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OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

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SUBJECT: Estimating Malathion Points of Departure (PODs) or Average Blood Concentrations for Developmental Neurotoxicity Assessment using a Physiologically Based Pharmacokinetic-Pharmacodynamic (PBPK-PD) Model

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FROM: Dan Hoer, Ph.D., Physical Scientist
Cody Addington, Ph.D., Physical Scientist
Risk Assessment Branch VIII (RAB8)
Health Effects Division (HED; 7509T)

THROUGH: Brandall Ingle, Ph.D., Lead Biologist
Risk Assessment Branch VIII (RAB8)
Health Effects Division (HED; 7509T)

TO: Cecilia Tan, Ph.D., Senior Scientist
Immediate Office (IO)
Health Effects Division (HED; 7509T)

Estimating Malathion Points of Departure (PODs) or Average Blood Concentrations for Developmental Neurotoxicity Assessment using a Physiologically Based Pharmacokinetic-Pharmacodynamic (PBPK-PD) Model

Prepared by OPP/HED/RAB8 on 10-April-2023

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Introduction

To replace the default uncertainty factors associated with interspecies extrapolation from an animal point of departure (POD), the registrant developed a physiologically based pharmacokinetic-pharmacodynamic (PBPK-PD) model to predict human PODs based on a maximum of 10% acetylcholinesterase (AChE) inhibition in humans. A review of the model code, model modifications, and determination of updated points of departure (PODs) for malathion was conducted by the Agency and reported in *"Report on Malathion and Dimethoate Physiologically-Based Pharmacokinetic (PBPK) Model Review and Point of Departure (POD) Calculations"* (D. Hoer, C. Addington, B. Ingle, TXR 0058366, 12/01/2021).

The rat version of the model was subsequently used by Agency scientists to calculate average blood concentrations at *in vivo* PODs based on 10% AChE inhibition to compare with half-maximal activity concentration (AC_{50}) values from a battery of *in vitro* assays that assess processes critical to development of the nervous system (hereafter referred to as the DNT NAM battery). This comparison evaluated the relative sensitivity of activity in the DNT NAM battery to AChE inhibition.

Herein we briefly describe how to use the model files to estimate PODs based on AChE inhibition as well as average blood concentrations for comparison to AC_{50} values from the DNT NAM battery. This document is meant to serve as a technical resource for understanding the storage of the model files and their use.

Code Storage

To simplify storage and distribution of the model code, it has been organized into a pdf portfolio; this format allows for organization and dissemination of multiple file types in a single, uncompressed unit. The portfolio is organized into folders based on target organism (human or rat) and then within each organism there are subfolders for each modeled exposure pathway (e.g., dietary, dermal, and inhalation). For the rat, there is an additional subfolder for calculating the average blood concentration. Each subfolder contains several files: scenario files, a parameter file, and the main PBPK-PD model. The scenarios require both the parameter and main model to be in the same directory to function properly. Therefore, one can extract the files at the organism level or the exposure pathway level and retain all the necessary files for proper operation. Extraction of the scenarios individually requires that the user also extract the model and parameters to the same directory.

For conciseness, the scenario names contain an organism (human or rat), number, output (POD; average blood concentrations are calculated in batch), and chemical (parent or oxon). A description of the conditions simulated by each numbered scenario file is shown in Tables 1 through 4. Average blood concentrations were only calculated using the rat model and are calculated in batch using the scenario file entitled "run_pod_blood_conc.R"; a description of simulated conditions are shown in Table 5. The numbers shown in Tables 1 through 5 correspond to the scenario numbers in the filenames of the model code; for example, the scenario file named 'HUMAN_3_PARENT_POD.R' will determine the AChE-based POD for acute dietary exposure to malathion in human children 3-5 years of age (i.e., scenario 3 in Table 1).

Table 1. Numbered simulations of human dietary exposure to malathion or malaoxon in food. All food scenarios assume a single exposure per day. Acute scenarios simulate 2 days of time with chemical exposure occurring only on the first day, whereas steady state scenarios model 123 days of daily exposure.

Scenario Number	Population Subgroup	Exposure Frequency	Bodyweight (kg)	Parent, Oxon, or Both
1	All infants (<1 year old)	Acute	4.8	Both
2	Children (1-2 years)	Acute	12.6	Both
3	Children (3-5 years)	Acute	18.7	Both
4	Children (6-12 years)	Acute	37.1	Both
5	Youth (13-19 years)	Acute	67.3	Both
6	Adults (20-49 years)	Acute	81.5	Both
7	Adults (50-99 years)	Acute	81.2	Both
8	Female (13-49 years)	Acute	72.9	Both

Scenario Number	Population Subgroup	Exposure Frequency	Bodyweight (kg)	Parent, Oxon, or Both
9	All infants (<1 year old)	Steady State	4.8	Both
10	Children (1-2 years)	Steady State	12.6	Both
11	Children (3-5 years)	Steady State	18.7	Both
12	Children (6-12 years)	Steady State	37.1	Both
13	Youth (13-19 years)	Steady State	67.3	Both
14	Adults (20-49 years)	Steady State	81.5	Both
15	Adults (50-99 years)	Steady State	81.2	Both
16	Female (13-49 years)	Steady State	72.9	Both

Table 2. Numbered simulations of human dietary exposure to malathion and malaoxon in drinking water. Drinking water scenarios with individuals 12 years old or younger assume 6 exposure events per day; scenarios with individuals 13 or older assume 4 exposure events per day. As in food exposure, acute scenarios simulate 2 days of time with chemical exposure occurring only on the first day, whereas steady state scenarios model 123 days of daily exposure.

Scenario Number	Population Subgroup	Exposure Frequency	Bodyweight (kg)	Parent, Oxon, or Both
17	All infants (<1 year old)	Acute	4.8	Both
18	Children (1-2 years)	Acute	12.6	Both
19	Children (3-5 years)	Acute	18.7	Both
20	Children (6-12 years)	Acute	37.1	Both
21	Youth (13-19 years)	Acute	67.3	Both
22	Adults (20-49 years)	Acute	81.5	Both
23	Adults (50-99 years)	Acute	81.2	Both
24	Female (13-49 years)	Acute	72.9	Both
25	All infants (<1 year old)	Steady State	4.8	Both
26	Children (1-2 years)	Steady State	12.6	Both

Scenario Number	Population Subgroup	Exposure Frequency	Bodyweight (kg)	Parent, Oxon, or Both
27	Children (3-5 years)	Steady State	18.7	Both
28	Children (6-12 years)	Steady State	37.1	Both
29	Youth (13-19 years)	Steady State	67.3	Both
30	Adults (20-49 years)	Steady State	81.5	Both
31	Adults (50-99 years)	Steady State	81.2	Both
32	Female (13-49 years)	Steady State	72.9	Both

Table 3. Numbered simulations of human inhalation and incidental oral exposure to malathion or malaoxon in residential and occupational settings. All scenarios simulate 123 days of time with chemical exposures occurring as outlined in the table.

Scenario Number	Population Subgroup	Exposure Frequency	Bodyweight (kg)	Parent, Oxon, or Both
33	Occupational Worker: Inhalation	8.3 L min^{-1} 5 days $week^{-1}$ 8 hours day^{-1}	69	Both
34	Occupational Worker: Inhalation	16.7 L min^{-1} 5 days $week^{-1}$ 8 hours day^{-1}	69	Both
35	Occupational Worker: Inhalation	26.7 L min^{-1} 5 days $week^{-1}$ 8 hours day^{-1}	69	Both
36	Occupational Worker: Inhalation	28.8 L min^{-1} 5 days $week^{-1}$ 8 hours day^{-1}	69	Both
37	Residential Handler: Inhalation	10.7 L min^{-1} 7 days $week^{-1}$ 1 hour day^{-1}	69	Both
38	Aerial Mosquitocide: Inhalation, Adult	10.7 L min^{-1} 7 days $week^{-1}$ 1.5 hour day^{-1}	69	Both
39	Aerial Mosquitocide: Inhalation, Child	5.5 L min^{-1} 7 days $week^{-1}$ 1.5 hour day^{-1}	11	Both
40	Non-Occupational Bystander: Inhalation, Ambient Air (Max Conc), Adult	10.7 L min^{-1} 24 hours day^{-1} 1 day	69	Both

Scenario Number	Population Subgroup	Exposure Frequency	Bodyweight (kg)	Parent, Oxon, or Both
41	Non-Occupational Bystander: Inhalation, Ambient Air (Max Conc), Child	5.5 L min^{-1} 24 hours day^{-1} 1 day	11	Both
42	Non-Occupational Bystander: Inhalation, Ambient Air (Avg. Conc), Adult	10.7 L min^{-1} 24 hours day^{-1} 7 days $week^{-1}$	69	Both
43	Non-Occupational Bystander: Inhalation, Ambient Air (Avg. Conc), Adult	5.5 L min^{-1} 24 hours day^{-1} 7 days $week^{-1}$	11	Both
44	Non-Occupational Bystander: Inhalation, Ambient Air (Max Conc), Adult	10.7 L min^{-1} 2 hours day^{-1} 1 day	69	Both
45	Non-Occupational Bystander: Inhalation, Ambient Air (Max Conc), Child	5.5 L min^{-1} 2 hours day^{-1} 1 day	11	Both
46	Mosquitocide: Incidental Oral Children 1 to <2	7 days $week^{-1}$ 1.5 hours day^{-1}	11	Both

Table 4. Numbered simulations of human dermal exposure to malathion or malaoxon in residential and occupational settings. All scenarios simulate 123 days of time with chemical exposures occurring as outlined in the table. The dermal absorption factor for human scenarios was set to 1% over 8 hours.

Scenario Number	Population Subgroup	Exposure Frequency	Bodyweight (kg)	Parent, Oxon, or Both
47	Occupational Handler & Post-Application	5 days $week^{-1}$ 8 hours day^{-1}	69	Both
48	Residential Handler	7 days $week^{-1}$ 1.1 hours day^{-1}	69	Both
49	Residential Post-Application: Gardens, Adult	7 days $week^{-1}$ 2.2 hours day^{-1}	69	Both

Scenario Number	Population Subgroup	Exposure Frequency	Bodyweight (kg)	Parent, Oxon, or Both
50	Residential Post-Application: Gardens, Child	7 days $week^{-1}$ 1.1 hours day^{-1}	32	Both
51	Residential Post-Application: Pick Your Own, Adult	7 days $week^{-1}$ 5 hours day^{-1}	69	Both
52	Residential Post-Application: Pick Your Own, Child	7 days $week^{-1}$ 1.9 hours day^{-1}	32	Both
53	Residential Post-Application: Spray Drift, Mosquitocide Aerial and Ground, Adult	7 days $week^{-1}$ 1.5 hours day^{-1}	69	Both
54	Residential Post-Application: Spray Drift, Mosquitocide Aerial and Ground, Child	7 days $week^{-1}$ 1.5 hours day^{-1}	11	Both

Table 5. Numbered simulations of rat oral, dermal, and inhalation exposure to malathion used for calculating average blood concentrations. Simulated doses shown are AChE-based PODs from the previous Malathion risk assessment (S. Shelat, 09 June 2016, D414107). Scenario conditions are established to match the *in vivo* study from which the POD was derived. The dermal absorption factor for rat scenarios was set to 2.7% over 8 hours, to match the dermal absorption determined in the *in vivo* rat study. All steady state exposures were simulated for 32 days.

Scenario Number	Population Subgroup	Exposure Frequency	Bodyweight (kg)	Parent, Oxon, or Both	BMD_{10} for AChE inhibition in rat (mg/kg bw/day)[a]
1	Rat Dietary	Acute	0.25	Parent	450[b]
2	Rat Dietary	Steady State 32 days	0.25	Parent	25[c]
3	Rat Inhalation	5 days $week^{-1}$ 6 hours day^{-1} 32 days	0.25	Parent	53[d]
4	Rat Dermal	7 days $week^{-1}$ 6 hours day^{-1} 32 days	0.25	Parent	2200[e]

[a] BMD_{10} values were obtained from the 2016 draft risk assessment unless otherwise noted (S. Shelat, D414107, 09-JUN-2016).

[b] The AChE-based POD used for simulations for acute oral exposure was the lowest observed adverse effect level (LOAEL) based on 17% AChE inhibition in adult female rats (MRID 45566201). This value was selected

because the PBPK-PD rat model simulates adult individuals and available BMD_{10} value from the 2016 DRA was from studies using PND11 rat pups (S. Shelat, 09 June 2016, D414107).

[c] The value used for simulations of steady state oral exposure was the mean BMD_{10} from *in vivo* steady-state oral exposure (S. Shelat, D414107, 09-JUN-2016).

[d] The BMD_{10} from the 90-day inhalation study in male rats (0.17 mg/L/day) was converted from a concentration in inhaled air (0.17 mg/L) to a dose to match the units in dermal and oral exposures (0.17 mg/L × 12.9 L/hr × 6 hrs/day ÷ 0.25 kg, where 12.9 L/hr was the modeled rat respiration rate, 6 hrs/day was the exposure duration in the study, and 0.25 kg was the modeled rat bodyweight).

[e] Since the available BMD_{10} from the 2016 DRA was determined from a rabbit *in vivo* study, the AChE-based POD used for simulations was predicted using the rat PBPK-PD model.

Model Operation and Use

The model submitted to the Agency on behalf of the registrant was built in R and can be run from the command line or from RStudio. The model scenarios use packages outside of base R (e.g., “parallel”, “dplyr”, “tidyr”, and “readxl”), which, prior to running any scenarios, will need to be installed by the user.

These can be installed via the RStudio GUI or via the install.packages() command in the terminal. Further, as discussed above, the scenario files need to be in the same directory as the model and parameter files to function properly. The structure utilized in the pdf portfolio is such that the user can extract the files at the organism level or the exposure pathway level and retain all the necessary files for proper operation. Extraction of the scenarios individually requires that the user also extract the appropriate model and parameters to the same directory.

The model is run using the only the scenario files; that is to say, the scenario files will source the main model and the associated parameter files automatically without need for user intervention. In order to generate an average blood concentration or POD value, the user will run the scenario file of interest and the script will print the resulting POD to the terminal (in mg kg bw^{-1} day^{-1}) along with the minimum AChE activity (as a percentage; i.e., 90% activity would indicate 10% inhibition) for POD scenarios. For average blood concentration calculations, the scenario will read scenario-specific parameters from an Excel file entitled “malathion_rat_pods.xlsx”; this file is stored in the average blood concentration subfolder and requires no modification to reproduce the appropriate values. When calculating the average blood concentrations, the scenario script will automatically calculate the average blood concentration for all scenarios shown in Table 5 and will output the results in a comma delimited text file entitled "updated_malathion_rat_pods.csv". This file will be saved in the directory where the model and scenario files are saved on the user’s machine. The output contains all the provided scenario data as well as the calculated average blood concentration and simulated minimum AChE activity (as a percentage).

The scenario does not, by default, set the directory to the location of the file being used. If using RStudio, pasting the command “setwd(dirname(rstudioapi::getActiveDocumentContext()$path))” below the “rm(list = ls())” command in a given scenario will set the working directory to the location of the file in use; this command is in place in the scenario files, but it is currently commented out. This will not work outside of RStudio and the user will need to manually set the working directory using the setwd() command.

As stored in the portfolio, the scenarios are set up to recreate the POD or average blood concentration estimates shown in other Agency memos (e.g., *"Report on Malathion and Dimethoate Physiologically-Based Pharmacokinetic (PBPK) Model Review and Point of Departure (POD) Calculations"* (D. Hoer, C. Addington, B. Ingle, TXR 0058366, 12/01/2021)). No user modifications are required to reproduce the appropriate values.