Evaluation of Carbaryl and Malathion Human Studies For Their Proposed Application in a Physiologically-Based Pharmacokinetic Model for Risk Assessment

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List of Acronyms

AChE: Acetylcholinesterase
ADME: Absorption, Distribution, Metabolism, and Excretion
AOP: Adverse Outcome Pathway
DCA: Malathion Dicarboxylic Acid
DER: Data Evaluation Record
DMDTP: Dimethyl Dithiophosphate
DMP: Dimethyl Phosphate
DMTP: Dimethyl Thiophosphate
ECG: Electrocardiography
HED: Health Effects Division
HSRB: Human Studies Review Board
MCA: Malathion Monocarboxylic Acid
MOA: Mode of Action
NMC: *N*-Methyl Carbamate
OP: Organophosphate
OPP: Office of Pesticides Program
PBPK: Physiologically-Based Pharmacokinetic
PD: Pharmacodynamic
PND: Postnatal Day
PoD: Point of Departure
RBC: Red Blood Cell

1.0 Background and Introduction

The purpose of this report is to present an overview of two different human studies to the Human Studies Review Board (HSRB) for evaluation of their individual scientific validity. The human studies that are presented are single oral dose studies for the active ingredients carbaryl and malathion. The following are the citations for the human studies discussed in this report.

- Carbaryl citation:
  - May, D.G., *et al*. (1992). Cimetidine-Carbaryl Interaction in Humans: Evidence for an Active Metabolite of Carbaryl. *J Pharmacol Exp Ther*. **262** (3) 1057-1061.
- Malathion citations:
  - Aston, L.S. (2000). Determination of residues of malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in human urine. Pacific Toxicology Laboratories, 6160 Variel Avenue, Woodland Hills, CA 91367. PTL119801. October 11, 2000. MRID 45244601. Unpublished.
  - Gillies, D., Dickson, J. (2000). A randomised double blind ascending single oral dose study with malathion to determine the no effect level on plasma and RBC cholinesterase activity. Inveresk Research, Elphinstone Research Centre, Tranent, EH 32 2NE, Scotland. ICR 013177. March 20, 2000. MRID 45125602. Unpublished.

These studies are discussed and evaluated in this document. In addition, this document explains how the studies will be used to evaluate physiologically-based pharmacokinetic (PBPK) models applicable to each[1] as well as how the Office of Pesticide Programs (OPP) Health Effects Division (HED) proposes to use these data in risk assessment.

Agency reviews of these studies have been summarized in the following data evaluation records (DERs).

- US EPA. (2016). Data Evaluation Record. Carbaryl Human Study. TXR# 0057550.
- US EPA. (2016). Data Evaluation Record. Malathion Human Study. TXR# 0053266.

1.1 Physiologically-Based Pharmacokinetic (PBPK) Modeling

The relationship between an external dose of a chemical and its internal concentration is determined by a set of chemical properties (*e.g.*, solubility in blood and tissues, volatility, binding affinity to proteins) and properties of the biological system (*e.g.*, tissue volumes, blood flows, metabolic capabilities). Computational models that describe these chemical, physiological, and biochemical characteristics that are needed to predict a chemical's absorption, distribution, metabolism, and excretion (ADME) behaviors are commonly referred to as physiologically-based pharmacokinetic (PBPK) models. Since PBPK models incorporate relevant biology that determines the ADME processes, they are not only useful for predicting

[1] To clarify, EPA will consider the recommendations of the January 2017 HSRB as part of the basis of an evaluation of the PBPK models for both carbaryl and malathion that EPA will complete. This evaluation and the associated PBPK models will undergo external peer review in calendar year 2017.

internal dosimetry related to a certain chemical within specific testing conditions (*e.g.*, dose ranges, species, tissues), but also for extrapolating outside of the studied conditions. Such capability makes PBPK models particularly useful in risk assessment where extrapolation to varied exposure conditions is needed (e.g., consideration of doses well below those for which data are available). PBPK models have been used to assist high-to-low dose, route-to-route, and interspecies extrapolations necessary for estimating human risk on the basis of animal toxicity studies or *in vitro* bioactivity data. The physiological structure of PBPK models also allows for examining the effects of changing physiology, such as aging, early life-stage, or pregnancy.

The Agency recognizes the value of using PBPK models to predict internal target tissue doses for risk assessment applications based on the assumption that similar tissue responses arise from equivalent target tissue doses across species and exposure conditions. The 2006 EPA report on *Approaches for the applications of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment* (US EPA, 2006) states that "PBPK model analysis is accepted as a scientifically sound approach to estimating the internal dose of a chemical at a target site and as a means to evaluate and describe the uncertainty in risk assessments." The report also states that PBPK models intended for risk assessment applications should be evaluated for "model purpose, model structure, mathematical representation, parameter estimation, computer implementation, and predictive capacity." Specifically, for evaluating a model's predictive capacity, the primary goal is to examine whether the model and corresponding parameters can adequately simulate the available time course of tissue/blood data over a range of doses. This process includes both model calibration, which means adjusting relevant parameter values to fit the available data, and comparison to data that are not used to calibrate the model. A PBPK model that simulates human ADME behaviors is often constructed without being calibrated using human data. Often times, a parallelogram (or allometric scaling) approach is used to build a PBPK model for humans from an animal model that has been calibrated and evaluated using available data. In the rare case that human *in vivo* data are available, these observed tissue/blood concentrations can be compared to model simulations to evaluate a human PBPK model's predictive capacity. In the event in which model simulations do not agree with human data, the modelers may decide to use the human data to calibrate the model which is the case in this situation for carbaryl and malathion.

Specifically, for carbaryl, the data from the presented human study are only expected to be used for establishing confidence in the human version of the model by comparing these human data with model simulations. Time courses of both carbaryl concentrations in plasma and red blood cell (RBC) AChE inhibition data will be used to evaluate the predictive capability of the carbaryl PBPK-PD model. For malathion, the data from the presented human study are used for the same purpose of examining whether the model can adequately simulate the human data. Time courses of metabolites concentrations in urine, but not RBC AChE inhibition data, will be used to evaluate the predictive capability of the malathion PBPK-PD model. If there are large discrepancies between observed data and model simulations, these human data will be used to adjust model parameters to calibrate the human version of the model for each chemical.

1.2 Acetylcholinesterase Inhibition: Organophosphates and *N*-Methyl Carbamates

The information presented below is intended to provide context pertaining to how the PBPK models that are under development will be used in risk assessment. The two chemicals mentioned in the introduction that are the subject of this HSRB review are from two different classes of pesticides, but both are known acetylcholinesterase inhibitors. Carbaryl is a *N*-methyl carbamate (NMC) pesticide and malathion is an organophosphate (OP) pesticide. There are similarities and important differences between these two classes. The initiating event in the adverse outcome pathway (AOP)/mode of action (MOA) for both classes is inhibition of the enzyme acetylcholinesterase leading to accumulation of acetylcholine and ultimately to neurotoxicity in the central and/or peripheral nervous system. However, the AOPs/MOAs are differentiated by their action upon the active site of the enzyme, which results in clear differences in the timing and duration of inhibition between the two classes.

NMCs inhibit AChE via carbamylation of the serine residue located in the active site of the enzyme (see Figure 1). The NMC inhibition of AChE occurs via carbamylation of the serine hydroxyl group results in a reversible binding process thus allowing for rapid reactivation of the enzyme. The NMCs, therefore, have a unique mode of action that results in rapid onset and recovery of the enzyme. The time to peak inhibition for NMCs is typically between 15 to 45 minutes while complete recovery of the enzyme is achieved within minutes to hours (US EPA, 2007). Therefore, for NMCs, including carbaryl, repeated daily exposure does not result in an increased inhibition of AChE since enzyme recovery is complete before the next acute exposure, and only acute exposure durations are a concern for neurotoxic effects.

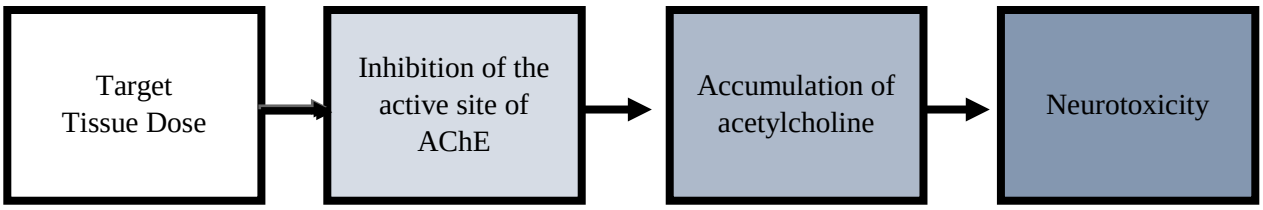

Figure 1. Adverse outcome pathway for OPs and NMCs.

In contrast, OPs inhibit AChE via phosphorylation of the serine hydroxyl group at the active site of the enzyme, which results in an irreversible inhibition of the bound enzyme. Inhibition occurs within a few hours and continues until new, uninhibited enzymes are produced. This results in the OPs exhibiting a phenomenon known as steady-state AChE inhibition. After repeated dosing with an OP at the same dose level, the degree of AChE inhibition comes into equilibrium with the production of new, uninhibited enzyme. At this point, the amount of AChE inhibition at a given dose remains consistent across duration. Therefore, acute and steady state exposure durations are of concern for OPs.

2.0 Chemical Specific Reviews

For carbaryl and malathion, a summary of toxicological effects is presented below followed by a summary of the studies under review. Additional information can be found in the associated DERs.

2.1 Carbaryl

Carbaryl is one of the most widely used broad spectrum insecticides in agriculture, professional turf management, professional ornamental production, and in the residential lawn and garden markets. For carbaryl, most of the toxicological studies were conducted in laboratory animals; however, studies are available that involved direct dosing of humans with carbaryl.

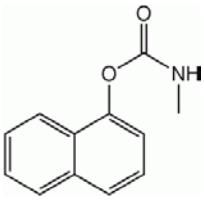

Figure 2. Structure of Carbaryl.

2.1.1 Summary of Toxicological Effects

Based on the available data in laboratory animals, the nervous system is the primary target for carbaryl and AChE inhibition is the most sensitive non-cancer endpoint in multiple species, durations, lifestages, and routes. Oral, dermal, and inhalation studies that evaluate AChE inhibition are available for carbaryl. Inhibition has been shown to be rapid, but also reversible within minutes to hours. Therefore, repeated daily exposure does not result in an increased inhibition of AChE since enzyme recovery is complete before the next acute exposure. As a result, acute exposures are the exposure duration of concern for carbaryl neurotoxicity.

The carbaryl database for neurotoxicity is complete, with acceptable acute, subchronic, and developmental neurotoxicity studies in animals. In the acute and subchronic neurotoxicity studies in rats, clinical signs of toxicity were observed that were consistent with AChE inhibition, including decreased motor activity, tremors, altered gait, decreased body temperature, decreased arousal, pinpoint pupils, increased salivation, and decreased grip strength. Increased inhibition of RBC, plasma, blood, and brain AChE was measured at the same doses that induced clinical signs. Other effects in rodents, mostly at higher doses, included cataracts, degeneration of sciatic nerve and muscle, pneumonitis, hepatocyte hypertrophy, and kidney disease.

Data are available describing the time to peak AChE inhibition and dose-response of carbaryl brain and blood AChE inhibition in several studies in rats. The time from exposure to peak AChE inhibition is well understood for carbaryl. Studies have found that peak inhibition occurs within 30-60 minutes in adults and within 15-45 minutes in postnatal day (PND) 17 pups (E. Reaves, 2007). The enzyme recovery half-lives estimated using the AChE inhibition time-course data were 5.4 hours for PND 17 pups and 1.8 hours for adult rats. AChE activity was recovered

to pre-treatment levels in adult and juvenile rats within 24 hours. This is consistent with a metabolism study that found slower metabolism of carbaryl in juveniles than in adults (Moser et al., 2013). A comparative cholinesterase study is available for carbaryl that directly compares the time to peak inhibition and magnitude of AChE inhibition in the young (both PND 11 and PND 17 pups) to adults. The data demonstrate that PND 11 pups are more sensitive to carbaryl than adult rats.

The complete toxicology database is available for carbaryl, including studies assessing developmental, reproductive, and carcinogenic effects. However, these studies are not relevant to the development or evaluation of the PBPK model.

2.1.2 Summary of Human Study

In a human study evaluating AChE inhibition resulting from carbaryl exposure (May, D.G., *et al*., 1992), four non-smoking, drug-free normal males were dosed with carbaryl and at another time with carbaryl following pre-treatment with cimetidine. Cimetidine is an H_2-histamine receptor blocker that is known to reduce the activity of cytochrome P450 isozymes. Pre-treatment with cimetidine was conducted to allow for the evaluation of the contribution of cytochrome P450s to carbaryl metabolism. *In vitro* assays were also conducted; however, these data will not be used for the PBPK model development, and therefore, are not discussed in this White Paper. Details of the *in vitro* studies can be found in the study data evaluation record.

Carbaryl concentrations were measured in plasma at 0, 10, 20, 30, 45, 60, 90, 120, 150, 210, and 240 minutes following exposure, and the area under the plasma concentration-time curve was estimated. Oral clearance was calculated. RBC AChE activity was determined at each time point. This study did not measure any metabolites of carbaryl.

Following oral administration of 1 mg/kg carbaryl, the mean peak plasma concentration was 0.26 ± 0.14 μg/ml at 30 minutes after exposure. The mean plasma half-life for carbaryl was 0.79 ± 0.47 hours in this study. The apparent oral clearance for carbaryl (5.4 ± 2.0 L/min) was greater than would be expected for liver blood flow. The maximal reduction in RBC AChE activity (27 ± 12%) was measured at 30 minutes after dosing. The RBC AChE activity recovery half-life was approximately 2.6 ± 1.5 hours, which was slower than the plasma half-life.

One week after the first dose of carbaryl, the same four male subjects were treated with 200 mg of cimetidine every 8 hours for 3 days. On the third day, 1 mg/kg of carbaryl was administered one hour after the last dose of cimetidine. Pre-treatment with cimetidine reduced the clearance by 50% which resulted in a 2-fold increase in peak plasma concentration. These findings support the hypothesis that the main site of metabolism for carbaryl is the liver by drug-metabolizing enzymes that can be inhibited by cimetidine. Despite the increased systemic availability, the concentration of carbaryl required to achieve a 20% inhibition of AChE activity increased by over an order of magnitude in the presence of cimetidine.

For the purposes of the PBPK model, the carbaryl concentrations measured in the plasma and AChE inhibition data from this study will be used to evaluate the predictive capability of the

model. There were no scientific issues identified during the review of this study (TXR# 0057550).

2.1.3 Utility of Carbaryl Human Data for PBPK Model

For the carbaryl PBPK-pharmacodynamic (PD) model for humans, the predictive capability of the model will be evaluated using published time-concentration data of carbaryl in human plasma, as well as AChE inhibition over time (May, D.G., *et al*., 1992). Specifically, the PBPK-PD model will simulate carbaryl exposure following a single oral dose at 1 mg/kg to predict carbaryl concentrations in plasma during and after exposure, up to 4 hours. The model will also predict RBC AChE inhibition over the same period of time. Preliminary simulations have shown reasonable agreement between model predictions and observed data. Thus, it is likely that no further adjustment of model parameters will be needed as the model developers continue to refine the model to account for life-stage differences. The OPP plans to use the human PBPK-PD model to derive points of departure (PoDs) based on 10% RBC AChE inhibition for various exposure scenarios (e.g., dietary exposure, drinking water exposure, residential exposure). As described earlier, when fundamental biochemical processes are properly described in a PBPK model, its primary power lies in its capability to extrapolate to species of interest and exposure scenarios for which no pharmacokinetic data are available to characterize the relationship between external doses and internal dose metrics. In the past, the Agency has used toxicity studies conducted with laboratory animals for selecting PoDs for the carbaryl human health risk assessment. The reference dose for risk assessment is based on an external dose that has been administered to an animal and the default uncertainty factors to account for potential inter- and intra-species differences. The use of the PBPK-PD model for carbaryl allows for the derivation of an internal dose metric, which is a more accurate assessment of dose leading to an adverse effects. This refinement reduces the uncertainty in extrapolation, and therefore, the uncertainty factors incorporated into the reference dose used in risk assessment.

2.2 Malathion

Malathion is a non-systemic, wide spectrum OP insecticide. It is used in the agricultural production of a wide variety of food/feed crops to control insects such as aphids, leafhoppers, and Japanese beetles. It is also used in residential settings and in wide-area mosquitocide applications. For malathion, most of the toxicological studies were conducted in laboratory animals; however, studies are available that involved direct dosing of humans with malathion.

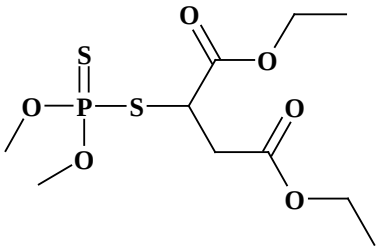

Figure 3. Structure of Malathion

2.2.1 Summary of Toxicological Effects

Malathion acts by inhibiting AChE in nerve cells, which is consistent with other OPs. This inhibition leads to accumulation of acetylcholine and ultimately to neurotoxicity in the central and/or peripheral nervous system. Clinical signs of neurotoxicity (such as, tremors, salivation, urogenital staining, and decreased motor activity) can be found throughout the database of experimental animal toxicity studies at doses higher (10-fold) than those causing AChE inhibition.

Based on the available animal data, RBC AChE inhibition is the most sensitive endpoint for all lifestages evaluated (adults, juveniles, pregnant dams, and fetuses) after oral and dermal exposure. There are no sex differences regarding AChE inhibition. However, endpoints from portal of entry effects (histopathological lesions of the nasal cavity and larynx) are shown to be more sensitive than AChE inhibition after inhalation exposure.

Malathion, like some other OPs, requires metabolic activation to its oxon metabolite (malaoxon) to inhibit AChE, with subsequent metabolism that leads to detoxification. Generally, absorption and distribution are rapid with extensive metabolism and no accumulation in the tissues. The steady state AChE inhibition is achieved after 11-15 days of exposure.

The complete toxicology database is available for malathion, including studies assessing developmental, reproductive, and carcinogenic effects. However, these studies are not relevant to the development or evaluation of the PBPK model.

2.2.2 Summary of Human Study

In a randomized double-blind study (Gillies, D. and Dickson, J., 2000; Aston, L.S., 2000), human volunteer subjects (27 males, 7 females, aged 18-50 years) were administered a single oral dose (0.5, 1.5, 5.0, 10.0, or 15.0 mg/kg in gelatin capsules) of malathion (Lot No. 50913-01, 95.4% chemical purity) after completing a light breakfast. Control subjects (11 males, 3 females) received a placebo (lactose in gelatin capsule). The study was conducted in 7 treatment blocks with ascending single oral dose of malathion. Vital signs and electrocardiography (ECG) were measured on day -1, pre-dose (0) and at 2,4, 8, and 24 hours after dosing. Blood samples were collected for hematology, clinical chemistry, plasma and RBC AChE activity measures, as well as plasma levels of malathion and its metabolite malaoxon. Urine samples were collected on all volunteers from 12 hours before dosing to 48 hours after dosing at -12-0, 0-12, 12-24, and 24-48 hour intervals and analyzed to evaluate the magnitude of five malathion metabolites: malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP).

Results of the double-blind study with human volunteer subjects showed that there were no significant treatment-related changes in vital signs, ECGs, hematology, clinical chemistry, urinalysis or physical examination in any subject during the study. There were no significant treatment-related effects on RBC and plasma AChE activities up to the highest dose tested (15 mg/kg). Although the statistical analysis on AChE activity was considered inadequate, it does not change the conclusion of the study. At this time, the plasma and RBC AChE data are not

appropriate for use as a point of departure for risk assessment. Plasma levels of malathion and malaoxon were both below quantifiable levels (<102 ng/mL and 99.8 ng/mL for malathion and malaoxon, respectively) 1-12 hours after dosing in all male and female volunteers who received 15 mg/kg malathion or placebo, respectively.

Urine samples collected from the volunteers were analyzed with focus on the detection and quantitation of malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in human urine. The analyses showed that excretion of malathion residues occurred primarily in the first 12 hours and generally increased with dose. MCA was the most prevalent urinary component excreted over 48 hours followed by DMTP, DCA, DMP, and DMDTP.

Two additional studies (Scott, D. L., et al., 1999) described and validated the analytical methods used for determination of malathion and malaoxon in human plasma and verified storage stability of malathion and malaoxon in plasma samples in support of the study described above. The GC method was validated by demonstrating adequate accuracy, precision, detection limits and limits of quantitation. Limit of detection was 0.0002 ppm for DCA and MCA, and 0.0125 ppm for DMP, DMTP, and DMDTP. Limit of quantitation was 0.020 ppm for DCA and MCA, and 0.025 ppm for DMP, DMTP, and DMDTP. Based upon established criteria for column efficiency, precision, resolution, detection and quantitation limits, and intraday accuracy and precision, the method was considered valid and appropriate.

For the purposes of the PBPK model, the only data from the malathion study that will be used are the urinary metabolite concentration data. The AChE inhibition data ~~measured~~ will not be utilized. There were no scientific issues identified during the review of this study (TXR# 0053266).

2.2.3 Utility of Malathion Human Data for PBPK Model

For the malathion PBPK-PD model for humans, the AChE inhibition data reported in the study will not be utilized. Instead, the predictive capability of the model will be evaluated using published urinary metabolite concentration data, specifically for MCA and DMP, as well as plasma levels of malathion and malaoxon (Gillies, D. and Dickson, J., 2000; Aston, L.S., 2000). The model will predict the time course of MCA and DMP concentrations in urine, and these predicted values will be compared to the observed data to examine whether the human model is capable of predicting malathion metabolism based on metabolism rates measured from the *in vitro* system. The model will also predict the time-concentrations profiles for malathion and malaoxon in plasma, which will be compared to the observed data. In the case which model predictions do not agree with the observed data, the urinary metabolite data and plasma concentrations for malathion and malaoxon (Gillies, D. and Dickson, J., 2000; Aston, L.S., 2000) may be used to calibrate the model by adjusting model parameters (*e.g.*, elimination rate constants) to fit the human data. Similar to the case of carbaryl, the OPP plans to use the human PBPK-PD model to derive PoDs based on 10% RBC AChE inhibition. The use of the human PBPK-PD model provides a scientifically sound basis to extrapolate across species, routes, and life-stages compared to the use of uninformative default uncertainty factors.

3.0 Summary

The Agency recognizes the value of using PBPK models to predict internal target tissue doses for risk assessment applications based on the assumption that similar tissue responses arise from equivalent target tissue doses across species and exposure conditions. Currently, there are PBPK models under development for carbaryl and malathion that will allow for the derivation of an internal dose metric, reducing the uncertainty from extrapolating data from animals to humans for human health risk assessment. In 2017, the PBPK models for both carbaryl and malathion will undergo external peer review, and the findings of the January 2017 HSRB will be used as part of the basis of the evaluation. The Agency's review determined that there were no scientific issues with the human studies.

4.0 References

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