

SRBACH=RBCHE\*VBL\*HCT !RBC

SKACH=KACHE\*VK !kidney

!BuChE

SBBUC=BBUCE\*VBr !brain

SHBUC=HBUCE\*VH !liver

SBLBUC=BLBUCE\*VBL\*(1-HCT) !plasma

SKBUC=KBUCE\*VK !kidney

!CaE

SBRCE=BRCE\*VBr !brain

SHECE=HECE\*VH !liver

SPLOCE=PLOCE\*VBL\*(1-HCT) !plasma

SKACE=KECE\*VK !kidney

!calculation of esterase binding sites (umol)

!AChE

IBCE=SBACH/TRCE !total brain AchE (umol)

IHCE=SHACH/TRCE !total liver AchE (umol)

IBLCE=SBLACH/TRCE !total plasma AchE (umol)

IRBCE=SRBACH/TRCE !total RBC AChE (umol)  
 IKCE=SKACH/TRCE !total kidney AchE (umol)

!BuChE  
 IBBE=SBBUC/TRBE !total brain BuChE (umol)  
 IHBE=SHBUC/TRBE !total liver BuChE (umol)  
 IBLBE=SBLBUC/TRBE !total plasma BuChE (umol)  
 IKBE=SKBUC/TRBE !total kidney BuChE (umol)

!CaE  
 IBCR=SBRCE/TRCR !total brain CaE (umol)  
 IHCR=SHECE/TRCR !total liver CaE (umol)  
 IBLCR=SPLOCE/TRCR !total plasma CaE (umol)  
 IKCR=SKACE/TRCR !total kidney CaE (umol)

!calculation of enzyme synthesis rates in tissues (umol/hr)

!liver

KSHCE=IHCE\*KDHCE !AChE  
 KSHBE=IHBE\*KDHBE !BuChE  
 KSHCR=IHCR\*KDHCR !CaE

!brain

KSBCE=IBCE\*KDBCE !AChE  
 KSBBE=IBBE\*KDBBE !BuChE  
 KSBCE=IBCE\*KDBCE !CaE

!kidney

KSKCE=IKCE\*KDKCE !AChE  
 KSKBE=IKBE\*KDKBE !BuChE  
 KSKCR=IKCR\*KDKCR !CaE

!plasma

KSDLCE=IBLCE\*KDBLCE !AChE  
 KSDLBE=IBLBE\*KDBLBE !BuChE  
 KSDLCE=IBLCE\*KDBLCE !CaE

!RBC

KSRBCE=KDRBCE\*IRBCE !AChE

! variable enzymology

VMAXMALL=VMLvv+VMAXMALL  
 VMAXMALK=VMHCPvv+VMAXMALK  
 VMAXMALBR=VMHHCOvv+VMAXMALBR  
 VMAXMALMOX=VMCBCPvv+VMAXMALMOX  
 VMOXDMPPL=VMCBCOvv+VMOXDMPPL  
 VMOXDMPB=VINTTvv+VMOXDMPB  
 VMOXDMPK=VINTOvv+VMOXDMPK

!-----

!dosing

!oral dose molar conversion with fractional absorption

ODOSEMAL=ORALMAL\*(BWT/MWMAL)\*FA ! MAL dose conversion (ug/kg to umol)

ODOSEMOX=ORALMOX\*(BWT/MWMOX)\*FAO ! malaoxon dose conversion (ug/kg to umol)

ODMALCHILD=ODOSECHILD\*(BWT/MWMAL)\*FA !MAL dose conversion for child (ug/kg to umol)

!oral dose

DISCRETE DOSEX

ODOSE=ODOSE+DORAL(indx)\*(BWT/MWMAL)\*FA ! MAL dose molar conversion (ug/kg to umol)

ODOSEO=ODOSEO+DORALO(indx)\*(BWT/MWMOX)\*FA ! oxon dose molar conversion (ug/kg to umol)

ADOSEM=ADOSEM+AMTMDOSE(indx)\*FA/MWMAL ! MAL dose conversion (ug to umol)

ADOSEO=ADOSEO+AMTDOSE(indx)\*FA/MWMOX ! oxon dose conversion (ug to umol)

indx=indx+1

SCHEDULE DOSEX .AT. DT(indx)

END

!dermal

DISCRETE DOSEON1

INTERVAL DOSEINT = 1440.0 !Interval to repeat dosing (hr); set to 1440 for 60 days

SCHEDULE DOSEOFF1 .AT. (TIME+TCHNG)

IF (Time.LE.TCHNG) THEN

DZONE=1

ENDIF

END

DISCRETE DOSEOFF1

DZONE = 0.0

END

!inhalation

DISCRETE DOSEON2

INTERVAL DOSEINT = 1440.0 !Interval to repeat dosing (hr); set to 1440 for 60 days

SCHEDULE DOSEOFF2 .AT. (TIME+TCHNG1)

IF (Time.LE.TCHNG1) THEN

INZONE=1

ENDIF

END

DISCRETE DOSEOFF2

INZONE=0.0

END

!oral exposure for child

DISCRETE DOSEON4

INTERVAL DOSEINT = 1440.0 !Interval to repeat dosing (hr); set to 1440 for 60 days

SCHEDULE DOSEOFF4 .AT. (TIME+TCHNG4)

IF (Time.LE.TCHNG4) THEN

OZONE=1

ENDIF

END

DISCRETE DOSEOFF4

ONZONE=0.0

END

!drinking H2O

constant volume=0.688557 !L/day consumed

CONSTANT EVENTS=6 !times/day to drink

DERIVATIVE

!-----

!malathion (parent compound)-----

!-----

!oral dose for child exposure

constant W4 = 21 !days exposed per year

constant D4 = 7 !days per week exposed

constant P4 = 1.5 !hr per day exposed

ODCHILD = ODMALCHILD\*pulse(0,year\*24,W4\*24)\*pulse(0,7\*24,D4\*24)\*pulse(0,24,P4)

ODCHILD2 = ODCHILD\*OZONE

!stomach

RSTOM = -kas\*Astom-ksi\*Astom+Amuc\*kmuc !rate of change in stomach (umol/hr)

ASTom = ODOSE+ADOSEM+ODOSEMAL + integ(RStom,0.0)+ODCHILD2 !amount in stomach (umol)

!intestine

RInst = ksi\*ASTom - kai\*AIntM !rate of change in intestine (umol/hr)

AIntM=integ(RInst,0.0) !amount in intestine (umol)

CINTM = AIntM/VINT !concentration in intestine (umol/L)

!liver

RALM=QH\*CBL-QH\*CVLM+kai\*AIntM+kas\*ASTom-RALM1-RALM2 !rate of change in liver (umol/hr)

ALM = integ(RALM,0.0) !amt in liver (umol)

CLM = ALM/VH !concentration in liver (umol/L)

CVLM = CLM/PMaLL !concn in venous blood leaving liver (umol/L)

!Liver metabolism of Mal by CYPs--oxidation

$RALM1 = (V_{maxMalMox} \cdot CVLM) / (K_{mMalMox} + CVLM)$  !rate of metabolite formation (umol/hr)  
 $ALM1 = \text{integ}(RALM1, 0.0)$  !amt oxon formed (umol)

!Liver--metabolism of MAL to MCA/DCA by CaE  
 $RALM2 = V_{MAXMALL} \cdot CVLM / (K_{MMALL} \cdot (1 + CVLO/KI) + CVLM)$  !rate of metabolites formed (umol/hr)  
 $ALM2 = \text{integ}(RALM2, 0.0)$  !amount of metabolites formed (umol)

!kidney  
 $RAKM = QK \cdot CBL - QK \cdot CVKM - RAKM2$  !rate of change in kidney (umol/hr)  
 $AKM = \text{integ}(RAKM, 0.0)$  !amt in kidney (umol)  
 $CKM = AKM / VK$  !concn in kidney (umol/L)  
 $CVKM = CKM / PMaK$  !concn in venous blood leaving kidney (umol/L)

!kidney--metabolism of MAL to MCA/DCA by CaE  
 $RAKM2 = V_{MAXMALK} \cdot CVKM / (K_{MMALK} \cdot (1 + CVKO/KI) + CVKM)$  !rate of metabolites formed (umol/hr)  
 $AKM2 = \text{integ}(RAKM2, 0.0)$  !amount of metabolites formed (umol)

!fat  
 $RAFM = QF \cdot CBL - QF \cdot CVFM$  !rate of change in fat (umol/hr)  
 $AFM = \text{integ}(RAFM, 0.0)$  !amt in fat (umol)  
 $CFM = AFM / VF$  !concn. in fat (umol/L)  
 $CVFM = CFM / PMaF$  !concn. in venous blood leaving fat (umol/L)

!slowly perfused  
 $RASM = QS \cdot CBL - QS \cdot CVSM$  !rate of change in slowly perfused (umol/hr)  
 $ASMaI = \text{integ}(RASM, 0.0)$  !amt in slowly perfused (umol)  
 $CSM = ASMaI / VS$  !concn. in slowly perfused (umol/L)  
 $CVSM = CSM / PMaIS$  !concn. in venous blood leaving slowly perfused (umol/L)

!brain  
 $RABrM = QBr \cdot CBL - QBr \cdot CVBrM - RABRM2$  !rate of change in brain (umol/hr)  
 $ABrM = \text{integ}(RABrM, 0.0)$  !amt. in brain (umol)  
 $CBrM = ABrM / VBr$  !concn in brain (umol/L)  
 $CVBrM = CBrM / PMaBr$  !concn. in venous blood leaving brain (umol/L)

!brain--metabolism of MAL to MCA/DCA by CaE  
 $RABRM2 = V_{MAXMALBR} \cdot CVBrM / (K_{MMALBR} \cdot (1 + CVBrO/KI) + CVBrM)$  !rate of metabolites formed (umol/hr)  
 $ABRM2 = \text{integ}(RABRM2, 0.0)$  !amount of metabolites formed (umol)

!rapidly perfused tissues  
 $RARM = QR \cdot CBL - QR \cdot CVRM$  !rate of change in rapidly perfused (umol/hr)  
 $ARM = \text{integ}(RARM, 0.0)$  !amt in rapidly perfused (umol)  
 $CRM = ARM / VR$  !concn in rapidly perfused (umol/L)  
 $CVRM = CRM / PMaIR$  !concn in venous blood leaving rapidly perfused (umol/L)

! Dermal exposure  
constant year = 364 !days  
constant w2 = 21 !days exposed per year  
constant d2 = 5 !days exposed/week  
constant p2 = 8 !hr exposed/day  
DDOSE2 = DDOSE\*pulse(0,year\*24,w2\*24)\*pulse(0,7\*24,d2\*24)\*pulse(0,24,p2)  
DDOSE3 = DDOSE2\*DZONE

ASURF=integ(RASURF,0.0)+DDOSE3 !amt at skin surface (umol)  
CSURF=ASURF/VLIQ !concn at skin surface (umol/L)  
RASURF=-((((KPL\*SA/1000)\*CSURF))\*DZONE) !rate of change at skin surface (umol/hr)

RADL=(((KPL\*SA/1000)\*CSURF))\*DZONE)-RADLL !rate of uptake into skin tissue from skin surface (umol/hr)  
ADL=INTEG(RADL,0.0) !amout of uptake (umol)

RADLL=ADL\*PSKL !rate of absorption (umol/hr)  
ADLT=INTEG(RADLL,0.0) !total amt absorbed (umol)  
CADERM=ADL/VSKCC !concn absorbed (umol/L)

!Inhalation Exposures  
constant w3 = 21 !days exposed per year  
constant d3 = 5 !days exposed per week  
constant P3 = 8 !hr exposed per day  
CIM = (concmgm/1000)\*pulse(0,year\*24,w3\*24)\*pulse(0,7\*24,d3\*24)\*pulse(0,24,P3) !mg/L

CI=CIM/MWMAL\*1000\*INZONE !inhaled dose concentration (from mg/L to umol/L)

!lung --update with Cecelia's modifications--make sure this is correct  
RALINH = QALV \* FRACIN \* CI !rate of uptake into lung (umol/hr)  
RALU=QC\*(CV-CBL)-(QALV\*CLEX) + RALINH !rate of change in lung (umol/hr)  
ALU=INTEG(RALU,0.0) !amt in lung (umol)  
CLU=ALU/VL !concn in lung (umol/L)

ALUINH=INTEG(RALINH,0.0) !amt absorbed in lung (umol)  
INMG=ALUINH\*MWMAL/BWT/1000 !amt absorbed (mg/kg)

!for swallowing part of an inhaled dose  
RLUGI=FRLUGI\*CI\*QALV-AMUC\*KMUC !rate of transfer from lung to GI (umol/hr)  
AMUC=INTEG(RLUGI,0.0) !amount transferred (umol)  
RMUCOS = AMUC\*KMUC !rate of absorption from GI tract (umol/hr)  
ALUNGDOSE = INTEG(RMUCOS, 0.0) !amount absorbed in GI tract (umol)

!amt exhaled  
CLEX = CBL / PLAIR !concn exhaled (umol/L)  
RAEX = QALV \* CLEX !rate of exhalation (umol/hr)

AEX = INTEG(RAEX, 0.0) !amt exhaled (umol)

!plasma

RABL=QC\*(CV-CBL)+RADLL !rate of change in mixed blood (arterial & venous; umol/hr)

ABL=INTEG(RABL,0.0) !amount in mixed blood (umol)

CBL=ABL/VBL !concn in mixed blood (umol/L)

CV=(QH\*CVLM+QF\*CVFM+QK\*CVKM+QS\*CVSM+QBr\*CVBrM+QR\*CVRM)/QC !concentration in venous blood (umol/L)

!-----

!oxon

!-----

!stomach

RAStomO = -ksiO\*ASTomO-kasO\*ASTomO !rate of change in stomach (umol/hr)--do we have the drinkingwater here??

ASTomO = integ(RASTomO,0.0)+ODOSEO+ADOSEO+ODOSEMOX !amount in stomach (umol)

!intestine

RAIntO = -kaiO\*AINTO+ksiO\*ASTomO !rate of change in intestine (umol/hr)

AINTO = integ(RAIntO,0.0) !amount in intestine (umol)

CINTO = AINTO/VINT !concn in intestine (umol/L)

!liver

RALO = QH\*(CBLO-CVLO)+kaiO\*AINTO+kasO\*ASTomO-RALOD+RALM1-(RHPCE+RHPBE+RHPCR)!rate of change in liver (umol/hr)

ALO = integ(RALO,0.0) !amt in liver (umol)

CLO = ALO/VH !concentration in liver (umol/L)

CVLO = CLO/PMOXL !concn in venous blood leaving liver (umol/L)

!oxon detox--catalytic

RALOD = (VMOXDMPL\*CVLO)/(KMOXDMPL\*(1+CVLO/KI)+CVLO)

AALOD = integ(RALOD,0.0)

!AChE activity--liver

RHCE=KSHCE-AHCE\*(KDHCE+KIHCE\*CLO)+HOCE\*KRHCE ! rate of AChE activity (umol/hr)

AHCE=INTEG(RHCE,IHCE) ! liver AChE activity (umol)

RNHCE=KSHCE-ANHCE\*KDHCE ! rate of baseline AChE activity (umol/hr)

ANHCE=INTEG(RNHCE,IHCE) ! Amt of baseline AChE (umol)

RHOCE=AHCE\*KIHCE\*CLO-HOCE\*(KAHCE+KRHCE) ! rate of AChE inhibition (umol/hr)

HOCE=INTEG(RHOCE,0.0) ! amt of liver AChE inhibited (umol)

RHPCE=AHCE\*KIHCE\*CLO ! rate of oxon consumption (umol/hr)

AHPCE=integ(RHPCE,0.0) ! amt of oxon consumed (umol)



!BuChE activity--liver

RHBE=KSHBE-AHBE\*(KDHBE+KIHBE\*CLO)+HOBE\*KRHBE ! rate of BuChE activity (umol/hr)

AHBE=INTEG(RHBE,IHBE) ! liver BuChE activity (umol)

RNHBE=KSHBE-ANHBE\*KDHBE ! rate of baseline BuChE act  
(umol/hr)

ANHBE=INTEG(RNHBE,IHBE) ! Amt of baseline BuChE (umol)

RHOBE=AHBE\*KIHBE\*CLO-HOBE\*(KAHBE+KRHBE) ! rate of BuChE inhibition  
(umol/hr)

HOBE=INTEG(RHOBE,0.0) ! amount of liver BuChE inhibited  
(umol)

RHPBE=AHBE\*KIHBE\*CLO ! rate of oxon consumption  
(umol/hr)

AHPBE=integ(RHPBE,0.0) ! amt of oxon consumed (umol)

!CaE activity--liver

RHCR=KSHCR-AHCR\*(KDHCR+KIHCR\*CLO)+HOCCR\*KRHCR ! rate of CaE activity (umol/hr)

AHCR=INTEG(RHCR,IHCR) ! liver CaE activity (umol)

RNHCR=KSHCR-ANHCR\*KDHCR ! rate of baseline CaE act (umol/hr)

ANHCR=INTEG(RNHCR,IHCR) ! Amt of baseline CaE (umol)

RHOCCR=AHCR\*KIHCR\*CLO-HOCCR\*(KAHCR+KRHCR) ! rate of CaE inhibition (umol/hr)

HOCCR=INTEG(RHOCCR,0.0) ! amount of liver CaE inhibited  
(umol)

RHPCR=AHCR\*KIHCR\*CLO ! rate of oxon consumption  
(umol/hr)

AHPCR=integ(RHPCR,0.0) ! amt of oxon consumed (umol)

!liver B-esterase (AChE, BuChE, and CaE) activity

HBE=AHCE+AHBE+AHCR ! total B-esterase=AChE+BuChE+CaE  
(umol)

IHE=100\*(HBE/(ANHCE+ANHBE+ANHCR)) ! % total B-esterase inhibition

HCE=100\*(AHCE/ANHCE) ! % AChE inhibition

HBES=100\*(AHBE/ANHBE) ! % BuChE inhibition

HCR=100\*(AHCR/ANHCR) ! % CaE inhibition

!fat

RAFO = QF\*CBLO-QF\*CVFO !rate of change in fat (umol/hr)

AFO = integ(RAFO, 0.0) !amt in fat (umol)

CFO = AFO/VF !concn. in fat (umol/L)

CVFO = CFO/PMOXF !concn. in venous blood leaving fat (umol/L)

!slowly perfused

RASO = QS\*CBLO-QS\*CVSO !rate of change in slowly perfused (umol/hr)

ASO = integ(RASO, 0.0) !amt in slowly perfused (umol)

CSO = ASO/VS !concn. in slowly perfused (umol/L)

CVSO = CSO/PMOXS !concn. in venous blood leaving slowly perfused (umol/L)

!rapidly perfused tissues  
RARO = QR\*CBLO-QR\*CVRO !rate of change in rapidly perfused (umol/hr)  
ARO = integ(RARO,0.0) !amt in rapidly perfused (umol)  
CRO = ARO/VR !concn in rapidly perfused (umol/L)  
CVRO = CRO/PMOXR !concn in venous blood leaving rapidly perfused (umol/L)

!brain  
RABrO = QBr\*CBLO-QBr\*CVBrO-RABROD-(RBPCE+RBPBE+RBPCR)!rate of change in brain (umol/hr)  
ABrO = integ(RABrO,0.0) !amt. in brain (umol)  
CBrO = ABrO/VBr !concn in brain (umol/L)  
CVBrO = CBrO/PMOXR !concn. in venous blood leaving brain (umol/L)

!oxon detox--catalytic  
RABROD = (VMOXDMPB\*CVBrO)/(KMOXDMPB\*(1+CVBrO/KI)+CVBrO)  
AABRO = integ(RABROD,0.0)

!AChE activity--brain  
RBCE=KSBCE-ABCE\*(KDBCE+KIBCE\*CBrO)+BOCE\*KRBCE ! rate of AChE activity (umol/hr)  
ABCE=INTEG(RBCE,IBCE) ! brain AChE activity (umol)  
RNBCE=KSBCE-ANBCE\*KDBCE ! rate of baseline AChE act  
(umol/hr)  
ANBCE=INTEG(RNBCE,IBCE) ! Amt of baseline AChE (umol)  
RBOCE=ABCE\*KIBCE\*CBrO-BOCE\*(KABCE+KRBCE) ! rate of AChE inhibition  
(umol/hr)  
BOCE=INTEG(RBOCE,0.0) ! amount of brain AChE inhibited  
(umol)  
RBPCE=ABCE\*KIBCE\*CBrO ! rate of oxon consumption  
(umol/hr)  
ABPCE=integ(RBPCE,0.0) ! amt of oxon consumed (umol)

!BuChE activity--brain  
RBBE=KSBBE-ABBE\*(KDBBE+KIBBE\*CBrO)+BOBE\*KRBBE ! rate of BuChE activity (umol/hr)  
ABBE=INTEG(RBBE,IBBE) ! brain BuChE activity (umol)  
RNBBE=KSBBE-ANBBE\*KDBBE ! rate of baseline BuChE act  
(umol/hr)  
ANBBE=INTEG(RNBBE,IBBE) ! Amt of baseline BuChE (umol)  
RBOBE=ABBE\*KIBBE\*CBrO-BOBE\*(KABBE+KRBBE) ! rate of BuChE inhibition  
(umol/hr)  
BOBE=INTEG(RBOBE,0.0) ! amount of brain BuChE  
inhibited (umol)  
RBPBE=ABBE\*KIBBE\*CBrO ! rate of oxon consumption  
(umol/hr)  
ABPBE=integ(RBPBE,0.0) ! amt of oxon consumed (umol)

!CaE activity--brain  
RBCR=KSBCR-ABCR\*(KDBCR+KIBCR\*CBrO)+BOCR\*KRBCR ! rate of CaE activity (umol/hr)  
ABCR=INTEG(RBCR,IBCR) ! brain CaE activity (umol)

RNBCR=KSBCR-ANBCR\*KDBCR ! rate of baseline CaE act  
 (umol/hr)  
 ANBCR=INTEG(RNBCR,IBCR) ! Amt of baseline CaE (umol)  
 RBOCR=ABCR\*KIBCR\*CBrO-BOCR\*(KABCR+KRBCR) ! rate of CaE inhibition (umol/hr)  
 BOCR=INTEG(RBOCR,0.0) ! amount of brain CaE inhibited  
 (umol)  
 RBPCR=ABCR\*KIBCR\*CBrO ! rate of oxon consumption  
 (umol/hr)  
 ABPCR=integ(RBPCR,0.0) ! amt of oxon consumed (umol)

!brain B-esterase (AChE, BuChE, and CaE) activity  
 BBE=ABCE+ABBE+ABCR ! total B-esterase=AChE+BuChE+CaE (umol)  
 IBE=100\*(BBE/(ANBCE+ANBBE+ANBCR)) ! total B-esterase inhibition  
 BCE=100\*(ABCE/ANBCE) ! % AChE inhibition  
 BBES=100\*(ABBE/ANBBE) ! % BuChE inhibition  
 BCR=100\*(ABCR/ANBCR) ! % CaE inhibition

!kidney  
 RAKO = QK\*CBL0-QK\*CVKO-RAKOD -(RKPCE+RKPBE+RKPCR) !rate of change in kidney (umol/hr)  
 AKO = integ(RAKO,0.0) !amt in kidney (umol)  
 CKO = AKO/VK !concn in kidney (umol/L)  
 CVKO = CKO/PMOXX !concn in venous blood leaving kidney (umol/L)

!oxon detox--catalytic  
 RAKOD = (VMOXDMPK\*CVKO)/(KMOXDMPK\*(1+CVKO/KI)+CVKO)  
 AAKOD = integ(RAKOD,0.0)

! AChE activity--kidney  
 RKCE=KSKCE-AKCE\*(KDKCE+KIKCE\*CKO)+KOCE\*KRKCE ! rate of AChE activity (umol/hr)  
 AKCE=INTEG(RKCE,IKCE) ! kidney AChE activity (umol)  
 RNKCE=KSKCE-ANKCE\*KDKCE ! rate of baseline AChE act  
 (umol/hr)  
 ANKCE=INTEG(RNKCE,IKCE) ! Amt of baseline AChE (umol)  
 RKOCE=AKCE\*KIKCE\*CKO-KOCE\*(KAKCE+Krkce) ! rate of AChE inhibition  
 (umol/hr)  
 KOCE=INTEG(RKOCE,0.0) ! amount of kidney AChE  
 inhibited (umol)  
 RKPCE=AKCE\*KIKCE\*CKO ! rate of oxon consumption  
 (umol/hr)  
 AKPCE=integ(RKPCE,0.0) ! amt of oxon consumed (umol)

! BuChE activity--kidney  
 RKBE=KSKBE-AKBE\*(KDKBE+KIKBE\*CKO)+K0BE\*KRKBE ! rate of BuChE activity (umol/hr)  
 AKBE=INTEG(RKBE,IKBE) ! kidney BuChE activity (umol)  
 RNKBE=KSKBE-ANKBE\*KDKBE ! rate of baseline BuChE act  
 (umol/hr)  
 ANKBE=INTEG(RNKBE,IKBE) ! Amt of baseline BuChE (umol)

$RKOB = AKBE * KIKBE * CKO - KOBE * (KAKBE + KRKBE)$  ! rate of BuChE inhibition  
 (umol/hr)  
 $KOBE = INTEG(RKOB, 0.0)$  ! amount of kidney BuChE  
 inhibited (umol)  
 $RKPBE = AKBE * KIKBE * CKO$  ! rate of oxon consumption  
 (umol/hr)  
 $AKPBE = integ(RKPBE, 0.0)$  ! amt of oxon consumed (umol)

! CaE activity--kidney  
 $RKCR = KSKCR - AKCR * (KDKCR + KIKCR * CKO) + KOCCR * KRKCR$  ! rate of CaE activity (umol/hr)  
 $AKCR = INTEG(RKCR, IKCR)$  ! kidney CaE activity (umol)  
 $RNKCR = KSKCR - ANKCR * KDKCR$  ! rate of baseline CaE act  
 (umol/hr)  
 $ANKCR = INTEG(RNKCR, IKCR)$  ! Amt of baseline CaE (umol)  
 $RKOCR = AKCR * KIKCR * CKO - KOCCR * (KAKCR + KRKCR)$  ! rate of CaE inhibition  
 (umol/hr)  
 $KOCCR = INTEG(RKOCR, 0.0)$  ! amount of kidney CaE  
 inhibited (umol)  
 $RKPCR = AKCR * KIKCR * CKO$  ! rate of oxon consumption  
 (umol/hr)  
 $AKPCR = integ(RKPCR, 0.0)$  ! amt of oxon consumed (umol)

!kidney B-esterase (AChE, BuChE, and CaE) activity  
 $KBE = AKCE + AKBE + AKCR$  ! total B-esterase = AChE + BuChE + CaE (umol)  
 $IKE = 100 * (KBE / (ANKCE + ANKBE + ANKCR))$  ! % total B-esterase inhibition  
 $KCE = 100 * (AKCE / ANKCE)$  ! % AChE inhibition  
 $KBES = 100 * (AKBE / ANKBE)$  ! % BuChE inhibition  
 $KCR = 100 * (AKCR / ANKCR)$  ! % CaE inhibition

!blood  
 $RABLO = QC * (CVO - CBLO) - (RBLPBE + RBLPCR + RBLPCE + RRPBCE)$  !rate of change in blood (umol/hr)  
 $ABLO = integ(RABLO, 0.0)$  !amount in blood (umol)  
 $CBLO = ABLO / VBL$  !concn in blood (umol/L)

$CVO = (QH * CVLO + QF * CVFO + QS * CVSO + QR * CVRO + QBr * CVBrO + QK * CVKO) / QC$  !concn in venous blood  
 (umol/L)

! AChE activity--plasma  
 $RBLCE = KSBLC - ABLCE * (KDBLCE + KIBLCE * CBLO) + BLOCE * KRBLCE$  ! rate of AChE activity (umol/hr)  
 $ABLCE = INTEG(RBLCE, IBLCE)$  ! plasma AChE activity (umol)  
 $RNBLCE = KSBLC - ANBLCE * KDBLCE$  ! rate of baseline AChE act  
 (umol/hr)  
 $ANBLCE = INTEG(RNBLCE, IBLCE)$  ! Amt of baseline AChE (umol)  
 $RBLOCE = ABLCE * KIBLCE * CBLO - BLOCE * (KABLCE + KRBLCE)$  ! rate of AChE inhibition (umol/hr)  
 $BLOCE = INTEG(RBLOCE, 0.0)$  ! amount of plasma AChE  
 inhibited (umol)

$RBLPCE = ABLCE * KIBLCE * CBLO$  ! rate of oxon consumption  
 (umol/hr)  
 $ABLPCE = \text{integ}(RBLPCE, 0.0)$  ! amt of oxon consumed (umol)

! BuChE activity--plasma  
 $RBLBE = KSBLBE - ABLBE * (KDBLBE + KIBLBE * CBLO) + BLOBE * KRBLBE$  ! rate of BuChE activity (umol/hr)  
 $ABLBE = \text{INTEG}(RBLBE, IBLBE)$  ! plasma BuChE activity (umol)  
 $RNBLBE = KSBLBE - ANBLBE * KDBLBE$  ! rate of baseline BuChE act  
 (umol/hr)  
 $ANBLBE = \text{INTEG}(RNBLBE, IBLBE)$  ! Amt of baseline BuChE (umol)  
 $RBLOBE = ABLBE * KIBLBE * CBLO - BLOBE * (KABLBE + KRBLBE)$  ! rate of BuChE inhibition  
 (umol/hr)  
 $BLOBE = \text{INTEG}(RBLOBE, 0.0)$  ! amount of plasma BuChE  
 inhibited (umol)  
 $RBLPBE = ABLBE * KIBLBE * CBLO$  ! rate of oxon consumption  
 (umol/hr)  
 $ABLPBE = \text{integ}(RBLPBE, 0.0)$  ! Amt of oxon consumed (umol)

! CaE activity--plasma  
 $RBLCR = KSBLCR - ABLCR * (KDBLCR + KIBLCR * CBLO) + BLOCR * KRBLCR$  ! rate of CaE activity (umol/hr)  
 $ABLCR = \text{INTEG}(RBLCR, IBLCR)$  ! plasma CaE activity (umol)  
 $RNBLCR = KSBLCR - ANBLCR * KDBLCR$  ! rate of baseline CaE act  
 (umol/hr)  
 $ANBLCR = \text{INTEG}(RNBLCR, IBLCR)$  ! Amt of baseline CaE (umol)  
 $RBLOCR = ABLCR * KIBLCR * CBLO - BLOCR * (KABLCR + KRBLCR)$  ! rate of CaE inhibition  
 (umol/hr)  
 $BLOCR = \text{INTEG}(RBLOCR, 0.0)$  ! amount of plasma CaE  
 inhibited (umol)  
 $RBLPCR = ABLCR * KIBLCR * CBLO$  ! rate of oxon consumption  
 (umol/hr)  
 $ABLPCR = \text{integ}(RBLPCR, 0.0)$  ! amt of oxon consumed (umol)

!plasma B-esterase (AChE, BuChE, and CaE) activity  
 $BLBE = ABLCE + ABLBE + ABLCR$  !total B-esterase=AChE+BuChE+CaE  
 (umol)  
 $IBLE = 100 * (BLBE / (ANBLCE + ANBLBE + ANBLCR))$  ! % Total B-esterase inhibition in plasma  
 $BLCE = 100 * (ABLCE / ANBLCE)$  ! % AChE inhibition in plasma  
 $BLBES = 100 * (ABLBE / ANBLBE)$  ! % BuChE inhibition in plasma  
 $BLCR = 100 * (ABLCR / ANBLCR)$  ! % CaE inhibition in plasma

! AChE activity--RBCs  
 $RRBCCE = KSRBCE - ARBCE * (KDRBCE + KIRBCE * CBLO) + RBCOCE * KRRBCE$  ! rate of AChE activity  
 (umol/hr)  
 $ARBCE = \text{INTEG}(RRBCCE, IRBCE)$  ! RBC AChE activity (umol)  
 $RNRBCCE = KSRBCE - ANRBCE * KDRBCE$  ! rate of baseline AChE act  
 (umol/hr)  
 $ANRBCE = \text{INTEG}(RNRBCCE, IRBCE)$  ! Amt of baseline AChE  
 (umol)

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RRBOCE=ARBCE*KIRBCE*CBLO-RBCOCE*(KARBCE+KRRBCE)          ! rate of AChE inhibition
(umol/hr)
RBCOCE=INTEG(RRBOCE,0.0)                                     ! amount of RBC AChE inhibited
(umol)
RRBPCE=ARBCE*KIRBCE*CBLO                                     ! rate of oxon consumption
(umol/hr)
ARBPCE=integ(RRBPCE,0.0)                                     ! amt of oxon consumed (umol)

!RBC B-esterase (AChE only)activity
RBCCE=100*(ARBCE/ANRBCE)                                     ! % AChE inhibition in RBCs
RBCPERC=100-RBCCE                                           ! % inhibited
versus inhibition

!-----
!metabolite excretion
!MCA + DCA metabolites--compartment 1
RAMCA = RAKM2+RABRM2+RALM2          !total rate of metabolite formation (umol/hr)
AMCA = integ(RAMCA, 0.0)             !amount of MCA+DCA (umol)
RA1 = RAMCA-RAMCAex                  !rate of change of MCA+DCA in compartment 1 (umol/hr)
A1 = integ(RA1,0.0)                  !amount of MCA+DCA in compartment 1 (umol)
CBMCA = A1/V1                        !concentration of MCA+DCA in compartment 1 (umol/L)
RAMCAex = A1*KE1                     !rate of MCA+DCA excretion (umol/hr)
AMCAex = integ(RAMCAex,0.0)          !amount of MCA+DCA excreted (umol)

end !dynamic

!-----
!mass balance--update this
TMASSMAL=ASTOM+AINTM+ALM+AKM+AFM+ASMaI+ABRM+ARM+ABL+ALU    !umol
TMASSMOX=ASTOMO+AINTO+ALO+AFO+ASO+AKO+ARO+ABRO+ABLO        !umol
TMASSMCA=A1+AMCAEX                                           !umol
TMASSODT=AALOD+AAKOD+AABRO                                   !umol
TMASSOXONNC=AHPCE+AHPBE+AHPCE+ABPCE+ABPBE+ABPCR+AKPCE+AKPBE+AKPCR+ABLPCE+ABLPB
E+ABLPCE+ARBPCE      !non-catalytic oxon detox (umol)
TMASSTOTAL=TMASSMAL+TMASSMOX+TMASSMCA+TMASSODT+TMASSOXONNC    !umol--total
better add up to what went in

TDOSE=ODOSE+ODOSEMAL+ODOSEMOX+ADOSEM+ADOSEO+ODOSEO+ODCHILD2+ALUINH+ALUNGDO
SE-AEX+ADLT+ADL+ASURF    !total dose (umol)
MASSBAL=TDOSE/(TMASSTOTAL+1E-99) !should equal 1 (or really close to it)

end !derivative
end !program

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