RESPONSE TO EPA'S DRAFT BIOLOGICAL EVALUATION FOR MALATHION

FINAL REPORT

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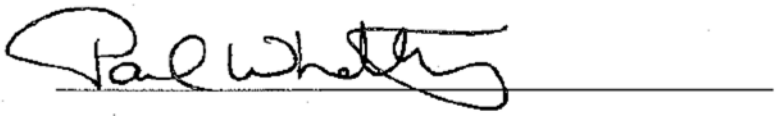
[signature]: 

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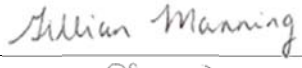
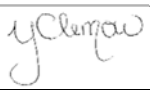
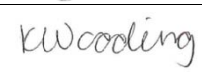
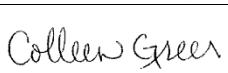
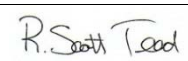
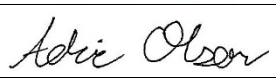
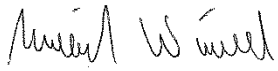
Gillian E. Manning, Environmental Risk Analyst II		June 10, 2016
Yvonne H. Clemow, Environmental Risk Analyst II		June 10, 2016
Katie L. Wooding, Environmental Risk Analyst II		June 10, 2016
Colleen D. Greer, Environmental Risk Analyst II		June 10, 2016
R. Scott Teed, Senior Scientist		June 10, 2016
Adric D. Olson, Environmental Risk Analyst II		June 10, 2016
Michael F. Winchell Senior Environmental Modeler / GIS Specialist		June 10, 2016
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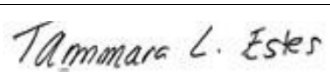
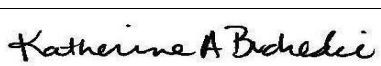
Tammy L. Estes Senior Research Scientist		June 10, 2016
Katherine Budreski Senior GIS Specialist		June 10, 2016

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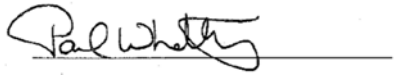
Paul Whatling Senior Registration Manager FMC Corporation EPA Agent for Cheminova A/S		June 10, 2016

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EXECUTIVE SUMMARY

The Environmental Protection Agency (EPA or "the Agency"), in conjunction with the Fish and Wildlife Services (FWS), National Marine and Fisheries Service (NMFS) and United States Department of Agriculture (USDA) prepared draft Biological Evaluations (BEs) for three pilot chemicals: chlorpyrifos, diazinon and malathion. These draft BEs are the first ever national assessments of the potential effects of pesticides to listed species (threatened and endangered) attempted by the federal government.

Cheminova A/S (hereafter referred to as "Cheminova") is the sole manufacturer and primary registrant in the United States for the technical form of malathion (CAS Registry Number 121-75-5). All other registrants of technical malathion obtain their material from Cheminova and all end-use products registered in the United States are produced from Cheminova's technical malathion. In 2015, Cheminova A/S and Cheminova, Inc., were acquired by FMC Corporation (FMC). Cheminova Inc.'s end-use product registrations are in the process of being transferred to FMC. The registrations currently held by Cheminova A/S will be transferred to FMC in the near future. When these transfers are accomplished, FMC will supplant Cheminova as the "applicant". Although we often only refer to Cheminova in this document, the comments contained herein were developed on behalf of Cheminova and FMC and reflect the positions of both companies.

On April 11th, 2016, EPA released the draft BEs for public comment in support of registration review for these pesticides. This date marked the start of a 60-day public comment period. The comment period for submission of comments on the draft BEs ends on June 10th, 2016. On April 29th, 2016, a 120-day extension to the comment period was requested by Dow AgroSciences LLC, Makhteshim Agan of North America, Inc. (Adama) and Cheminova to commence after EPA corrected various missing and broken links and provided other missing information that should have been provided with release of the draft BEs. Extension requests were also submitted to EPA by Edward M. Ruckert, representing the American Mosquito Control Association (May 10th, 2016), CropLife America (May 6th, 2016) and James Callan, representing 39 grower groups (May 9th, 2016). EPA responded on May 17th, 2016 by denying the extension. Given their size and complexity, this denial compromised our ability to thoroughly evaluate the draft BEs. In the justification, EPA cited a court-mandated deadline that they and the Services are working under, as well as the early release of parts of the draft BEs in December, 2015 (allowing for some review prior to the official comment period). Notably, substantial changes made to the draft documents posted in December required additional efforts by affected parties to identify and evaluate modifications made to the documents, supporting models, the missing data, broken links, and other errors in the draft BEs. In addition, the court-mandated deadline is not a reasonable excuse for not allowing a fair and substantive review of the draft BEs by affected parties.

Registration and/or re-registration of pesticides under the *Federal Insecticide, Fungicide, and Rodenticide Act* (FIFRA) constitutes a federal action under the *Endangered Species Act* (ESA). Under ESA Section 7, in some circumstances EPA must consult with the Fish and Wildlife

Service and/or National Marine Fisheries Service ('the Services') to ensure that a pesticide's registration is not likely to jeopardize the continued existence of federally endangered and threatened species (hereafter, 'listed species') or result in the destruction or adverse modification of designated critical habitat. Therefore, these draft BEs purport to provide initial nationwide assessments of the potential effects of the pilot pesticides to listed species and their designated critical habitat. Potential effects to candidate and proposed species and critical habitat proposed for listing under Section 7 of the ESA were also considered.

In the draft BE for malathion (EPA, 2016a), EPA followed the Interagency Interim Approaches (Agencies, 2013), a process agreed to by EPA, the Services and USDA to implement some of the recommendations from the National Academy of Science's National Research Council ("NRC") report "Assessing Risks to Endangered and Threatened Species from Pesticides" (NRC, 2013). The NRC recommended a three step process to evaluate potential risk and satisfy EPA's consultation obligations under Section 7 of the ESA. At each step, EPA assigns a risk finding to each species and/or critical habitat (i.e., Step 1: 'No Effect/May Affect' determination, Step 2: 'Not Likely to Adversely Affect (NLAA)/Likely to Adversely Affect (LAA)'). Under this procedure, species and/or critical habitat receiving a 'MA/NLAA' finding are to be subject to informal consultation with the Services to determine concurrence. Species and/or critical habitat that considered MA/LAA enter Step 3, where a formal consultation occurs with the Services is to occur. A biological opinion is generated by the Services with the goal of making a 'Jeopardy/No Jeopardy' finding for listed species and 'Adverse Modification/No Adverse Modification' determination for their designated critical habitat. The draft BEs for the three pesticides are one of two pilots being used to evaluate the Interagency Interim Approaches. Lessons learned from this process will be used by EPA and the Services to modify the Interim Approaches for future, broader use in registration review and otherwise.

EPA's draft BE attempted to evaluate risk to malathion exposure for all ESA listed species, proposed species, and candidate species in the United States. For malathion, EPA reached the MA/LAA determination for 1725 out of 1782 assessed species (i.e., 97%) and 787 of the 795 assessed critical habitats (99%), suggesting that formal consultation and biological opinions are required for almost all species/ critical habitats evaluated. Aside from being biologically implausible in light of the fact that malathion has been used for decades without the predicted devastating effects on wildlife, completing formal consultations on this scale is a near impossible undertaking for the Services. While it is recognized that considerable effort went into the development of the draft BE, it is clear that using the Interagency Interim Approaches (Agencies, 2013) has resulted in a cumbersome, inefficient, and scientifically indefensible process for screening pesticides posing significant risks to endangered species for in-depth evaluation by the Services through the formal consultation process.

Cheminova has serious concerns regarding the effects determinations for listed species potentially exposed to malathion presented in the draft BE. This response document contains initial comments on the technical aspects of the draft malathion BE with focus on assessed aquatic and terrestrial species and exposures. Particular emphasis is given to methods, data used, and assumptions made. The comments are not comprehensive given the limited

timeframe for the review, and the obstacles imposed by the deficiencies prompted an official request for extension of the 60 day comment period. The EPA stated in the letter denying extension of the comment period that the “interim approach is subject to further refinement, and there will be further opportunities for stakeholder feedback in the future.” Given the insufficient time for a detailed review of the draft malathion BE, Cheminova expects to be provided future opportunities for stakeholder feedback to provide additional comments on the Interim Approaches to the BEs, should that process not be abandoned.

EPA’s draft BE falls far short of being scientifically defensible. One major concern Cheminova has with the draft malathion BE is that in contrast to the NRC (2013) recommendations, risk quotients (RQs) were used to determine risk designations in Step 2. RQs can eliminate the negligible risk scenarios, freeing up resources to use probabilistic approaches for the remaining species. However, an ecological risk assessment does should not/cannot conclude on the results of a cursory RQ screen. The NRC (2013) specifically stated that “[Risk quotients] are not scientifically defensible for assessing the risks to listed species posed by pesticides or indeed for any application in which the desire is to base a decision on the probabilities of various possible outcomes.” The NRC conclusion is consistent with recommendations in the EPA agency-wide guidelines for ecological risk assessment (EPA, 1998), which are cited in the NRC report to point out the importance of the explicit treatment of uncertainty during problem formulation (including distributions of values ignored in risk quotients that are better described by probability statements). In direct contrast to this the EPA has maintained its use of RQs, and as will be demonstrated in Section 5, bases species and habitat calls on the most conservative RQs. In contrast to the use of RQs the NRC (NRC, 2013) recommended “using probabilistic approaches that require integration of the uncertainties (from sampling, natural variability, lack of knowledge, and measurement and model error) into the exposure and effects analyses by using probability distributions rather than single point estimates for uncertain quantities. The distributions are integrated mathematically to calculate the risk as a probability and the associated uncertainty in that estimate. Ultimately, decision-makers are provided with a risk estimate that reflects the probability of exposure to a range of pesticide concentrations and the magnitude of an adverse effect (if any) resulting from such exposure.”

Other key concerns identified include:

- A major lack of transparency necessary for evaluation and reproduction of results;
- Use of toxicological measures of effects or attributes that were not empirically linked to apical ecological risk assessment endpoints (mortality, growth and reproduction),
- Many studies selected by EPA as threshold values were not evaluated for data quality and relevance, and when evaluated, many evaluations did not follow EPA’s own study quality criteria. Use of threshold values from studies deemed invalid by the Agency, or deemed acceptable for quantitative use when criteria for quantitative use were not met;
- Compounding of conservatism of “upper bound” exposure estimate inputs, resulting in unrealistically high deterministic exposure estimates;

- Species calls and critical habitat calls are made assuming that all label uses can be made anywhere in the United States, without drawing any distinctions between use patterns, timing of application, locations and co-occurrence;

- Disparities between exposure durations leading to effects in toxicological studies used to generate thresholds, and field exposure estimates;

- Numerous serious transcription and calculation errors that affected risk designations,

- Use of the newly developed aquatic bin conceptual models resulted in physically impossible malathion EECs for numerous scenarios;

- An assumption that adulticide applications may be made anywhere in the United States as justification for the potential malathion exposure of almost all listed species, when data clearly show this is not the case;

- With the exception of the Agency's overly conservative RQs, other lines of evidence were not directly considered in species and critical habitat calls in the weight-of-evidence tools (e.g., incident reports, field studies, monitoring data, etc.); and,

- EPA gave equivalent "weights" to exceedances of thresholds associated with direct effects to survival, growth or reproduction as they did to exceedances of sublethal thresholds not necessarily linked to adverse effects on individual fitness (e.g., endpoints for avoidance behavior, AChE inhibition, etc.).

Combined, the draft BE estimates nothing less than totally unrealistic, unsupportable catastrophic predictions for the majority of listed species. Yet, as shown with the Kirtland's warbler example, the size of the Kirtland's warbler population is currently at its historical maximum, which is nearly 10 times larger than it was at the time of listing and close to twice as large as the threshold stated in the primary objective (FWS, 2012a). The evidence with respect to the recovery and health of the Kirtland's warbler population in the US is clearly inconsistent with the catastrophic risk finding for this species of the highly conservative draft BE for malathion. Similar anomalies between reality and the risks predicted by EPA exist for numerous listed species.

This document will also be supplemented by a report containing a problem formulation, a Step 1 and a Step 2 ESA that will be submitted to EPA after the public comment period (Teed et al., 2016). These documents will provide an alternate, more scientifically defensible and resource-efficient approach than is reflected in EPA's draft BE for malathion. In addition, Cheminova is submitting contemporaneously with these comments three refined effects determinations for malathion conducted on the Kirtland's warbler, the California tiger salamander and the delta smelt to provide additional examples on how individual listed species assessments should be conducted to screen out cases that do not warrant to formal consultation with the Services (Step 3). Cheminova had previously submitted a refined effects determination for the California Red-

legged frog (CRLF) (Breton et al., 2013a [MRID 49211702]). Cheminova's effects determinations demonstrate that when higher tier assessments are carried out using the best available scientific data, realistic exposure and effects assumptions and consideration of all lines of evidence, the conclusion of these effects determinations are quite different and reflective of real world observations than those predicted by EPA. Such 'refined' assessments should be conducted once potential risks are identified using highly conservative risk approaches (e.g., risk quotients) in screening-level ecological risk assessments (e.g., NRC, 2013; EPA, 1998, 2004, 2013).

Cheminova believes that the exercise of creating the three draft BEs currently under review has demonstrated that the Interagency Interim Approaches tested by them should be set aside. The current draft does not provide a scientifically sound basis on which to proceed as to malathion and, Cheminova understands, for either chlorpyrifos or diazinon. To the extent EPA tries to continue the existing process, Cheminova requests an earnest and comprehensive review of the comments detailed herein. EPA also must correct obvious errors and oversights, particularly in the weight-of-evidence approach taken in the draft BE (which is nonsensical), use refined probabilistic methods that better characterize risk to listed species and/or their critical habitats on which they depend, and address the many other issues that even our limited review has identified.

Cheminova looks forward to the opportunity for continued participation in the development of an efficient approach to evaluating ESA listed species and their critical habitat in the registration/re-registration process under FIFRA.

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1 INTRODUCTION

The Environmental Protection Agency (EPA or "the Agency"), in conjunction with the Fish and Wildlife Services (FWS), National Marine and Fisheries Service (NMFS) and United States Department of Agriculture (USDA) prepared draft Biological Evaluations (BEs) for three pilot chemicals: chlorpyrifos, diazinon and malathion. These draft BEs are the first case studies for national assessments of the potential effects of pesticides to listed species (threatened or endangered) carried out by the federal government. On April 6th, 2016, the EPA released the draft BEs for review. This date marked the start of a 60-day public comment period.

Cheminova A/S (hereafter referred to as "Cheminova") is the sole manufacturer and primary registrant in the United States for the technical form of malathion (CAS Registry Number 121-75-5). All other registrants of technical malathion obtain their material from Cheminova and all end-use products registered in the United States are produced from Cheminova's technical malathion. In 2015, Cheminova A/S and Cheminova, Inc., were acquired by FMC Corporation (FMC). Cheminova Inc.'s end-use product registrations are in the process of being transferred to FMC. The registrations currently held by Cheminova A/S will be transferred to FMC in the near future. When these transfers are accomplished, FMC will supplant Cheminova as the "applicant". Although we often only refer to Cheminova in this document, the comments contained herein were developed on behalf of Cheminova and FMC and reflect the positions of both companies.

Cheminova contracted Intrinsik Environmental Sciences, Inc. (hereafter referred to as Intrinsik) and Stone Environmental (hereafter referred to as Stone) to assist in the review and evaluation of the portions of the malathion draft BE pertaining to the assessment of risk to aquatic and terrestrial listed species (or species that have an aquatic or terrestrial component of their life cycle). This document contains Intrinsik's and Stone's comments to date.

Under the *Endangered Species Act* (ESA, 1973), Federal agencies are responsible for ensuring that their actions do are not likely to jeopardize listed species. The EPA is responsible for registering pesticides under the *Federal Insecticide, Fungicide, and Rodenticide Act* (FIFRA), and accordingly ensuring that pesticide registration does not result in unreasonable adverse effects to the environment. Registration of a pesticide is considered a federal action. Given the potential intersection of obligations of the Services (FWS and NMFS), in a consultative role, with the obligations of EPA under the two Acts with respect to listed species, the agencies collectively requested that the National Research Council examine the scientific and technical issues associated with assessing risk to listed species. In 2010, a Committee on Ecological Risk Assessment under FIFRA and ESA was formed under the auspices of the National Academy of Science, and after review of the issues, provided extensive guidance to the agencies in a final report entitled "Assessing Risks to Endangered and Threatened Species from Pesticides" (NRC, 2013). Later that same year, the agencies produced an Interagency Interim Approaches document for "National-Level Pesticide Endangered Species Act Assessments" that focussed

on approaches to the first two of three steps in the assessment process (Figure 1-1)("Interagency Interim Approaches").

The recently released draft BEs attempt to provide national assessments of the potential risk of chlorpyrifos, diazinon and malathion (the pilot chemicals) to listed species and their designated critical habitat, accounting for both Step 1 and Step 2 in the pilot process detailed in Figure 1-1. In this process, species/critical habitat that receive a "No Effect" (NE) determination in Step 1 are screened out from further analysis, and species/critical habitat that receive a "Not Likely to Adversely Affect" (NLAA) determination in Step 2 are sent to the Services for concurrence. Only species/critical habitats that receive a "Likely to Adversely Affect" (LAA) determination in Step 2 are carried forward for additional analyses (Step 3: "formal consultation" with the Services).

On April 29th, 2016, a 120-day extension to the comment period was requested by Dow AgroSciences LLC, Makhteshim Agan of North America, Inc. (Adama) and Cheminova because the 60-day comment period was deemed by these registrants as insufficient for review of the contents of the draft BEs which (1) exceed 12,000 pages, and contain links to Excel files and model output files with millions of lines of data, and (2) contained a number of omissions and errors (including broken links), making comprehensive review impossible. Extension requests were also submitted to EPA by Edward M. Ruckert, representing the American Mosquito Control Association (May 10th, 2016), CropLife America (May 6th, 2016) and James Callan, representing 39 grower groups (May 9th, 2016). The request for extension was denied by EPA in a formal letter sent via e-mail on the 17th of May, 2016 to the counsel of the registrants (David B. Weinberg and David E. Menotti). In the justification, the Agency cited a court-mandated deadline under which they and the Services are working, as well as the early release of parts of the draft BEs in December, 2015 (allowing for some review prior to the official comment period). However, substantial changes made to the draft documents posted in December required additional efforts by affected parties to identify and evaluate modifications made to the documents, supporting models, the missing data, broken links, and other errors in the draft BEs. In addition, the court-mandated deadline is not a reasonable excuse for not allowing a fair and substantive review of the draft BEs by affected parties.

Given the limited time available for public comment, this document contains only Intrinsik's and Stone's preliminary review and evaluation of the portions of the malathion draft BE pertaining to the assessment of risk to aquatic and terrestrial listed species (or species that have an aquatic or terrestrial component of their life cycle). The Agency stated in the letter denying extension of the comment period that the Interagency Interim Approaches is subject to further refinement, and there will be further opportunities for stakeholder feedback in the future." Given insufficient time for a detailed review of the malathion draft BE, Cheminova expects to use future opportunities for stakeholder feedback to provided additional comments on the Interim Approaches to the draft BEs, with focus on how it applies to malathion and other Cheminova pesticide products.

For malathion, EPA reached the MA/LAA determination for 1725 out of 1782 assessed species (i.e., 97%) and 787 of the 795 assessed critical habitats (99%), suggesting that biological

opinions would be required for almost all species/ critical habitats evaluated. Aside from being biologically implausible in light of the fact that malathion has been used for decades without the predicted devastating effects on wildlife, completing formal consultations on this scale is a near impossible undertaking for the Services. While it is recognized that considerable effort went into the development of the draft BE, it is clear that using the Interagency Interim Approaches (Agencies, 2013) has resulted in a cumbersome, inefficient, and scientifically indefensible process for screening pesticides posing significant risks to endangered species for in-depth evaluation by the Services through the formal consultation process.

Cheminova has serious concerns regarding the effects determinations for listed species potentially exposed to malathion made in the draft BE. Many, but by no means are all of the errors and deficiency addressed in this report. Most notably, the processes used in the draft BEs to determine risk designations, and ultimately species and critical habitat “calls” (i.e., effects determinations) lacked the transparency necessary for reproduction of results and a robust review of the assessments. Ultimately, the approach is absurdly conservative, with species calls being driven by extreme and unrealistic exposure estimate exceedance of No-Observed-Effects-Levels, with clear disparity between exposure durations in toxicity tests and field estimates. This risk quotient approach is in direct contrast with the NRC (2013) recommendations, which called for probabilistic methods, such as those employed by Cheminova for the three refined effects determinations for malathion conducted on the Kirtland’s warbler, the California tiger salamander and the delta smelt (Moore et al., 2016, Breton et al. 2016a,b), which are being submitted contemporaneously with this document. Cheminova had previously submitted a refined effects determination for the California Red-legged frog (CRLF) (Breton et al., 2013a [MRID 49211702]). Cheminova’s effects determinations demonstrate that when higher tier assessments are carried out using the best available scientific data, realistic exposure and effects assumptions and consideration of all lines of evidence, the conclusion of these effects determinations are quite different and reflective of real world observations than those predicted by EPA. Such ‘refined’ assessments should be conducted once potential risks are identified using highly conservative risk approaches (e.g., risk quotients) in screening-level ecological risk assessments (e.g., NRC, 2013; EPA, 1998, 2004, 2013).

EPA does not present all the available data (e.g. toxicity, physical-chemical properties and fate) and their associated evaluations in the draft malathion BE. All of the regulatory data should be made available to the Services as part of the consultation, not just those data that EPA have deemed acceptable.

This Document will also be supplemented by an alternative problem formulation, and a Step 1 and Step 2 ESA that will be submitted to EPA after the public comment period (Teed et al., 2016). These Documents are far from being representative of a fully realistic approach to risk assessment in the FIFRA/ESA context, but it should provide the basis for development of an alternate, more scientifically defensible and resource-efficient approach than is reflected in EPA’s draft BE for malathion.

This response document first addresses the exposure assessment conducted by EPA (Sections 2.0 and 3.0), followed by the effects assessment (Section 4.0) and the Agency's effects determinations (Section 5.0) for listed aquatic and terrestrial species in the draft malathion BE. It concludes with a summary of the overarching problems identified in the portion of the draft BE dealing with the aquatic and terrestrial listed species (Section 6.0).

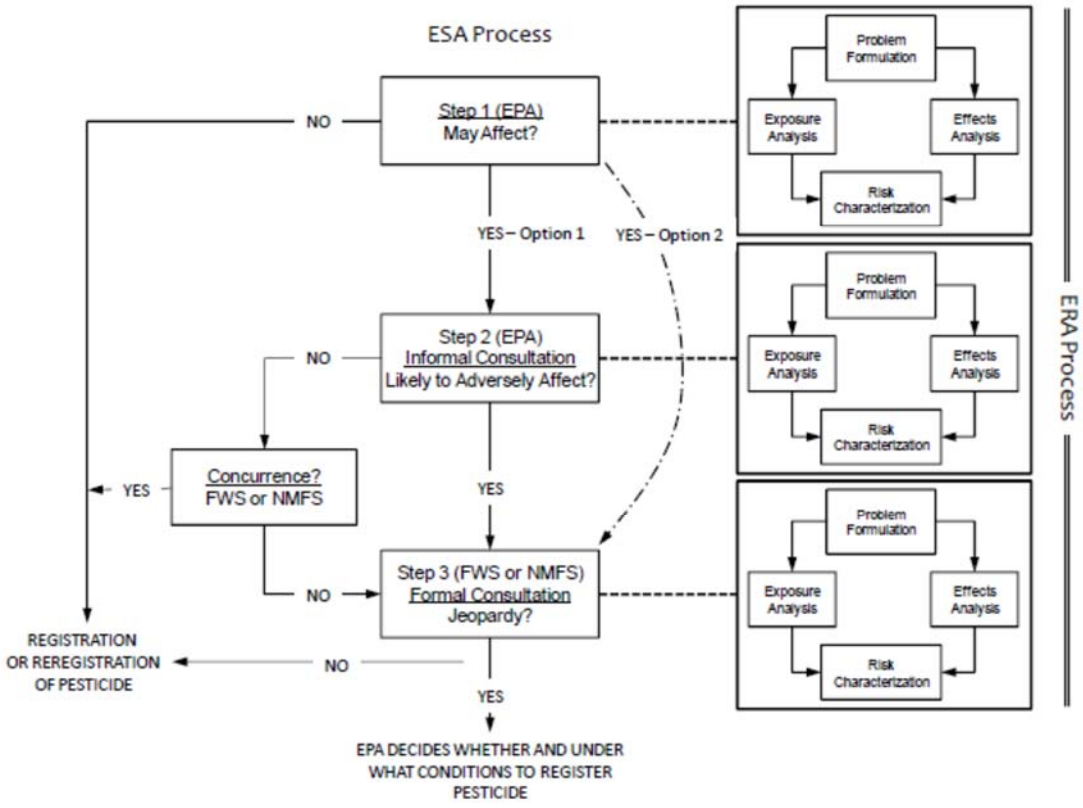

Figure 1-1 **Relationship between the ESA process and the ERA process (Figure S-1 from NAS (2013))**

2 METHODS FOR ESTIMATING EXPOSURE OF TERRESTRIAL ORGANISMS TO MALATHION

To estimate exposure of listed terrestrial plants, vertebrates (i.e., birds, mammals and herptiles) and invertebrates to malathion, EPA relies on the TEDtool that combines terrestrial models from EPA's standard screening-level toolbox including: T-REX, T-HERPS, AgDRIFT and Terrplant. In general, EPA fails to provide adequate description on many of their exposure estimate approaches, and often points instead to the individual model user manual located on the EPA webpage (https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment). In some cases, however, EPA's method within the TEDtool is not in line with the appointed model or there are calculation errors in the TEDtool worksheets. Additionally, the draft BE lacks transparency in the selection of input parameters for modeling purposes, and in the rational and use of all exposure estimates. Moreover, there are serious deficiencies with the models used in EPA's standard toolbox. These deficiencies have been commented on numerous occasions in previous responses to EPA risk assessments, and in some depth herein. See Breton et al., (2013a,b [MRID 49211702, 49211701]; 2014b,c [MRID 49400601, 49400501]) for additional comments on the models within the EPA's standard toolbox.

Much of the information provided by EPA on their draft BE exposure estimates are presented in Chapter 3 (Exposure Assessment), Attachment 1-7 (Methodology for Estimating Exposures to Terrestrial Animals (mammals, birds, reptiles, amphibians and invertebrates), Attachment 1-16 to 1-20 (Biological information on listed birds, mammals, herptiles) and the TEDtool root files (TEDtool_v1.0_alt.xlsx and TEDtool_v1.0.xlsx). This section contains general comments on EPA's methodology for terrestrial vertebrates, plants and invertebrates as well as spray drift estimates that apply to all taxa (Sections 2.1 through 2.4), as well as chemical specific comments and results (Section 2.5 and 2.6). The general comments are organized by taxa (Section 2.1 – Terrestrial vertebrates, Section 2.2 – Terrestrial plants, Section 2.3 – Terrestrial invertebrates).

2.1 Terrestrial Vertebrates

EPA uses similar approaches in estimating exposure of listed terrestrial vertebrates to malathion for birds, mammals and herptiles. As such, the comments presented below generally apply to all vertebrate species and are organized by exposure route. Comments on dietary exposure estimates are presented in Section 2.1.1; Drinking water comments are presented in Section 0; and comments on dermal and inhalation exposure routes are located in Sections 2.1.3 and 2.1.4, respectively.

2.1.1 Dietary Exposure

Comment 1
Attachment 1-7, Section 1 (Introduction)

EPA states that, “Dietary requirements as well as body weights of specific species are provided in appendices x-y.” There is no appendix x, nor is there an appendix y, in the Biological Evaluation Chapters for Malathion.

Comment 2
Attachment 1-7
Section 1 (Introduction)

TEDtool_v1.0_alt.xlsx and TEDtool_v1.0.xlsx
Worksheet: Min and max rate dietary concentration results

The Agency states that two types of exposure estimates are used, concentration-based and dose-based. In their TEDtool, EPA uses both dose-based and concentration-based (dietary) exposure estimates to estimate risk by comparing dose-based exposure estimates to dose-based effects thresholds (mg a.i./kg bw) and concentration-based (dietary) exposure estimates to concentration-based (dietary) effects thresholds. Despite the comparison of values with matching units (i.e. mg a.i./kg diet), however, concentration-based “exposure estimates” should not be compared to concentration-based effects metrics (e.g., an LC50 in mg a.i./kg diet). This approach is flawed for a number of reasons. First, the diet used in the laboratory toxicity test supporting the effects metric is not likely to be equivalent to the diet of potential receptors in the wild. That is, the diets are unlikely to have the same gross energy and assimilation efficiency. Second, the actual dietary exposure of receptors in the wild is dependent on their food intake rate. Thus, a concentration in food is not an exposure estimate. Food intake rates vary between species, and between individuals within a species, are generally allometric with body mass, and also depend on the metabolizable energy of the food. As such, it is inappropriate to assess risk to wildlife based on estimated concentrations in potential feed items by direct comparison to effects metrics in units of mass active ingredient per mass diet. It is appropriate to compare dose based exposure estimates (i.e. mg a.i./kg bw) with dose-based effect metrics (i.e. mg a.i./kg bw).

Comment 3
Attachment 1-7
Section 4b (Concentrations in invertebrates)

EPA estimates concentrations in soil-dwelling invertebrates to assess exposure of terrestrial vertebrates consuming soil-dwelling invertebrates. In their description of their approach to estimating concentrations in soil-dwelling invertebrates the Agency reports using a partitioning approach to estimating pesticide concentrations in earthworms, which are used to represent soil-dwelling invertebrates. The following equation is presented for estimating earthworm concentration (Equation 2 in Attachment 1-7; Equation 2-1 herein):

$$C_E = \frac{C_{w(pore)} * L * K_{ow}}{\rho_E}$$

Equation 2-1

Where,

C_E	=	Chemical concentration in earthworm (mg a.i./kg)
$C_{w(pore)}$	=	Concentration of the pesticide in pore water (mg/L)
L	=	Lipid fraction in earthworm
ρ_E	=	Density of earthworm kg/L

EPA presents their method for estimating soil pore water concentrations for use in estimating earthworm concentrations. The approach is reportedly based on the Tier I rice model, and the following equations are provided (Equations 3 and 4 of Attachment 1-7):

$$C_{w\ (pore)} = \left(\frac{A_{rate} * 11.2}{d_w + d_{soil}(\theta_{soil} + \rho_b * K_{OC} * f_{OC(soil)})}\right)$$

Equation 2-2

Where,

A_{rate}	=	Application rate from label (lb a.i./A)
d_w	=	Depth of puddle water (cm)
d_{soil}	=	Depth of soil at equilibrium with water (cm)
θ_{soil}	=	Porosity of soil
ρ_b	=	Bulk density of soil (kg/L)
K_{OC}	=	Organic carbon: water partitioning coefficient
$f_{OC(soil)}$	=	Fraction of organic carbon in soil

$$\theta_{soil} = 1 - \frac{\rho_b}{\rho_p}$$

Equation 2-3

Where,

ρ_p	=	Density of soil particles kg/L

First, EPA does not provide a reference for this approach. However, the method is clearly derived from the fugacity approach often applied by the Agency in ecological risk assessments of pesticides (e.g., EPA, 2007; EPA, 2008; EPA, 2009a,b,c,d), and applied in the on-line übertool (http://qed.epa.gov/ubertool/earthworm). Notably, however, the equation provided in Attachment 1-7 for chemical concentration in earthworm, C_E, does not agree with the equation provided in the algorithms of the on-line übertool (http://qed.epa.gov/ubertool/earthworm/algorithms). In the algorithms provided in the on-line übertool the equation for C_E is a function of soil and water concentration, and is derived from the fugacity capacities of earthworm, soil and water, as follows:

$$C_E = C_S * \left(\frac{Z_E}{Z_S}\right) + C_W * \left(\frac{Z_E}{Z_W}\right)$$

Equation 2-4

Where,

C_E = Chemical concentration in earthworm tissue (mol/m^3)
C_S = Chemical concentration in soil (mol/m^3)
Z_E = Fugacity capacity of pesticide in earthworms (mol/m^3 Pa)
Z_S = Fugacity capacity of pesticide in soil (mol/m^3 Pa)
C_W = Chemical concentration in pore water of soil (mol/m^3)
Z_W = Fugacity capacity of pesticide in pore water (mol/m^3 Pa)

$$C_E = K_{OW} * L * \left(\frac{C_S}{K_d * \rho_b}\right) + C_W$$

Equation 2-5

Where,

K_{OW} = Octanol to water partitioning coefficient
L = Lipid fraction of earthworm
K_d = Soil partitioning coefficient (cm^3/g)

This general approach is based on the method presented by Mackay and Paterson (1981). However, in both the approach described in Attachment 1-7 and in the on-line übertool algorithms, the estimation of earthworm soil concentration has been oversimplified. Equation 2 in Attachment 1-7, implies that the only environmental medium concentration influencing earthworm uptake is that of soil pore water. However, the overall fugacity of the system is necessary for an accurate estimate of earthworm concentration in the fugacity modeling approach (Mackay and Paterson, 1981). The fugacity of the system is a function of the total amount of the pesticide added to the system, the volumes of each of the system components, as well as their associated fugacity capacities (Mackay and Paterson, 1981). In a system presumed to have no inflows or outflows, at equilibrium with respect to a certain chemical, there is a common prevailing fugacity, *f*, for that chemical. This common fugacity, in Pa, can be calculated with the following equation (Mackay and Paterson, 1981):

$$f = \frac{M_T}{\sum Z_i V_i}$$

Equation 2-6

Where,

M_T = Total amount of the chemical in the system (mol)
Z_i = Fugacity capacity of the *i*th compartment in the system (mol/m^3 Pa)
V_i = Volume of the *i*th compartment in the system (m^3)

Then, concentration in each compartment, C_i, in mol/m^3, can be related to fugacity by (Mackay and Paterson, 1981):

$$C_i = Z_i f$$

Equation 2-7

Thus, the fugacity capacity of each component of the system must be estimated. Accordingly, the concentration in earthworms may be estimated with the following equation:

$$C_E = Z_E * \left(\frac{M_T}{\sum Z_i V_i}\right)$$

Equation 2-8

The system in which earthworms are found is made up of at least four components: soil, air, water and earthworms (unless the soil is entirely saturated). According to the Agency's on-line übertool (http://qed.epa.gov/ubertool/earthworm), the fugacity capacities of soil, water and earthworms can be estimated with the following equations:

$$Z_S = \frac{k_d * \rho_b}{H}$$

Equation 2-9

$$Z_W = \frac{1}{H}$$

Equation 2-10

$$Z_E = \frac{L * K_{OW}}{H}$$

Equation 2-11

Where *H* is Henry's Law constant in m^3 Pa/mol.

These expressions are consistent with the estimates provided in Mackay and Paterson (1981), except that for biota, such as earthworm, the estimate is presented as (Mackay and Paterson, 1981):

$$Z_E = \frac{BCF * \rho_E}{H}$$

Equation 2-12

In Equation 2-11 the Agency has assumed that the lipid fraction of the earthworm multiplied by the K_{ow} is equivalent to the BCF, assuming earthworm has a density of 1 g/cm^3.
The water fraction is thought to have a negligible effect on *BCF* for l*og* K_{OW} greater than 2 (Jager, 1998). So, the Agency's assumption may be reasonable for malathion, but may not be for other pesticides considered.

Notably, the Agency disregards air as a separate component in the system in both Attachment 1-7 and their on-line übertool (http://qed.epa.gov/ubertool/earthworm). Further, in Attachment 1-7 EPA disregards the soil fugacity capacity as well, and presumes that the system consists of only soil pore water and earthworms, which is inconsistent with the approach presented in the algorithms of the Agency's on-line übertool (http://qed.epa.gov/ubertool/earthworm.

EPA should make note when methods differing from their standard toolbox are used to estimate exposure for any species. In the case of estimating concentrations in earthworms, a discussion on übertool vs BE approach should be provided with appropriate justification.

Comment 4
Attachment 1-7
Section 6 (Concentrations in aquatic organisms)

EPA states that concentrations in aquatic organisms will be estimated with empirical BCFs using water concentration estimates generated with the Pesticide Root Zone Model (PRZM5) and the Variable Volume Water Model (VVWM). The Agency does not specify which water concentration estimates will be used. That is, they do not specify what assumptions will be made regarding model parameters, nor do they specify an averaging period, or whether, for example, a mean or percentile of the estimated data will be used. Since this information is also not presented in Chapter 3 (exposure assessment), it seems that this information has been omitted, making it impossible to reproduce EPA's estimates.

Comment 5
Attachment 1-7
Section 4c (Concentrations in vertebrates)

The Agency states: “The method for estimating upper-bound and mean concentrations of pesticides in mammals, birds, reptiles, and amphibians is described in detail in the T-HERPS manual. For mammals serving as prey, the most conservative prey item is used (i.e., the 15 g mammal that consumes short grass). For birds, the small bird (20 g) that consumes 100% arthropods was selected because this is the most common dietary item among birds (Appendix D of TIM manual). A 2 g animal that consumes 100% arthropods is selected to represent reptile and amphibian prey. For carrion, residues in large mammals (1000 g) consuming short grass are used as a surrogate.”

The T-HERPS manual (EPA, 2008) explains that the exposure of herptiles consuming vertebrate prey is estimated by (1) dietary concentration: estimating the concentration of pesticide in the prey based on total daily intake, and (2) dose: assuming that the predator consumes the total daily intake of its prey in mg a.i.

First, dietary concentrations are not exposure estimates. Refer to Section 2.1.1, Comment No. 2.

Second, the rationale for the selection of prey is unclear. For mammals, “the most conservative” prey is selected, where as for birds, the standard small bird is selected, with the “most common” diet. These two approaches are inconsistent. The smaller prey will not necessarily provide the most conservative dose. On a mg a.i./kg basis, smaller prey may carry higher concentrations, but because of their small size, they may carry a lower total load of pesticide than larger prey. The Agency does not acknowledge this, nor account for this. No justification is provided for selecting large mammals (1000 g) as carrion prey.

Finally, EPA does not account for the fact that when a predator ingests vertebrate prey, it does not consume their total daily intake, it consumes their body burden. A prey’s body burden can be considerably different from their total daily intake depending on their foraging habits, the absorption, distribution, metabolism, and excretion (ADME) rates of the pesticide.

Comment 6
Attachment 1-7
Section 5 (Concentrations in soil, for estimating concentrations in earthworms)

The Agency describes how soil concentrations will be estimated using the pore water concentration calculated in the earthworm fugacity modeling. The pore water concentration estimate is to be multiplied by the K_{OC} and f_{OC} to get mg a.i./kg dw soil.

Critically, this approach relies on the assumption that the soil is saturated. The volume of soil pore water affects both the concentration in the soil pore water and the concentration in the soil.

Further, this approach, and other approaches presented by the Agency in Attachment 1-7 do not account for any interception by the crop, which would be considerable for most organophosphate insecticides such as malathion which are generally applied to crop canopies to control pests.

Comment 7
Attachment 1-7
Section 7 (Doses received by birds, mammals, reptiles and terrestrial-phase amphibians)
First paragraph

In their description of terrestrial exposure approaches, it was not made clear how EPA planned on assessing various routes of exposure. The Agency states that, “Dose-based exposures (units: mg a.i./kg-bw = µg a.i./g-bw) include four different routes: diet, drinking water, dermal and inhalation. Essentially, a dose based exposure is calculated by multiplying the relevant ingestion rate by the concentration in the relevant medium.” EPA goes on to say, “It should be noted that these methods are intended to be conservative in nature and the individual doses should not be added together. For instance, it is assumed that 100% of the daily diet of an individual is consumed on the treated field. If that individual consumes other food items, the total amount of food consumed would be overestimated. EECs generated using this approach are also based on the assumption that the individual is on the treated field 100% of the time; however, it is expected that the individual will spend time off of the field, receiving lower exposures.”

As previously noted, it is unclear what the Agency is trying to convey. Based on the first sentence of the second paragraph (“It should be noted that these methods are intended to be conservative in nature and the individual doses should not be added together.”), we might presume that the Agency is suggesting that estimated doses from diet, drinking water, dermal and inhalation should not be summed. However, the example provided is perplexing. The example text does not describe a summing of doses, nor a lack thereof. So, the doses that “should not be added together” remain ill defined.

Based on the WoE tools that were not available for review until April 11, 2016 (after several communications between Steven Snyderman (EPA) and Sara Rodney (Intrinsik)), it is apparent that EPA did not add together separate exposure estimates for the different routes of exposure. As described above, their description and rational for this approach was not made clear throughout the text of the BEs, but should be clarified in the revised BE.

Comment 8
Attachment 1-7
Section 7 (Doses received by birds, mammals, reptiles and terrestrial-phase amphibians)

The Agency states that turtle egg exposure to spray drift will be assessed, as well as the extent to which eggs will be exposed if they are buried.

EPA is not explicit about how the exposure of buried eggs will be determined, nor do they refer to where more information can be found on turtle egg exposure estimates. Further, it is not clear how these exposure estimates will be considered in the risk assessment given the paucity of toxicity studies examining the toxic effects associated with the direct spray of pesticides on eggs.

Comment 9
Attachment 1-7
Section 7a (Doses received by birds, mammals, reptiles and terrestrial-phase amphibians): Diet

EPA states that "food ingestion rates vary by type of vertebrate and water content of the diet." This statement is supported by Equation 6 in Attachment 1-7 (Equation 2-13 herein):

$$IR_{food} = \frac{a1 * BW^{b1}}{1 - FW_k}$$

Equation 2-13

Where,

IR_{food}	=	Food intake rate (g ww/d)
a1	=	Alpha parameter of allometric regression model
BW	=	Body weight of species (g)
b1	=	Beta parameter of allometric regression model
FW_k	=	Fraction of water in dietary item *k*

The Agency provides tables of values for FW_k, and *a1* and *b1* (Tables A 1-7.4 and A 1-7.5, respectively, in Attachment 1-7).

This is an oversimplification. Food ingestion rates are a function of more than simply the species and water content of the diet. Food intake depends on the size and growth stage of the individual, the season, and the metabolizable energy available in the various feed items within their diet (which is a function of gross energy and assimilation efficiency).

The food ingestion rate model and associated parameter estimates have two principal limitations with their use. First, the data and analyses on which they are based are outdated, and have been updated and revised at least twice since the original publication (Nagy et al., 1999; Nagy, 2005). The first and most extensive update (Nagy et al., 1999) involved the addition of data for 12 eutherian mammals (increasing the eutherian mammal dataset by 26%), 45 birds (nearly doubling the avian dataset), and 20 reptiles (increased the reptilian dataset by almost 60%). Second, the equations extracted from Nagy (1987) by the Agency (EPA, 1993) are based on deterministic assumptions regarding metabolizable energy, such that:

(1) variability in gross energy and assimilation efficiency are not accounted for,

(2) only one type of dietary item is considered, or alternatively a general “estimated” metabolizable energy is applied for omnivores, and frugivorous birds, to all food without accounting for actual variability in the proportions of various feed items in the diet, and

(3) food intake estimates do not account for uncertainty in field metabolic rate, gross energy, assimilation efficiency or dietary composition.

As noted by NRC (2013), probabilistic approaches that account for variability in model parameters such as gross energy and assimilation efficiency (by sampling, natural variability, lack of knowledge and model error) should be used in an ESA. Probabilistic approaches (compared to RQs) provide a better understanding of the range of potential exposure and therefore magnitude of risk decision makers need to fully understand species risk implications. Thus, EPA has failed to consider the NRC (2013) recommendations in their exposure assessment.

Comment 10
Attachment 1-7
Section 7a (Doses received by birds, mammals, reptiles and terrestrial-phase amphibians): Diet
Table A 1-7.4

In Table A 1-7.4, EPA presents fractions of water in fresh food items reportedly obtained from EPA (1993). Most of the estimates presented in the table were confirmed to be from EPA (1993), or were noted as coming from other sources (e.g., pollen and nectar estimates). However, we could not confirm the estimates for aquatic plants, benthic invertebrates and zooplankton, which either were not reported (e.g., zooplankton) in EPA (1993), or the values do not agree with those presented in Table A 1-7.4. For instance, the fraction moisture for aquatic plants is listed in the table as 0.80. However, in EPA (1993), algae from three studies were reported to be 84% water on average with a standard deviation of 4.7%; aquatic macrophytes from three studies were on average 87% water with a standard deviation of 3.1%. Emergent vegetation was reportedly more variable due to drying, 45 to 85%, with submerged vegetation maintaining high water content (data from three studies; see EPA (1993) for original references). The selection of 80% moisture for aquatic plants seems to be an under estimate for submerged vegetation, but may be a gross over estimate for some emergent plants. The Agency should consider approaches accounting for these differences in submerged and emergent vegetation.

Comment 11
Attachment 1-7
Section 7a (Doses received by birds, mammals, reptiles and terrestrial-phase amphibians): Diet
Table A 1-7.5

In Table A 1-7.5, EPA presents parameters used to calculate food intake rates for vertebrates based on EPA (1993).

As discussed in Comment No. 9 herein, these parameters are based on outdated data and analyses.

The parameters identified as applying to “mammals not in the Rodentia order” in fact apply to “all eutherians” assessed in Nagy (1987). The analysis that produced these parameter estimates was in fact based primarily on rodent data (33 of 46 data points). Parameters for eutherians not in the Rodentia order were not reported for food intake rate by Nagy (1987; only the field metabolic rate equation was provided for this guild). Further, the parameters presented for reptiles and amphibians, are based on an allometric regression model fit to insectivorous iguanids, excluding the five herbivorous iguanids reported in Nagy (1987), and also, as stated, excluding the 20 additional reptiles, including non-iguanids that were published in 1999 (Nagy et al., 1999).

Comment 12
TEDtool_v1.0_alt.xlsx and TEDtool_v1.0.xlsx
Worksheet: Min and max dose rate
Column: F (Body Weight)

Attachment 1-16 through Attachment 1-19 (Biological Information on Listed Species)
Supplemental Information 1

EPA does not provide sufficient guidance and are inconsistent in their approach for developing body weight estimates used for modeling purposes (assumed body weights are presented in the min and max dose rate worksheets) in the BE. In their Supplemental Information (1) (for Attachments 1-16 through 1-19) “Instructions for extracting biological information for listed mammals/birds/reptiles/terrestrial amphibians”, EPA notes in terms of exposure modeling that…“If all other parameters are kept equal, decreases in the species BW parameter result in increases in risk. Therefore, for all listed birds/mammals/reptile/terrestrial-phase amphibians, the lowest available BW value is used”. This suggests that the assumed body weight of a species highly influences the potential estimated risk of the species. However, in these attachments, EPA provides little instruction beyond “the lowest value” on selecting body weights (BW) for use in the exposure assessment. It is not clear if the lowest value should be the lowest average of combined or separate sexes, or the lowest of all available ranges. In reviewing the biological information in Supplemental Information 3 for all terrestrial taxa, it seems that EPA is not consistent with their BW selection. It appears as though BWs selected represent a variety of statistical measures and sources. For example, some body weights are reported as one value from one source, with no indication of sex, life stage, or statistical parameters while others are clearly the lowest reported value in a range, and others the average of a particular sex. Additionally, some body weights were estimated using reported means and standard deviations. It was not clear as to which situations would warrant the estimation of a body weight parameter, given the availability of data (i.e., even one value from one source). For example, average body weights for male and female Florida salt marsh voles (*Microtus pennsylvanicus dukecambelli*) were reported as 44.2 ± 6.29 and 44.0 ± 10.25 g, respectively. A range of 34-54 g was

estimated for this species using the mean and standard deviation. The lowest (estimated) body weight of 34 g was used for exposure modeling purposes. It is not clear why an estimated lower bound value was used instead of the mean female body weight of 44.0 g, where for many other species, only a mean value was assumed. As such the methods for deriving and selecting an appropriate body weight were not consistent for all species. This is inappropriate, given the influence that body weight can have on exposure estimates.

Comment 13
Attachment 1-19 (Biological Information on Listed Species of amphibians)

According to EPA's draft BE's, apparently there is a paucity of data on the body weights of several species of listed Salamanders. Because body weights are necessary for estimating risk to a species, an estimated body weight was used for species for which data could not be located. In Attachment 1-19, an approach for estimating body weights for salamander species in the Plethodontidae family is suggested.

"For terrestrial adults of listed salamander species in the Plethodontidae family, including: *Plethodon sp*., *Batrachoseps aridus* (desert slender salamander) and *Phaeognathus hubrichti* (red hills salamander), the following regression determined from data published by Heatwole and Heatwole (1962)[1] can be used: BW = (L-29)/8.5, where: BW = body weight in g and L = length in mm. This regression, was derived by K. Garber using the figure in the 1962 article. The relationship between snout vent lengths (SVL) and BW from *Plethodon cinereus* is used for listed species within the same genus and family. If other sources are located with body weight data on these species or on the genera, the other sources can be used instead."

Body weight estimates using this approach were applied for 5/10 listed salamander species (Desert slender salamander, Berry cave salamander, Red Hills salamander, Jemez Mountains salamander, Shenandoah salamander). A few comments on this approach are provided below:

1) It is noted in the text above that the regression was derived using the figure as reported by Heatwole and Heatwole (1962) who collected *Plethodon cinereus* in Emmet and Cheboygan counties, Michigan. The study does not provide the raw data of the collected individuals, but presents a figure plotting male, female, juvenile and hatchling body weight and snout vent lengths. It is not clear which data were used by K. Garber to estimate the body weight regression, despite the instructions being specific for use to estimate weights of "adult" salamanders. There are upward of over 50 data points that are cluttered together on plot, so deciphering between them would have been incredibly tedious with a lot of uncertainty. Whether the data points were estimated from the figure in the study or recovered from the original author, the dataset should be made available in the BE.

[1] Heatwole, H. and A. Heatwole. 1962. Weight-length curve of the salamander, *Plethodon cinereus*. Journal of the Ohio Herpetological Society 3: 37-39.

2) The authors of the study note that the relationship between weight and length of *P. cinereus* is not a straight-line one. As such, it seems inappropriate to fit a linear regression model to the data.
3) Due to time constraints, we were unable to conduct a detailed search for all species specific body weights. However, Reinhart (2011) presents body weight ranges for male and female Red Hills Salamanders (*Phaenognathus hubrichti*) of 7-22 and 6-14 g, respectively. The lowest values from this range are 25 and 29 times higher than the body weight (0.24 g) estimated by EPA.
4) There are uncertainties with estimating body weights using a regression that are outside the range of the data used to create the regression. The body weight for male and female Jemez Mountain salamanders of 8.1 and 8.8 g, respectively, are way beyond the range of data provided in Heatwole and Heatwole (1962) who report body weights up to 2 g.

Due to the time constraints of a 60 day comment period, we were unable to critically evaluate all of the methods used for estimating body weights for other herptile, bird or mammal studies. However, the comments provided above on the approach taken for these salamander are just an indication that EPA could have made potential errors or inappropriate assumptions for other species. This can have a large influence on the potential risk outcome and should be carefully considered and addressed.

Comment 14
TEDtool_v1.0_alt.xlsx and TEDtool_v1.0.xlsx
Worksheet: Min and max rate doses
Column: V, W, X, Mortality, sublethal and lowest LD50 threshold

Column V, W, X are supposed to hold the body mass-adjusted dose-based effects metric for all listed terrestrial vertebrate species in the TEDtool. For birds, it is clear that the body mass scaling applied in T-REX is retained here. However, for herptiles, an exponent of 1 is applied in the avian body mass scaling equation. This is equivalent to a scaling factor of 2, and results in the test 1/million dose estimate being multiplied by the ratio of the body weights of the species being assessed and the test species. This leads to much lower effects metrics for herptiles which are typically smaller than the test species (compared to birds). There is no justification for this scaling factor anywhere in the document. It is presumed in error, where body mass scaling should have been omitted due to a lack of data supporting body mass scaling for terrestrial vertebrates potentially exposed to malathion in general, and moreover no data at all for terrestrial herptiles specifically.

2.1.2 Drinking Water

Comment 1
Attachment 1-7
Section 7b (Doses received by birds, mammals, reptiles and terrestrial-phase amphibians): Drinking water

The Agency states that Equation 3 (Equation 2-2 herein) will be used to estimate puddle concentrations for drinking water exposures. The depths of half an inch and six inches are to be used to estimate “upper bound” and “mean” doses.

EPA does not justify the selection of depths, nor how they actually reflect upper bound and mean exposures for drinking water.

The approach presented does not account for any degradation or dissipation of malathion, and assumes that the system is at equilibrium. Since malathion is not to be applied during, or in close proximity to rain events, and because malathion degrades rapidly in soil and water, it is implausible that wildlife would be exposed to concentrations as high as those estimated with Equation 3 in Attachment 1-7 (Equation 2-2 herein). Further, the approach does not account for any interception by crop foliage, which would be typical of malathion applications, which are made to crop foliage (with the exception of mosquitocide use for public health applications).

2.1.3 Dermal Exposure

Comment 1
Attachment 1-7
Section 7c (Doses received by birds, mammals, reptiles and terrestrial-phase amphibians): Dermal, Table A 1-7.8

In Table A 1-7.8, EPA presents parameters used to estimate the surface area of vertebrates. To estimate the surface area of turtles and tortoises available for potential dermal absorption, the Agency presents parameters for an allometric equation for soft-shelled turtles. However, there are no federally listed soft-shell turtles in the United States (ecos.fws.gov; accessed April 6th, 2016). For most hard shell turtles it is excessively conservative to assume that their entire surface area is a potential dermal absorption site. The possible exception in the United States is the leatherback sea turtle (*Dermochelys coriacea*) which lacks a bony shell. However, given this species’ marine habitat, direct spray from pesticides is improbable.

Comment 2
Attachment 1-7
Section 7c (Doses received by birds, mammals, reptiles and terrestrial-phase amphibians): Dermal, Equation 13

EPA provides the equation used to estimate dermal spray dose, which is a function of estimated animal surface area, the fraction assumed to be exposed to spray deposition (0.5), the dermal absorption factor, the dermal equivalency factor and the body weight of the animal.

First, the assumption that 0.5 of the surface area of the animal will be exposed to spray drift is likely a gross overestimate for many terrestrial vertebrates, given that most birds and mammals have limbs on their ventral side that account for a considerable amount of their overall dermal surface area. Second, the equation in no way accounts for any interception by fur, feathers, shell or scales which are assumed to considerably reduce dermal exposure (e.g., Suter, 2007). Notably, also, this is in contrast with the approach to generating the dermal contact dose in Attachment 1-7 (top of page A7 (PF)-13), where 0.079 is used to represent the fraction of the animal in contact with foliage. This number is reportedly based on the fraction of bird that is represented by unfeathered feet.

Comment 3
Attachment 1-7
Section 7c (Doses received by birds, mammals, reptiles and terrestrial-phase amphibians): Dermal, Equation 16

TEDTool_v1.0_alt.xlsx and TEDTool_v1.0
Worksheet: Min and Max rate doses
Column: O, Dermal dose–contact (upper bound)

The following equation is used in Column O for birds and mammals to estimate the upper bound dermal dose for contact exposure (with foliage).

$$D_{contact(t)} = \frac{C_{plant(t)} * F_{dfr} * R_{foliar\ contact} * 8 * (SA_{total} * 0.079) * 0.1}{BW} * F_{red}$$

Equation 2-14

Where,

$D_{contact(t)}$	=	Not explicitly defined in Attachment 1-7, but presumably contact dose (µg a.i./g bw; reportedly calculated on a daily time assuming eight hours of activity)
$C_{plant(t)}$	=	Concentration of the pesticide in crop foliage at time *t* (mg/kg)
F_{dfr}	=	Dislodgeable foliar residue adjustment factor (kg/m^2; default = 0.62).
$R_{foliar\ contact}$	=	Rate of foliar contact (default = 6.01; cm^2 foliage/cm^2 body surface per hour)
SA_{total}	=	Total surface area of bird (cm^2)
BW	=	Body weight (g)

This equation comes directly from the TIM technical manual (EPA, 2015a). In Attachment 1-7, and also in the TIM manual, the Agency states that “In this equation, a factor of 0.1 is used to generate $D_{contact(t)}$ value with units in µg a.i./g-bw.”

The description of the F_{dfr} value used in Equation 2-15 as described in the TIM manual suggests a major flaw in the $D_{contact(t)}$ equation (Equation 2-14).

In Section 6.2.1 of the TIM manual, it is stated that the F_{dfr} value is necessary because "total residues are commonly expressed in terms of mass of pesticide per unit fresh mass of vegetation, while dislodgeable residues are commonly expressed in terms of mass of pesticide per unit surface area of the vegetation". The following formula is then provided for calculating F_{dfr} on the basis of dislodgeable pesticide residues (DPRs) and total pesticide residues (TPR) measured immediately following application:

$$F_{dfr} = \frac{DPR}{TPR}$$

Equation 2-15

Where,

F_{dfr} = Fraction of dislodgeable foliar residues (kg/m^2)
DPR = Dislodgeable pesticide residues (mg/m^2)
TPR = Total pesticide residues (mg/kg)

In the absence of chemical specific data, the TIM manual indicates that a default value for F_{dfr} of 0.62 can be calculated by setting DPR to 28 mg/m^2 and TPR to 45 mg/kg. The TPR value is said to be "the mean for the total pesticide residue value on broadleaf plants." (no reference given). The Dislodgeable Pesticide Residue (DPR) value is stated to be "based on the Health Effects Division's default assumption that at day 0, the dislodgeable foliar residue value is 25% of the application rate (in lb a.i./A) (Section D.6.2 of Appendix D of USEPA, 2012b)". Note that this value was converted from lb a.i./A to mg/m^2." However, the conversion from 25% of the application rate (in lb a.i./A) to 28 mg/m^2 (with no mention of application rate) is clearly incorrect. Mathematically, 25% of the application rate (in lb a.i./A) would also equal 25% of the application rate (in mg/m^2 or any other unit) and cannot be estimated independently of the actual application rate.

Review of the actual HED document (EPA, 2012) clarifies that, contrary to what is stated in the TIM manual, field studies have been done to quantify dislodgeable residue amounts as a fraction of the application rate for various types of crops and various active ingredients. On the basis of these data, HED recommends that "when chemical-specific data are unavailable the recommended default value for the fraction of application rate as dislodgeable foliar residue for both liquid and solid formulations following application is 0.25 (25%)." This value is presented as the arithmetic mean of 60 measured values in Table D-20 of the HED document (EPA, 2012). Therefore, if the HED assumption of 25% application rate as dislodgeable foliar residues is a reasonable assumption for the NESA assessment, Equation 2-14 should be corrected to:

$$D_{contact(t)} = \frac{A_{rate} * F_{dfr} * R_{foliar\ contact} * 8 * (SA_{total} * 0.079) * 0.1}{BW} * F_{red}$$

Equation 2-16

Where,

$D_{contact(t)}$	=	Contact dose (µg a.i./g bw; reportedly calculated on a daily time step assuming eight hours of activity)
A_{rate}	=	Application rate in mg/m^2 (equivalent rate to be used in instances of multiple applications)
F_{dfr}	=	Fraction of dislodgeable foliar residues (unitless, default = 0.25, based on HED report).
$R_{foliar\ contact}$	=	Rate of foliar contact (default = 6.01; cm^2 foliage/cm^2 body surface per hour)
SA_{total}	=	Total surface area (cm^2) of bird
BW	=	Body weight (g)
F_{red}	=	Dermal route equivalency factor

The Agency erroneously calculated F_{red} as the oral LD50 divided by the log(dermal LD50) in column O of the "Min rate doses" and "Max rate dose worksheets in the Tedtool_v1.0.xlsx file, and alt file. They neglected to raise the estimated log(dermal dose) to the power of ten to get the correct estimate for F_{red} (i.e., oral LD50 divided by dermal LD50). This resulted in a lower dermal LD50 estimate than oral LD50, which is the exact opposite of what is expected based on the equation used to estimate dermal LD50 in Attachment 1-7 and the TIM Manual. See Equation 2-17.

$$logLD_{50(dermal)} = 0.84 + 0.62 * logLD_{50\ (oral)}$$

Equation 2-17

A worked through example will show the implication for the BE estimates.

We take the single application rate of 5.1 lb a.i./A, and consider the dermal contact exposure of the Northern aplomado falcon (*Falco femoralis septentrionalis*). EPA estimated a dermal contact dose of 14,857.8 mg a.i./kg. The upper bound dietary dose for a diet of arthropods was 110.3 mg a.i./kg bw. The estimated body weight is 325 g. The surface area based on the equation provided in Attachment 1-7 is 473.6 cm^2 (this is correctly calculated in the TEDtool for this species).

F_{red}, according to the Agency is 62.9, based on an estimated dermal LD50 of 2.16 mg/kg bw, supposedly corresponding to an oral LD50 of 136 mg/kg bw. However, following Equation 2-17 the dermal LD50 estimate should be 144.5 mg/kg bw. This would give an F_{red} of 0.94.

Using Equation 2-16 above we calculate the following:

First, 5.1 lb a.i./A = 2,313,319.2 mg a.i./A = 571.633 mg/m^2

$$D_{contact(t)} = \frac{(571.633\frac{mg}{m^2}) * (0.25) * \left(\frac{6.01\ \mathrm{cm^2 foliage}}{\mathrm{cm^2\ body\ surface\ per\ hour}}\right) * 8\ hour(473.6\ cm^2 * 0.079) * 0.1}{325\ g} * 0.94$$

$$D_{contact(t)} = \frac{74.4\ mg\ a.i.}{kg\ bw}$$

This value is 200 times lower than EPA's estimate for this species, and is notably lower than the upper bound dietary dose estimate of 110.3 mg a.i./kg bw for the bird consuming arthropods.

There are also a few other notable comments to be made on EPA's approach to estimate dermal exposure. First, multiplying the rate of foliar contact by eight, and then multiplying the residue concentration on the plant will significantly over estimate exposure over eight hours because it does not account for any dissipation of the foliar residues over the eight hour period. Dissipation of foliar residues can be quite rapid for some OP pesticides, including malathion. The estimated foliar half-life on forage/leafy crop is less than two days (1.86 days; Moore et al., 2014 [MRID 49389301]). The time of day during which an animal is active is also an important factor in estimating exposure. This is not accounted for in the Agency's dermal exposure estimation, nor in their estimation for oral routes (diet, drinking water and inhalation).

Second, dislodgeable foliar residues are generally washed off the surface of tested foliage using a solution (Korpalski et al., 2005). The amount of residue dislodged this way from a unit area of leaf is unlikely to be equivalent to the amount dislodged and transferred to animals via dermal contact with the same area. The fraction of dislodgeable residues transferred during contact is likely highly variable and dependent on the surface textures, degree of moisture at the interface and the forces acting at the interface between the leaf surface and the exposed skin. It is improbable that *all* dislodgeable residues will transfer on contact.

Comment 4
TEDTool_v1.0_alt.xlsx and TEDtool_v1.0.xlsx
Worksheet: Min and Max rate concentrations
Column(s): O and P (Upper bound and mean dermal contact dose)

In estimating the upper bound and mean dermal contact dose in the TEDtool, EPA selects the maximum residues from the min and max rate concentration worksheets. These residues correspond to those estimated for broadleaf plants assuming upper bound and mean RUDs of 135 and 45 mg a.i./kg ww per lb a.i./A, respectively. This is not appropriate given that residues vary depending on the crop they are found on. The residues found on plants, used in estimating dermal exposure should represent the corresponding use pattern (i.e., grass RUDs assumed for tall crop species like corn or wheat and broadleaf for leafy crops like lettuce).

2.1.4 Inhalation

Comment 1
TEDtool_v1.0_alt.xlsx; TEDtool_v1.0.xlsx
Worksheet: Min and Max Rate Doses
Column: R

When taxa-specific inhalation toxicity data are not available for birds, the relationship between rat acute oral and inhalation LD50 values were used to establish a route equivalency factor. The equation below was used:

$$F_{re} = \frac{LD_{50\ (oral;\ mammal)} * F_{AM}}{LD_{50(inhalation;\ mammal)}}$$

Equation 2-17

Where, F_{AM} is the relative rate difference between mammals and birds.

EPA states that to simplify the approach (in determining which F_{AM} value to assume for each listed species) the maximum available F_{AM} value of 3.4 will be used. In fact, however, in column R, the value of 2.8 was used, which corresponds to the factor for animals 30-50 g. Presumably this was done in error, as the actual calculations in the TEDtool do not match what is described in text.

Comment 2
Attachment 1-7
Section 7d (Doses received by birds, mammals, reptiles and terrestrial-phase amphibians): Inhalation

In describing the comparison between inhalation and oral toxicity, the Agency states that "Given [the] lack of knowledge relating reptiles and amphibians to mammals, it will be assumed that the oral and inhalation routes of exposure are equivalent for reptiles and terrestrial-phase amphibians. Therefore, a F_{AM} value of 1 will be used." Where F_{AM} is the relative diffusion rates across the pulmonary membrane.

If oral and inhalation rates are presumed to be equivalent for terrestrial herptiles, presumably F_{re} should be assumed to be one, not F_{AM}. That is, the LD50 oral is equal to the LD50 for inhalation converted to mg/kg bw. Setting F_{AM} to one does not make oral and inhalation exposures equivalent. See Equation 2-17 above.

Comment 3
Attachment 1-7
Section 7d (Doses received by birds, mammals, reptiles and terrestrial-phase amphibians): Inhalation

EPA describes how vapor inhalation exposure, below the crop canopy, will be calculated. Equation 24 (Equation 2-18 herein) reportedly estimates the concentration of the pesticide in the air at a particular time, based on a partitioning model between the foliage and the air.

$$C_{air\,(t)(vol)} = \frac{M_{pesticide}}{V_{air} + \left(\frac{m_{plant} * B_{vol}}{\rho_{plant}}\right)} \times e^{-rt}$$

Equation 2-18

Where,

$C_{air(t)(vol)}$	=	Air concentration
$M_{pesticide}$	=	The pesticide concentration on the field at time *t* (accounting for dissipation); function of application rate (mg)
V_{air}	=	The volume of air in 1 ha to a height equal to the height of the crop canopy (L)
m_{plant}	=	Not defined in Attachment 1-7
B_{vol}	=	Volume-based biotransfer factor (units not defined in Attachment 1-7)
ρ_{plant}	=	The density of the crop tissue (default = 0.77; kg/L)
r	=	Not defined in Attachment 1-7
t	=	Not explicitly defined in Attachment 1-7, but presumably time in hours

First, several model parameters are not defined in Attachment 1-7 making it difficult for the reader to reproduce results. Why is a first order degradation rate being applied in the model? Is it not true that $M_{pesticide}$ already accounts for dissipation? What value is to be assumed for *r*? What is m_{plant} (presumably the mass of crop on the one hectare field, but not defined in Attachment 1-7), and how will the Agency estimate m_{plant} for various use scenarios? When the TEDtool root files were reviewed, based on the equations in their min and max concentration tabs (column R), it appeared that the m_{plant} parameter was assumed to be 25,000 (presumably in kg/ha). Without any justification of this assumption it is impossible to determine if 25,000 kg/ha is an appropriate assumption to make.

Second, although the model is ambiguous, it seems as though EPA is assuming that all volatilized pesticide will be trapped beneath the crop canopy. This is obviously unrealistic and counter intuitive. If a pesticide is sprayed downward, interception will be greatest at the top of the canopy, which is exposed to the atmosphere. Thus, when intercepted pesticide volatilizes from the surface, much of it is expected to diffuse into the open atmosphere, as opposed to an illusory closed system beneath the canopy. In scenarios in which the crop does not cover the ground entirely (e.g., orchards), there is little constraint on airflow below the canopy, particularly at the time scales of hours and days. Thus, the Agency's approach seems overly conservative.

Additionally this approach is inconsistent with the approach taken by HED in their vapour drift risk assessments (EPA, 2013).

Comment 3
TEDtool_v1.0_alt.xlsx; TEDtool_v1.0.xlsx
Worksheet: Min and Max Rate Doses
Column: S

To estimate inhalation dose via volatilization, EPA uses the equation

$$D_{vapour(t)} = \frac{C_{air(t)(vol)} * V_{inhalation} * 24}{BW} * F_{re}$$

Equation 2-19

Here, the air concentration and respective volume of air respired is multiplied by 24, to estimate the dose over a 24 hour period. This assumption is overly conservative because it does not account for the fate and behavior (e.g. degradation) of the pesticide over a full day.

2.2 Terrestrial Plants

The comments in this section are focussed on Attachment 1-21 ("Biological Information on Listed Species of Plants and Lichens and Model Parameterization for Pesticide Effects Determinations") and files contained in the Weight of Evidence (WoE) Tools zip file for malathion available on the "Provision Models for Endangered Species Pesticide Assessments" website (https://www.epa.gov/endangered-species/provisional-models-endangered-species-pesticide-assessments#woe – Accessed April 11, 2016).

Comment 1
Chapter 3

The exposure assessment chapter (Chapter 3) of the biological evaluation of malathion itself does not contain a discussion of how exposure of listed plant species was assessed, nor are exposure estimates for terrestrial plants provided in this chapter. Exposure estimates were located in the WoE tool files, which are not provided, nor referenced, on the biological evaluation website (https://www.epa.gov/endangered-species/provisional-models-endangered-species-pesticide-assessments#woe). Determining the existence and location of these files took several communications with the Agency following the April 2016 release of the complete draft biological evaluations (email communications between Steven Snyderman (EPA) and Sara Rodney (Intrinsik), last communication dated April 11, 2016). Moreover, in their response to comments provided in the Request for Comment period extension, EPA attempted to clarify the location of the EECs for plants. Despite this, detailed discussion and summary of exposure data should be presented in the main document of the biological evaluations. This should be corrected for the final versions.

EPA is in the process of developing a new model for assessing risks to non-target plants (Audrey III; EPA, 2015b). This model has already been used in at least one ERA released by the EPA for public comment (EPA, 2015b). It is not clear why EPA did not use Audrey III in their BEs, given that the model was used in the sulfonylurea assessment conducted by EPA which was completed prior to the BE for Malathion. The EPA's provisional model, Audrey III, is based on a new, more refined conceptual model of plant exposure.

Comment 2
TEDtool_v1.0.xlsx
Worksheet: READ ME

The Agency states that the TED tool "integrates T-REX, T-HERPS, the earthworm fugacity model, TerrPlant and AgDrift" and goes on to provide a link to the full description of these models (http://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#terrestrial), implying that further details of how the TED tool works can be found there.

For terrestrial plants, however, the exposure calculations provided on the "Plant" worksheet of the TED tool do not exactly match the estimates provided by TerrPlant. Specifically, for exposures associated with drift, where TerrPlant estimates a single off-field drift exposure value in lb a.i./A, the TED tool estimates the "distance from edge of field where risk extends (ft)." Such differences should be mentioned in the text of the biological evaluation, and in the description of the model. The inaccuracy of the statement in the READ ME worksheet is misleading, and complicates validation of the Agency's work.

2.3 Terrestrial Invertebrates

Comment 1
Chapter 3
Section 3.2.1

In Chapter 3 (Measure of Terrestrial Exposure), EPA does not present a method for deriving EECs for listed terrestrial invertebrate species, nor are EECs presented for listed terrestrial invertebrate species. This comment was also made in the letter requesting extension to the comment period. EPA responded to this comment stating:

"Three types of EECs are calculated for terrestrial invertebrates: dietary based (mg a.i./kg-food), dose based (mg a.i./kg-bw) and in soil (mg a.i./kg-soil). EECs for different food items consumed by terrestrial invertebrates (e.g., broad leaves) are calculated in the same manner as described in Attachment 1-7 (see section 4). Dose-based EECs for terrestrial invertebrates that are above ground or soil-dwelling are also described in Attachment 1-7 (see section 4). The method for calculating exposures in soil is described in section 5 of Attachment 1-7. EECs are provided in the TED tools parameterized for each chemical (see response to previous comment for information on accessing this file) in the worksheets titled "Min rate concentrations" and "Max rate concentrations"."

However, the location of the “dose-based” concentrations is still unclear. There are no dose-based EECs located in the TEDtool for terrestrial invertebrates. Moreover, to estimate dose-based concentrations, an assumption of the species body weight is required (to convert mg a.i./kg diet to mg a.i./kg bw), there is no such information provided in Chapter 3, Attachment 1-7 or in the TEDtool.

Comment 2
TEDtool_v1.0_alt.xlsx and TEDtool_v1.0.xlsx
Worksheet: Max and Min Rate – Dietary Conc results

In their worksheets labeled “min and max rate – dietary concentration results”, EPA presents the “Number of exceedances of thresholds and endpoints for upper bound and mean EECs” where the number of dietary EECs within the year that exceed threshold values is presented. EPA compares the dietary EECs (as estimated in the “min and max rate concentration” worksheets) to effects thresholds (from the input page) for terrestrial invertebrates consuming a variety of dietary items (i.e., short grass, tall grass/nectar, broadleaf plants, seeds, arthropods (above ground), soil-dwelling arthropods, large mammals (carrion), and soil). In their counts for diets of arthropods (above ground) and soil dwelling invertebrates, EPA uses the dose based thresholds (in mg a.i./kg bw) to compare to the dietary exposure estimates (mg a.i./kg diet). This approach is incorrect, since dietary EECs and dose-based effects metrics are not the same measures and have differing units, and therefore cannot be directly compared. This is clearly a mistake in the TEDtool made by EPA, since reasoning for this discrepancy is not described anywhere in Chapter 3 and the EECs for other dietary items (i.e., short grass, tall grass/nectar, broadleaf plants, seeds/ fruits) are appropriately compared to the dietary based threshold values for terrestrial invertebrate species. Specifically, dose based thresholds are inappropriately used for diets of above ground arthropods (min and max rate for upper bound and mean EECs) and soil dwelling invertebrates (min and max rate for upper bound EECs only). See also Section 2.1.1, (Comment 2) for more details on why concentration-based exposure estimates should not be compared with dietary effects thresholds.

Comment 3
Attachment 1-7
Section 4b (Concentrations in invertebrates)

See Comment 3 presented above in Section 2.1.1 for additional details on EECs for soil-dwelling arthropods.

2.4 Spray Drift

Comment 1
Attachment 1-7
Section 3 (Spray drift)

The Agency presents Equation 1 in Attachment 1-7, which reportedly gives "the distance where the risk extends" based on "an analysis of the deposition curves generated in AgDrift (v. 2.1.1)". Equation 1 is (Equation 1 in Attachment 1-7; Equation 2-20 herein):

$$d_t = \frac{\left(\frac{c5}{F_{AR}}\right)^{\frac{1}{b5}} - 1}{a5}$$

Equation 2-20

Where F_{AR} is the fraction of the application rate that is equivalent to the threshold, and d_t is the distance where the risk extends. EPA makes reference to **Table A 1-7.1**, which is found on the subsequent page (page A7 (PF)-2) and contains numerical values for the parameters a5, b5 and c5 for aerial, ground and airblast application methods for a range of droplet size spectra.

First, EPA does not provide units for the distance formula presented in Equation 2-20.

Second, a reference for Equation 2-20 is not given. In the same paragraph a footnote is provided to AgDrift (v.2.1.1) that contains an outdated URL that is known to have contained a link for downloading the AgDrift model. The most recent AgDrift User's Manual (Teske et al., 2003) that is available in the regulatory version download (file name: agdrift_2.1.1.zip; retrieved from: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#atmospheric; March 29, 2016) contains the following equation used for Tier I ground sprayer assessment (Equation 2-21):

$$D(x) = \frac{c}{[1 + ax]^b}$$

Equation 2-21

Where *D(x)* is the deposition level relative to the nominal application rate, x is the downwind distance (in feet), and a, b and c are model parameters.

This equation can be rearranged to give Equation 2-20, as follows (assuming *x* in the User's Manual is d_t, and *D(x)* is F_{AR}):

$$[1 + ax]^b = \frac{c}{D(x)}$$

Equation 2-22

$$1 + ax = \sqrt[b]{\frac{c}{D(x)}}$$

Equation 2-23

$$x = \frac{\left(\frac{c}{D(x)}\right)^{\frac{1}{b}} - 1}{a}$$

Equation 2-24

Presumably then, the Agency obtained Equation 2-21 from the AgDrift User's Manual. However, in the User's Manual this equation applies to low boom ground sprayer applications, and describes models fit to empirical ground sprayer data only. It is unclear how EPA determined the three parameters for any of the application methods (ground, aerial or airblast), as even the parameter values for groundspray do not match those presented in the User's Manual. The Agency refers to an analysis of AgDrift output that is not presented, nor cited. Finally, EPA does not specify how many swaths the model and associated parameters (Equation 1 and Table A 1-7.1 in Attachment 1-7) apply to. In the AgDrift User's Manual, a, b and c parameters are estimated for a single swath only. AgDrift v.2.1.1 does not provide numerical values for *a, b* or *c* in any of the software's output.

Comment 2
TEDtool_v1.0_alt.xlsx and TEDTool_v1.0.xlsx
Worksheet: Max and Min Rate – Dietary Conc results

In these worksheets, EPA presents estimated spray drift distances (for upper bound EECs) for each taxa and diet assessed in the exposure assessment. These results, however, are not described in Chapter 3, nor do they seem to be accounted for in the final risk conclusions or calls for listed species (Chapter 4). Therefore, the purpose of these estimates is unknown.

2.5 Chemical Specific Comments on Selected Input Parameters

The comments provided below are focused on the chemical specific assumptions EPA made in their terrestrial exposure modeling. This section provides comments on the input parameters selected for use in the TEDtool (Section 0) as well as chemical specific results presented in the BE (Section 2.6). Some comments also apply to descriptions and references presented in Attachment 1-7 and Chapter 3. Comments are organized based on the chemical specific inputs for: residue unit doses (Section 2.5.1), foliar dissipation half-life (Section 2.5.2), aerobic metabolism half-life (Section 2.5.3), daily fraction retained (Section 2.5.4), Logkow (Section 2.5.5), and BCFs (Section 2.5.6).

In general, EPA fails to provide appropriate references for all chemical specific parameters in each location where the data is used. Moreover, EPA fails to provide discussion of studies and rational for selected parameters where appropriate. This makes review of and interpretation of results within the BE an oenerous task.

2.5.1 Residue Unit Doses (RUD)

Attachment 1-7
Section 4

TEDtool_v1.0_alt.xlsx and TEDtool_v1.0.xlsx
Worksheet: Min and max rate concentrations

In describing the method for estimating residues on plants and terrestrial arthropods (as dietary items consumed by terrestrial organisms), EPA refers the reader to the T-REX user manual for details. T-REX (v.1.3.1) uses Hoerger Kenaga (1972 [MRID 47299308]) nomograms (as modified by Fletcher et al. 1994) upper bound RUDs for specific plant feed items. These values have been characterized as being overly conservative (Trask et al., 2010) that rely on data reflective of outdated application practices and analytical methods (over 40 years old). As such, Cheminova, derived chemical specific RUDs for dietary items including: short grass, long/tall grass, forage/leafy crops, small fruit/seeds and large fruit (Moore et al., 2014 [MRID 49389301]). These data should be used in estimating on-field exposure to malathion (Table 2-1).

Table 2-1 Estimated RUDs for malathion on vegetation (mg a.i./kg ww per lb a.i/A)

Feed Item	*EPA Upper Bound RUD*[a]	*Cheminova's Malathion-Specific RUD*[b]	
		Mean	*95th Percentile*
Short grass	240	77.4	238
Long/tall grass	110	45.4	191
Forage/ leafy crops	135	28.3	90.0
Small fruit/Seeds	15	1.70	6.99
Large Fruit	NA	0.540	2.82

[a] Hoerger-Kenaga nomograms (Hoerger and Kenaga, 1972 [MRID 47299308]) as modified by Fletcher et al. (1994), and applied in T-REX v.1.4.1.
[b] As calculated by Moore et al. (2014 [MRID 49389301]).

For estimating concentrations in terrestrial arthropods, EPA uses upper bound and mean RUD values of 94 and 65 mg a.i./kg ww per lb a.i./A, respectively. The upper bound RUD represents the 90th percentile residue level on the 90th percentile field, derived via simulations with the data (EPA, 2012). This RUD represents a more scientifically defensible approach to estimating conservative exposure levels for arthropod consuming species that could be used for screening-level purposes. However, as noted by the NRC panel "model predictions can be only as accurate as the parameter estimates. If the relevant parameter values and their variances are poorly known the model predictions will be uncertain and difficult to use in decision making." As such, probabilistic residue estimates should be considered. Cheminova developed arthropod

RUDs (distinguishing between different arthropods groups) using residue data from EPA as well as registrant submitted data. The distributions for the residues are provided in Table 2-2, below.

Table 2-2 Summary of estimated lognormal distributions of insecticide RUDs for arthropods for use in refined risk assessment

Arthropod Type	*No. Trials*	*Mean of Natural logarithms of trial RUDs*	*Standard Deviation of Natural Logarithms of Trial RUDs*	*Percentiles of Estimate RUD Distribution (mg a.i./kg ww per lb a.i./A)*				
				5th	25th	50th	75th	95th
Flying Insects	7	0.154	1.60	0.0833	0.395	1.17	3.45	16.4
Orchard Crop-dwellers	19	2.56	0.99	2.54	6.63	12.9	25.1	65.5
Ground Crop-dwellers	15	2.02	1.21	1.03	3.34	7.56	17.1	55.3
Orchard Ground-dwellers	15	0.697	0.946	0.424	1.06	2.01	3.80	9.51
Ground Crop Ground-dwellers	20	1.16	1.15	0.477	1.46	3.18	6.91	21.2

Appendix B of EPA's T-REX User's Manual was used to identify studies known to contain pesticide residue data for arthropods (EPA, 2012b). ProQuest, a commercial bibliographic database, was used to search the open literature for other studies with the keywords: 'pesticide', 'residue' and 'insect' or 'arthropod' or 'cricket' or 'grasshopper' or 'beetle'. Eight articles were identified in the open literature that contained data meeting the criteria detailed below (Davis and French, 1969; Stromborg et al., 1982; Powell, 1984; Stromborg et al., 1984; Forsyth and Martin, 1993; Forsyth and Westcott, 1993; Brewer et al., 2003; Stahlschmidt and Bruhl, 2012). Also, Barber et al. (2005 [MRID 47841001]), a CropLife America (CLA) review of registrant studies investigating pesticide residues on arthropods, was used. Five Cheminova studies looking at residues of malathion and dimethoate on arthropods collected in ground crops and orchards were included in the analysis (Knäbe, 2004a,b [MRIDs 46525902, 46486401]; Hanebeck and Staedtler, 2011 [MRID 49086411]; Hanebeck and Henkes, 2011 [MRID 49348001]; Staedtler et al., 2011 [MRID 49086410]). See Breton et al. (2014b [MRID 49400601]; 2016c (in prep)) for a complete list of references. The dataset considered in Cheminova's analysis provides a more accurate estimation of arthropod RUDs than EPA's. Cheminova's dataset is more robust, and includes measured residues on various terrestrial invertebrates that allow for calculation of specific RUD estimates of different classes of arthropods. Further, the RUDs are based on only liquid formulations of organophosphate pesticides (not granular formulations).

2.5.2 Foliar Dissipation Half-life

TEDtool_v1.0_alt.xlsx and TEDTool_V1.0.xlsx
Worksheet: Inputs
Cell: C51 (Foliar dissipation half-life)

EPA presents a foliar dissipation half-life of 6.1 days, reported to be a "90th % mean on 37 malathion residue foliar persistence half-lives ranging from 0.3 to 10.9." from Willis and McDowell (1987).

Firstly, EPA did not present the data from Willis and McDowell (1987) that they considered in their DT50 estimate. Secondly, EPA did not consider any of the more recent plant residue studies that were submitted by the registrant to the agency. Using these data, Moore et al. (2014 [MRID 49389301] estimated malathion specific foliar DT50s of 2.28 days, 6.69 days and 3.80 days for foliar crops, small fruits/seeds/pods and large fruit, respectively. Thirdly, EPA used a foliar dissipation half-life to estimate degradation on terrestrial invertebrates in their terrestrial exposure modeling. This is not acceptable given that there are data to support an arthropod specific half-life estimate (Knäbe, 2004 [MRID 46525902]; Hanebeck and Staedtler, 2011 [MRID 49086411]; Staedteler et al., 2011 [MRID 49086410]). Breton et al., 2016c (in prep)) used these data to estimate an arthropod specific T90 of 3.54 days.

2.5.3 Aerobic Metabolism Half-life

TEDtool_v1.0_alt.xlsx and TEDTool_V1.0.xlsx
Worksheet: Inputs
Cell: C52 (Aerobic soil metabolism half-life)

An aerobic soil metabolism half-life of 1 day was used in EPA's terrestrial exposure modeling. However, there is no reference provided for this value in the TEDtool input parameters. In Chapter 3, EPA presents three MRIDs (41721701, 46769501 and 47834301) and state, "90th% mean on seven half-lives from registrant submitted guideline studies results in 0.5 day half-life input parameter (See Table 4). However, GLN 835.4100 stipulates soil moisture at 40%-60% of water holding capacity and malathion rapidly hydrolyzes. Non-guideline studies indicate that persistence is increased in soils with low moisture and low microbial activity (Walker and Stojanovic 1973). To account for the contribution of hydrolysis in metabolism studies, the input parameter is doubled."

Firstly, all three soil metabolism studies used by EPA are registrant submitted studies (Blumhorst, 1990 [MRID 41721701]; Knoch et al., 2001a [MRID 46769501]; Saxena (1998 [MRID 47834301]). EPA previously classified Saxena (1998 [MRID 47834301]) as unacceptable based on a number of factors (EPA, 2011a). Therefore, consistent with EPA policy, Cheminova removed this study from their half-life derivation calculation. Since EPA has concluded that the study by Saxena (1998 [MRID 47834301]) is invalid, it must omit it from its calculations.

Secondly, doubling of the soil metabolism half-life is unjustified and seemingly arbitrary for the following reasons:

1) Hydrolysis is embedded in the soil half-life, so subtracting it artificially removes malathions primary means of degradation in soil pore water. There is no other e-fate parameter for modeling in soil that simulates the hydrolysis process.
2) Typically, the soil half-life is the lumped dissolved and sorbed half-lives, (combined microbial and hydrolysis). The inclusion of degradation in the "sorbed-phase" naturally slows down the aerobic soil half-life. Therefore additional factors to slow the rate are unnecessary.
3) Dry, desert soil is not representative of an agricultural field and 40-60% moisture is typical of agricultural fields (and is part of the guidelines for soil metabolism studies in general), as such there is no reason to deviate from an empirically derived value calculated under those conditions.

Based on EPA's decision to make Saxena (1998 [MRID 47834301]) unacceptable for use in risk assessment, Cheminova calculated a mean soil half-life from five half-life estimates from the two available (and accepted) aerobic soil GLP studies (0.21 days from Blumhorst (1990 [MRID 41721701]); and, 0.17, 0.18, 0.25, and 0.25 days from Knoch (2001a [MRID 46769501])). The estimated 90th percentile upper confidence bound on the mean soil half-life is 0.24 days. To be consistent with the Agency's guidleines, the mean soil half-life of 0.24 days must be used as the aerobic soil metabolism input into EPA's environmental modeling for the malathion BE. See Section 3.2 below for further discussion on this parameters and its use in the aquatic assessment.

2.5.4 Daily Fraction Retained

Comment 1
TEDtool_v1.0_alt.xlsx and TEDTool_V1.0.xlsx
Worksheet: Min and Max Rate Concentrations
Column: J-M

EPA is not consistent in describing their approaches for estimating dietary exposure estimates and how they address metabolism of their daily intake. As such, it is difficult to identify their approach, without accessing and reviewing the calculations located in the TEDtool. Examples of the inconsistency are described below.

In estimating upper bound dietary concentration based EECs for terrestrial prey items (small and large mammals, small birds and small amphibians/reptiles), EPA accounts for the prey items body burden by multiplying the estimated daily dose by a "daily fraction that is retained in mammals" (a user input found on the inputs page). This method (depending on the data used to derive the fraction) presumably accounts for any degradation, excretion or metabolism of the consumed pesticide within the terrestrial vertebrate prey.

In Attachment 1-7 (Methodology for Estimating Exposures to Terrestrial Animals) or in Chapter 3 (Exposure Characterization for Terrestrial Mammals), EPA does not describe their use of the "fraction retained. In fact, when describing the estimation of concentrations on and in food or terrestrial dietary items, EPA only refers to the T-REX and T-HERPS manuals. EPA attempted to clarify the use and source of this parameter in their response to the request for an extension to the comment period (See Comment H-14 in their response (EPA, 2016b,c). However, to maintain full transparency (and efficiency for review), EPA should provide a discussion of the methodology and full references for each value or assumption presented in the TEDtool and throughout the document.

Both T-REX and T-HERPS models assume that the dietary dose within terrestrial vertebrate prey equals their total daily intake. Explicitly, the T-HERPS user guide states "Depuration of the pesticide from the prey item due to excretion or metabolism has not been included in the estimation. Therefore, the EECs for chemicals that are short-lived in an animal are expected to represent an over-estimate of exposure".

Moreover, in Chapter 3, EPA recognizes that metabolism does occur within the animal prey in their statement describing peak dietary concentrations (values presented in Table 3-12) on Page 3-39:

"As malathion residues on grass and insects dissipate, residues would be expected to decrease in terrestrial vertebrate prey. In addition, malathion residues would likely be metabolized by terrestrial vertebrates to the non-toxic metabolites, dicarboxylic acid (DCA) and monocarboxylic acid (MCA). Therefore, EECs in Table 3-12 represent conservative estimates of malathion concentrations in vertebrate prey".

EPA does not convey a clear description in their BE of how dietary concentrations in terrestrial vertebrate prey are estimated. Moreover, their calculations (found only in the TEDtool) contradict their described approaches in Chapter 3, Attachment 1-7 and associated T-REX and T-HERPS Manual. This should be clarified.

Comment 2
TEDtool_v1.0_alt.xlsx and TEDTool_V1.0.xlsx
Worksheet: Inputs
Cell: C53 and C54

On their User Inputs page, EPA includes the parameters "daily fraction retained in mammals" and "daily fraction retained in birds". These fractions of 0.27 and 0.81 are reported to be from studies only referenced as MRIDs 41367701 and 42715401. These studies correspond to registrant submitted data (Reddy et al., 1989 [MRID 41367701] and Cannon et al. 1993 [MRID 42715401]). However, EPA should provide full references for data they used in their assessments. Cheminova also has an additional study that should have been considered (Cannon et al. 1992 [MRID 42581401]).

Firstly, EPA does not describe in detail how these values are used in their calculations (See previous comment on terrestrial prey doses). Secondly, EPA does not provide a summary of these studies or present the data in which these values were derived from in their discussion on the estimation of dietary EECs. Thirdly, EPA only makes reference to these studies in a footnote in the Sea turtle analysis in Chapter 4, where they don't use the data quantitatively or describe how it was used quantitatively in the exposure analyses for other species. Moreover, the full references for these studies were difficult to find. The full references could only be found in Appendix 2-4 (Bibliography of Registrant submitted studies) of Chapter 2, where the studies are not even referenced. All of these issues make it extremely difficult to figure out what data EPA used and where the data came from. EPA attempted to clarify the use of this parameter in their response to the request for an extension to the comment period (See Comment H-14 in their response). However, to maintain full transparency (and efficiency for review), EPA should provide full references for each value or assumption presented in the TEDtool and throughout the document.

2.5.5 LogKow

TEDtool_v1.0_alt.xlsx and TEDTool_V1.0.xlsx
Worksheet: Inputs
Cell: C55 (LogKow)

In the TEDTool, EPA presents a Log Kow of 3.3, without a reference. In Chapter 3, a range of Kows (195-2000) is presented with a footnote indicating that these values were derived from registrant studies. Presumably EPA derived the Logkow of 3.3 using the highest reported Kow from this range. Nowhere else in the document is there reference to the estimated LogKow values used in EPA's terrestrial modeling. A registrant submitted study by Mangels (1987 [MIRD 40944108]) reports a LogKow of 2.75. This study should be considered by EPA and subsequently used in their BE.

2.5.6 Bioconcentration Factors (BCFs)

TEDtool_v1.0_alt.xlsx and TEDtool_v1.0.xlsx
Worksheet: Inputs
Cell(s): C59 – 66 (BCFs)

In their estimation of concentrations within aquatic taxa consumed by terrestrial organisms, EPA uses BCFs that were either empirically derived or generated using KABAM. As such, comments are provided below on each method and value.

Empirically derived BCFs**:** To estimate concentrations of malathion in the tissues of aquatic plants/algae, aquatic invertebrates and fish consumed by vertebrate species, EPA uses bioconcentration factors that relate the concentration of malathion within the organisms to environmental concentrations. Mean and upper bound BCFs of 23 and 131 µg a.i./kg ww per µg a.i./L, respectively, were reported to be empirically derived for aquatic plants in Chapter 3 (and TEDtool). This is in contrast to Attachment 1-7 where it states "Since no empirically based BCF

data are available for algae or aquatic plants exposed to malathion, the KABAM estimated BCFs for phytoplankton will be used to estimate concentrations of these chemicals in algae and aquatic plants". The inconsistency makes confirmation of the Agencies EECs difficult. Additionally, references for these studies are provided in the TEDtool in the form of ECOTOX and MRIDs. In chapter three, however, only the values are presented, with no discussion of the quality of the studies (i.e., acceptability, study conditions, species used etc).

The BCF of 131 µg a.i./kg ww per µg a.i./L is reported to be from MRID 43106401. This MRID corresponds to a group of documents (procedure and raw data) for a study conducted by Forbis and Leak, 1994a,b [MRID 43106401; 43106402] and Kammerer and Robinson, 1994 [MRID 43340301]. This is a registrant submitted study that actually reports a BCF for bluegill (*Lepomis macrochirus*) of 103. Because EPA fails to provide a discussion on the study and data used to determine the BCF, it is impossible to identify the discrepancy between the BCFs. As such, it seems that EPA reported a BCF of 131 from this study in error.

KABAM generated BCFs: There were no empirically generated data available for aquatic invertebrate BCFs. As such, EPA used KABAM to estimate a BCF of 72 µg a.i./kg ww per µg a.i./L. Other than stating that KABAM will be used, there is no discussion in the TEDtool or chapter 3 on any of the assumptions or data used for this modeling.

Water Concentrations**:** Cells C65-66 of the inputs page are listed water concentrations of 10 and 100 µg a.i./L. Firstly, there is no justification in the TEDtool or Chapter 3 for choosing these water concentrations other than stating that "EECs in aquatic habitats range from the parts per trillion to the parts per million". This is an incredibly large range (a million fold) in which the two arbitrarily chosen water concentrations may or may not represent actual concentrations found in the environment. Moreover, the assumed water concentration of 10 µg a.i./L is used for the two "minimum" exposure scenarios (5.1 lb a.i./A and 0.5 lb a.i./A) and a concentration of 100 µg a.i./L for the "maximum" exposure scenarios (2 lb a.i./A and 1.5 lb a.i./A with 3 applications and 7 day retreatment interval). There is no reason why a water concentration of 10 µg a.i./L would represent exposure based on a 5.1 lb a.i./A application and 100 µg a.i./L for 2 lb a.i./A. As such, it seems that the estimated dietary doses for organisms consuming aquatic taxa is more of a guess than a calculated effort to describe actual potential doses to terrestrial organisms consuming aquatic taxa.

2.6 Exposure Results

Comment 1
Chapter 3
Section 3.2; Table 3-12

In Table 3-12 of Chapter 3, EPA presents the mean and upper bound dietary EECs calculated for food items consumed by listed birds, terrestrial-phase amphibians or reptiles. When this table was compared to the appropriate TEDtool root files, approximately half of the values presented could be verified while the others could not. Those values from Table 3-12 that did

not match data in the TEDtool root files include EECs for terrestrial invertebrates (above ground and soil dwelling) for the multiple application scenario of 3 applications of 1.5 lb a.i./A; EECs for birds, mammals and amphibians/reptiles (insectivores) for all exposure scenarios. The values presented for these diets are in error.

Additionally, the ranges of EECs presented for aquatic dietary items (aquatic plants, invertebrates and fish) are incorrect. In Table 3-12 the ranges represent EECs based on water concentrations ranging from 0.01 to 100 µg/L. This range is inaccurate based on the actual water concentrations that were assumed in the terrestrial modeling (TEDtool root files) where only 10 µg/L was assumed for the "min" exposure scenario and 100 µg/L assumed for the "max" exposure scenario. Accounting for only these two water concentrations used in the terrestrial modeling exercise, the ranges should be corrected to 0.23 – 2.3 mg/kg ww for aquatic plants based on a mean and upper bound BCF of 23 µg/kg per µg/L; 0.72 – 7.2 mg/kg ww for aquatic invertebrates based on a mean and upper bound BCF of 72 µg/kg ww per µg/L; and 1.31 – 13.1 mg/kg for fish based on a mean and upper BCF of 131µg a.i./kg ww per µg/L, respectively.

Comment 2
TEDtool_v1.0_alt.xlsx and TEDtool_v1.0.xlsx
Worksheet: Min Rate Dose
Cell: I9

Based on EPA's approach for estimating upper bound and mean dietary concentrations for terrestrial organisms consuming aquatic taxa for the "min rate doses" EPA assumes a water concentration of 10 µg a.i./L. However, a mistake was made for the upper bound concentration in the diet of the Chiricaluna leopard frog (*Rana chiricahuensis*) where the water concentration of 100 µg a.i./L was used (Input cell C66) instead of 10 µg a.i./L (input cell C65). This mistake estimates an upper bound concentration in the diet that is 10x higher than it should be. See additional comments in Section 0 (Comments on Bioconcentration factors), presented on EPA's approach to estimating concentrations in aquatic organisms.

Comment 3
Chapter 3
Section 3.2, Table 3-12

The maximum upper bound concentration based EEC for short grass is calculated in the TEDtool to be 596 mg/kg diet, which represents the malathion residues found on short grass on the first day after the last application (3/3). In Table 3-12 of Chapter 3, EPA incorrectly reports this dietary EEC as 586 mg/kg diet. This appears to be a typographical error.

2.7 Summary of Concerns Regarding the Terrestrial Exposure Analysis

As described above, Cheminova has many concerns with the terrestrial exposure assessment conducted in the draft BE for malathion. The following list presents our most fundamental concerns:

- EPA fails to make use of best available chemical specific data in the draft BE. In many cases, they used out-of-date or generic input values to parameterize the terrestrial exposure models. Notably, all registrant commissioned data should be considered by EPA for use in the BE. For Malathion, a comprehensive list and review of these studies is presented in Breton et al. (2014a [MRID 49333901]).
- The exposure assessment was conducted using the newly developed TEDtool model that integrates many of EPA's standard toolbox models (i.e. T-REX, T-HERPS, TerrPlant, and earthworm fugacity model). EPA is inappropriately using this exposure tool for risk assessment before it has been fully evaluated, quality assured and peer reviewed. While EPA often refers to the user guides for details on the models within the TEDtool, in some cases, there are deviations between methods described in the model user guide versus what is actually being done within the TEDtool framework. These issues as well as the lack of sufficient information for reviewers to evaluate or replicate the terrestrial exposure assessment frustrates the review process, and at times precludes meaningful review altogether.
- EPA fails to comply with recommendations as per the NRC panel (NRC, 2013) to conduct probabilistic assessment wherever possible, thus leading to highly conservative results without context of the probability of risk (See Section 2.1.1, Comment 9 for an example).
- A number of hyper-conservative assumptions are employed without the consideration of realistic exposure scenarios, ultimately leading to an overly conservative exposure assessment.
- EPA inappropriately compares dietary concentration estimates (mg a.i./kg diet) to dietary effects thresholds to estimate risk (See Section 2.1.1, Comment 2 for an example), thus indicating nonsensical estimates of risk.
- Specific calculation errors are noted, including the major errors in the dislodgeable residue assumptions (derived from the TIM user manual) and in the estimation of the dermal equivalency factor used in the dermal exposure estimates for terrestrial vertebrates. These errors have a major impact on risk conclusions and lead to dermal exposure estimates that are orders of magnitude higher than corrected estimates (See Section 2.1.3, Comment 3 for an example).
- There is an error in the way EPA uses body mass scaling for terrestrial herptile species leading to exceedingly sensitive effects thresholds (See Section 2.1.1, Comment 14).

In short, there are a number of serious shortcomings in the draft BE terrestrial exposure assessment that lead to unrealistic (or completely wrong) exposure estimates for malathion. Cheminova also notes that EPA failed to comply with many of the recommendations as per NRC (2013), and as such the conclusions made on species risk designations based on this exposure assessment are highly questionable.

3 AQUATIC EXPOSURE MODELING

3.1 Spatial Data and Analysis

Document 1: Chapter 1, Problem Formulation
Section 1.4.1.1.a, Use Site Footprint

Comment 1:
Chapter 1.4.1.1.a indicates that as future National Agricultural Statistics Service (NASS) Cropland Data Layer (CDL) is published, up to 10 years of data will be used. As new data becomes available, the methodologies used and quality of data inputs has improved over time, often improving classification accuracy and reducing classification errors. The quality of older data should be considered prior to determining whether up to 10 years should be included in analysis.

Comment 2:
Rather than considering the reported acreage of the National Agricultural Statistics Service (NASS) Census of Agriculture (CoA) for expansion, the reported acreage less the bounds of error should be considered, as there are known and published errors associated with the dataset (page 1-38, paragraph 1 of the BE).

Comment 3:
The expansion method (page 1-38, paragraph 1) assumes that the National Agricultural Statistics Service (NASS) Census of Agriculture (CoA) is a more representative dataset than the National Agricultural Statistics Service (NASS) Cropland Data Layer (CDL). However, this database has known data errors and may be less accurate than NASS CDL in many cases. Additionally, cross-referencing target crops from NASS CoA to NASS CDL is not consistently a one-to-one relationship, resulting in inaccurate acreage comparisons.

Comment 4:
The expansion method (page 1-38, paragraph 1) results in expansion into arbitrary crop areas that may be disconnected from target crop areas.

Comment 5:
National Agricultural Statistics Service (NASS) Cropland Data Layer (CDL) has known data errors with varying degrees of data accuracy dependent upon crop class and include ‘spurious’ pixels (Budreski et al., 2015). Spurious pixels are small areas (single or a few pixels) that are misclassified as agricultural land or the incorrect crop class. Including multiple years of CDL data can artificially expand the use site footprint and result in potential use site areas that are not and have not historically been the target use.

- Attachment 1-3 of the BE indicates that methods have been employed to minimize data errors within the CDL. One method of reducing errors was done by expanding the crop footprint dataset into adjacent CDL 2014 Cultivated Layer pixels, which is based upon

the CDL layer and contains the same misclassified pixels as the source CDL layer. A more appropriate dataset for an expansion mask is available. The 2011 NLCD dataset has fewer instances of miss-classification and spurious pixels for the Cultivated Crop (Class 82) and Pasture/Hay (Class 81) classes due to additional data validation processing within the data development process and as evidenced in reported classification accuracy summaries. For these reasons, NLCD 2011 should be used as a mask (e.g., Classes 82 and 81) as opposed to the CDL 2014 Cultivated Layer.

- Attachment 1-3 states that 'Lumping classes reduces the likelihood of errors of omission and commission between similar crop categories.' Lumping classes addresses errors of omission, but does not address errors of commission, where pixels were incorrectly assigned to the target class. To address errors of commission, a probabilistic approach could be implemented to incorporate additional datasets as a weight of evidence, as well as, the number of years in target crop, and known accuracy statistics. For an example approach, refer to Budreski et al. (2015).

Comment 6:
For certain labeled uses, there are geographic restrictions. The crop footprint development process should include geographic restrictions where appropriate.

Comment 7:
The use site footprints for nursery uses were derived using a proprietary business database, Dun and Bradstreet. It is difficult to evaluate its use and conclusions drawn from the data without the knowledge of what the data looks like or the metadata associated with the dataset. Efforts should be made to make these data publicly available.

Document 1: Chapter 1, Problem Formulation
Section 1.4.1.1c: Action Area, Off-site transport area
Document 2: Attachment 1-4: Process for Determining Effects Thresholds
Section 1: Effects Thresholds for the Action Area (Step 1)

Comment 1:
The determination of the spray drift off-site transport distance component of the Action Area was based on spray drift modeling results and effects end points for the most sensitive aquatic or terrestrial species. For the aquatic species, the simulated EEC, due to spray drift, for the most sensitive aquatic habitat bin (Bin 5) was compared against the endpoint for the most sensitive taxa (aquatic invertebrates) to determine the spatial extent of the off-site transport distance. The use of both the most sensitive aquatic habitat, which many aquatic species do not occupy as habitat, and the effect endpoint that is several order of magnitude lower than is appropriate for many taxa, results in an Action Area that is scientifically inaccurate for many taxa and species . The extent of the spray drift based off-site transport zone and associated Action Area needs to be specific to the aquatic habitat characteristics a species occupies and based on relevant conservative effects endpoints.

Document 1: Chapter 1, Problem Formulation
Section 1.4.1.2. Species/Critical Habitat Locations

Comment 1: Section 1.4.1.2 states that species ranges used in the co-occurrence analysis were provided to EPA in the form of Geographic Information System (GIS) spatial files by the FWS and NMFS. These data have not been made available. Access to these data are required to appropriately evaluate the appropriateness of the data for a 'May Affect' determination.

Document 1: Chapter 1, Problem Formulation
Section 1.4.1.3, Overlap Analysis

Comment 1:
The Overlap Analysis was conducted using raster based zonal statistics. This method is limited to the pixel resolution (30m). A vector based analysis is a more appropriate approach to determine overlap of potential use sites and species habitat locations.

3.2 Comments on Chapter 3, Attachment 3-1 and Appendix 3-3

Document: Chapter 3, Exposure Characterization for Malathion
Section 1 Environmental Transport and Fate Characterization
Table 3-1. Chemical properties and environmental fate parameters for malathion and degradates with registrant submitted data.

Comment 1:
In Table 3-1, no sources of the environmental fate values are cited and only ranges of values are listed for several values. The ranges of these values do not match the ranges of values Cheminova has in the malathion set of acceptable environmental fate parameters (Breton et al. 2016c (In prep), Knopper et al., 2014 [MRID 4949901] and Reiss, 2013). Details about the individual environmental fate values, used to estimate the regulatory modeling environmental fate parameters including source and criteria for acceptability must be included in the documentation to allow for transparency and reproducibility.

Comment 2:
In Table 3-1, for malathion soil aerobic metabolism half-life, a range of 0.3- 11 days is reported. No sources were cited for this range. Cheminova recognizes two valid soil aerobic metabolism studies, Blumhorst (1990) and Knoch (2001a) for aerobic soil metabolism half-lives. The range of measured half-lives from these two studies is 0.17 – 0.25 days. The addition of unspecified soil longer half-life study significantly increases the t90 for regulatory modeling of malathion.

Comment 3:
In Table 3-1, for malaoxon, the reported soil aerobic metabolism half-life range is listed as <1 day. The actual laboratory reported range was 0.2 – 0.5 days (Hiler, 2012). Cheminova believes that the actual reported study range should have been reported here to be consistent with other parameters in the table.

Comment 4:
In Table 3-1, for malathion, the reported range of aerobic aquatic metabolism half-lives was listed as 0.5 – 10 days. Cheminova does not know where this range comes from Cheminova is aware of three GLP aerobic metabolism studies: Blumhorst (1991), Knoch (2001) and Hiler and Mannella (2012a [MRID 48906401]). The range of measured aerobic aquatic metabolism half-lives from these GLP studies if 0.3 – 3.3 days. Use of the longer water half-lives significantly increases the t90 for regulatory modeling of malathion.

Document: Chapter 3, Exposure Characterization for Malathion
Section 2.4.2 Spray Drift

Comment 1:
The BE states that, “While spray drift buffers reduce exposure to aquatic environments from direct deposition of finished spray on water via drift, they do not impact modeled estimates run-off received by the waterbody.” This is true that spray drift buffers do impact “modeled” estimates of runoff received by water bodies. However, in reality, a buffer of any kind between a treated field and receiving water will have some level of impact in reducing runoff contributions of pesticide to the receiving water. A well vegetated buffer, such as a grass filter strip, will have the most significant impact in reducing pesticide in runoff reaching a receiving water. Although the screening level exposure modeling in the BE does not account for any runoff reduction in malathion, there will in reality be a reduction in pesticide loading observed. This effectiveness of buffers in reducing off site pesticide transport has been well-documented (USDA, 2000; Poletika et al., 2009). In fact, malathion labels were required by the 2006 RED to include the following statement: “a level, well-mainted vegetative buffer strip between areas to which this product is applied and surface water features such as ponds, streams, and springs will reduce the potential for contamination of water from rainfall-runoff.” EPA should recognize the conservativeness of model runoff-based estimates resulting from lack of representation of buffer in the PRZM model. Accounting for, at a minimum, an unmaintained buffer between the edge of field and receiving water would more accurately reflect actual conditions.

Document: Chapter 3, Exposure Characterization for Malathion
Section 2.4.3 Application Timing

Comment 1:
The BE states that, “moving single application dates in which 100% of a watershed is treated in a single day in small increments can have a substantial impact on peak EECs and smaller impacts on chronic EECs. Though EEC differences can be substantial, changes of application day by less than one week should not be construed as a model refinement and should only be considered a demonstration of model sensitivity.” The rationale for why accounting for application date variability/uncertainty should not be considered as a model refinement is unclear. The selection of a single “worst case” date within a known application window is appropriate for initial screening level exposure modeling. To properly evaluate the likelihood of pesticide exposure, the uncertainty in assumptions that have significant influence on resulting

EECs needs to be accounted for. A probability distribution of application dates, even if the distribution is uniform, should be sampled in determining EECs exceedance probabilities in a refined exposure assessment. This level of analysis is appropriate for Step 2 in the endangered species risk assessment process.

Document: Chapter 3, Exposure Characterization for Malathion
Section 2.6.1: Ultra Low Volume Wide Area Uses

Comment 1:
For multiple application scenarios, the assumption is that the drift followed the same direction toward the same aquatic water body after each application. For very small water bodies (bins 2 and 5) this is highly improbable. The predicted EECs included carryover from previous applications and assumed the drift took the same direction pattern after each application.

Comment 2:
The input parameters and standard environmental scenarios for the aquatic modeling were not detailed in the document. Additionally, it is unclear if runoff was simulated in this process.

For mosquito adulticides, runoff is not a major source of malathion contamination in surface water. The drift is designed to stay airborne for great distances in order to be effective against adult mosquitoes. However, this type of application is not effective as a mosquito larvacide. Mosquito larva breed in standing water caused by runoff and in surface water bodies. For urban mosquito abatement, both ULV malathion applications are made for adulticide and additional non-ULV applications of other compounds are applied to runoff and surface water for larvacide effect.

Cheminova believes that runoff should not be included as a source of malathion contribution to aquatic bins in the modeling of malathion mosquito adulticide applications.

Comment 3:
It is not clear what environment fate parameters were used in the malathion adulticide modeling or what modeling tools were used to generate the EECs. Thus, the results are not reproducible.

Comment 4:
The study by Mickle et al. (2005) was not included in the list of studies evaluated for assessing drift deposition from ULV applications. The Mickle study is widely considered as high quality and includes an AgDisp8.26 calibrated equipment scenario for simulating ULV malathion applications.

Document: Chapter 3, Exposure Characterization for Malathion
Section 2.6.2: Homeowner Uses

Comment 1:
A new conceptual model for exposure in urban environments was presented. The new conceptual model was not backed up with data to show that they are realistic and representative of actual urban environments. Some specific aspects of the malathion urban conceptual model that could use some further justification or help in understanding the assumptions made include the following:

- The neighborhood consists of ¼ acre lots with 1000 ft^2 building footprints. How was this lot size and house size arrived at and how does it compare to neighborhoods throughout the US?
- It appears that the neighborhood only consists of ¼ acre house lots, and does not include additional roadway areas as previous EPA residential conceptual models have (EPA, 2010a). The residential conceptual model description should include whether other impervious and pervious areas, not specifically part of house lots, make up a portion of a residential watershed.
- The fence in this scenario leads to a very large application area. It is unclear what the 2 feet swath represents and whether the area treated is horizontal or vertical. In addition, it is unrealistic to have these fences surrounding every lot in a neighborhood.
- Patios and other treated areas are not to scale on map. 1,000 ft^2 would be the same size as the house footprint and nearly as large as the garden area. Given that this is a significant area, it should be better justified and represented to actual scale on the diagram to provide a better sense of the proportion of the lot these use sites occupy.
- Dimensions of important features on the conceptual model diagram would be helpful.

Comment 2:
The residential modeling approach does not account for run-on from rights of way into lawn, where infiltration would occur and reduce exposure potential.

Comment 3:
Total percent treated area with malathion is equivalent to 38.8% of the neighborhood. What is the likelihood that a residential watershed (of any size) would be 38.8% treated with malathion? Based on our understanding of malathion sales and residential use, this likelihood would be close to 0%. Is this an appropriate screening level scenario assumption?

Comment 4:
Much of the pervious area use sites used the “Right of Way” scenario which has a very high curve number (92) that approaches the runoff rate of an impervious surface. The “Right of Way” scenario should be replaced with a more appropriately conservative pervious scenario, such as the “Residential” scenario.

Comment 5:
Extrapolating the single lot conceptual model to watersheds of the size associated with Bin 3, Bin 4, and many Bin 2, Bin 6, and Bin 7 scenarios greatly exaggerates the density and percent treated area. This is because the approach does not account for the vast areas of the urban landscape that are not house lots (parks, shopping centers, major roadways, industrial parks) and that medium to larger watersheds will have many different types of land uses throughout them.

Document: Chapter 3, Exposure Characterization for Malathion
Section 2.7 Aquatic Modeling Input Parameters
Table 3-5. Input values used for Tier II surface water modeling with PRZM5/VVWM

Comment 1:
In Table 3-5, the reported DT50 values list revised kinetics values per recent NAFTA guidance, but detailed input and output per these analyses are not included in the report. Thus, these values are not reproducible.

Comment 2:
Hydrolysis is a significant pathway for degradation of malathion in water. At pH 7, the hydrolysis half-life is 6.21 days. In Table 3-5, EPA lists a water column half-life of 3.29 days with no hydrolysis to avoid double-counting based on the t90 value from five aerobic aquatic metabolism systems. Cheminova calculated a t90 of 3.4 days based six aerobic aquatic metabolism system values (Knoch (2001b [MRID 46769502]) and Hiler and Mannella (2012 [MRID 48986601])) with hydrolysis contribution removed and study temperature adjusted for each study value. Cheminova questions whether the values used to estimate the 3.29 day t90 value may have been adjusted to remove hydrolysis contribution and the proposed modeling should include the hydrolysis contribution.

Comment 3:
In Table 3-5, for soil aerobic metabolism, EPA arbitrarily doubled the regulatory estimated soil half-life arguing that “However, GLN 835.4100 stipulates soil moisture at 40%-60% of water holding capacity and malathion rapidly hydrolyzes. Non-guideline studies indicate that persistence is increased in soils with low moisture and low microbial activity (Walker and Stojanovic 1973). To account for the contribution of hydrolysis in metabolism studies, the input parameter is doubled”. First, this is not consistent with any of EPA existing policies. Secondly, in regulatory modeling, soil degradation is lumped with hydrolysis and microbial degradation. Further, aerobic soil metabolism studies are normally conducted in a 40%-60% soil moisture range representing normal agricultural growing conditions. While it is probably true that malathion degrades slower in drier conditions, these are the opposite conditions in which malathion would likely runoff into nearby surface water or leach into groundwater. It would simply state where it is. Then degradation would again speed-up when soil moisture increased again. So, this arbitrary adjustment is both against guidance and a scientifically unsound way to deal with conditions (very dry, abiotic surface) that have a low probability of occurrence and

apply for all model runs. Consistent with EPA policy, Cheminova has a recommended soil half-life of 0.24 days (Reiss, 2013).

Comment 4:
In Table 3-5, EPA lists a foliar half-life of 6.1 days based on the 37 data points from the Willis and McDowell (1987) study. In selection and use of this study from the open literature, EPA ignores a large amount of data generated in accordance with established test guidelines, validated methods, and GLPs. Cheminova is a member of the Agricultural Reentry Task Force (ARTF), which was assembled to address data worker and residential reentry data requirements for agricultural pesticides. The ARTF has conducted and submitted a number of foliar residue dissipation studies to EPA where malathion was the test material (MRIDs 45005910, 45491901, 45138202, 45491902, 45138201 and 455469501). These studies generally demonstrate that malathion residues dissipate at rates between 30 and 44% per day. In addition, Cheminova has submitted a turf transferable residue study on malathion (MRID 43945001) that demonstrated a half-life of 14.5 hours. Finally, Cheminova has compiled an extensive database of GLP foliar dissipation study data (Moore et al., 2014a [MRID 49389301]). The database contains different studies conducted on various field, row and orchard crops in which malathion was applied via non-ULV and/or ULV application (Moore et al., 2014 [MRID 49389301]). These residue trials include both residue decline studies and preharvest interval studies and have been conducted on a wide variety of fruits, vegetables, cereals, and forage items for non-ULV and ULV malathion end use products. Studies were conducted in accordance with the Commission Directive 96/68/EC amending Council Directive 91/414/EC and/or EPA Pesticide Assessment Guidelines, Subdivision N, Section 171-4. Using the data feed item (crop type) specific half-lives and RUDs were estimated that are specific to malathion (Moore et al., 2014 [MRID 49389301]). Cheminova estimated mean DT50 (in days) for long/tall grass (ULV: 5.18; non-ULV: 4.51), forage/leafy crops (ULV: 3.9; non-ULV: 1.86), small fruit (ULV: 6.01; non-ULV: 5.06) and large fruit (ULV: 6.14; non-ULV: 3.31). A detailed description of the data and methodology can be found in Moore et al. (2014 [MRID 49389301]).

Document: Chapter 3, Exposure Characterization for Malathion
Section 2.7 Aquatic Modeling Input Parameters
Table 3-6. PFAM Inputs for Malathion Use

Comment 1:
Table 3-6 lists some details about PFAM modeling. However, no details about the scenarios simulated are presented. Currently, PFAM does not have any publicly available environmental scenarios. Thus, the reported EECs from PFAM cannot be reproduced.

Document: Chapter 3, Exposure Characterization for Malathion
Section 2.7 Aquatic Modeling Input Parameters
Table 3-7. Summaries of registrant submitted and open literature malathion aerobic soil metabolism data

Comment 1:
In Table 3-7, EPA lists the sources used for aerobic soil metabolism study values. Several of these studies are either very old (e.g., Walker and Stojanovic 1973 and 1974) or field study data. The older studies were non-GLP and the limits of detection were probably much higher than current methods. The field studies were conducted with varying temperature, soil moisture and other environmental conditions which make the results incompatible as "baseline" values for modeling purposes. The reason the aerobic soil metabolism laboratory values are used for modeling is that they are conducted under controlled conditions and thus serve as "baseline" inputs into the models. Then, the models adjust for the varying environmental conditions per weather and environmental inputs. Additionally, the CALEPA 1994 study with the 4.2 – 6.9 reported soil half-life was associated with the medfly eradication program. This program used an unregistered malathion "bait" formulation. It is believed that the contents in the "bait" material itself caused degradation of malathion to slow. Cheminova strongly believes these values should be removed from the list of values used to estimate soil half-lives for malathion. Table 3-7 also includes a literature study by Howard, 1991 which examined malathion degradation as a function of soil moisture. EPA selected the average soil half-life from this study for inclusion in the list of soil half-lives. This is inappropriate because the average would be biased due to the long half-lives generated from the very dry soil values in the experiment. If any values from this study are included in the regulatory list, it should be the values associated with the 40% - 60% soil moisture range associated with Aerobic Soil Metabolism study guidelines. Additionally, since this is an older literature study (1991), it is unknown how well this study complies with current guidelines.

Document: Chapter 3, Exposure Characterization for Malathion
Section 2.8: Aquatic Modeling Results

Comment 1:
The BE states that, "EECs derived using the PRZM5/VVWM model for bin 3 (moderate flow aquatic bin) and bin 4 (high flow aquatic bin) ranged from 6.8-27,400 µg/L and 3.62-393,000 µg/L, respectively. ", and there was little confidence in these EECs. Reasons for the low confidence included:

- They exceeded malathion's solubility (145 mg/L; Cheminova A/S, 1988 [MRID 40966603])
- Bin 4 EECs were higher than Bin 3 EECs and Bin 3 EECs were higher than Bin 2 EECs
- The flowing bin EECs are several orders of magnitude higher than the static Bin EECs
- Maximum measured edge of field concentration for malathion was 146 µg/L
- Maximum modeled edge of field concentration for malathion in HUC2 15a was 603 µg/L

These are all good reasons to have low confidence in the modeled values. However, these issues also pertain to Bin 2 EECs and point towards problems with the overall flowing water modeling approach. Some of these reasons also apply to some of the static scenarios as well, which should cause concern for the parameterization of those static bin scenarios as well.

Comment 2:
The BE states that atrazine data from Wauchope (1978), edge of field runoff samples, would be used to represent Bin 2 estimates in deriving the qualitative approach to estimate Bin 3 and Bin 4 EECs from Bin 2 EECs. Edge of field runoff does not seem to be a suitable surrogate for Bin 2 headwater streams, which by definition, have flow rates of between 0.001 m^3/s and 1 m^3/s. Equating edge of field runoff to a headwater stream habitat sufficient for enabling the survival of aquatic species is not a good comparison.

Comment 3:
The 1 in 15 year peak diazinon concentrations are presented for Bin2, Bin 5, Bin 6, and Bin 7. A summary these results are shown in **Figure 3-1**. The BE reported that the highest measured edge of field concentration was 146 µg/L, while the highest malathion concentration detected in over 70,000 general surface water monitoring samples collected since 1988 was 22 µg/L. A total of 53 samples (0.08% of all samples taken) had malathion concentrations of greater than 1 µg/L. Looking across the four habitat bins shown, the modeled peak concentrations exceed both the highest measured edge of field concentration and highest measured surface water concentration in multiple HUC2s for all habitat bins. The most significant disparity between the highest measured concentrations and the modeled concentration occur for:

- Bin 2, Bin 5, and Bin 6 scenarios
- Scenarios in HUC2s with the larger assumed watersheds associated with each habitat.

For some use patterns and habitat bins, the peak annual receiving water body EEC can be higher than edge of field runoff concentration due to spray drift related exposure. Many of the peak Bin 5 EECs shown in Figure 3-1 are resulting from drift events for the use patterns with higher application rates. However, for many of the HUC2/habitat bin scenarios, the 1 in 15 year annual maximum peak concentrations are the result of runoff events. The unusually high EECs predicted in the BE's for Bin 2 are all resulting from events where the receiving water concentration is higher than the edge of field runoff concentration.

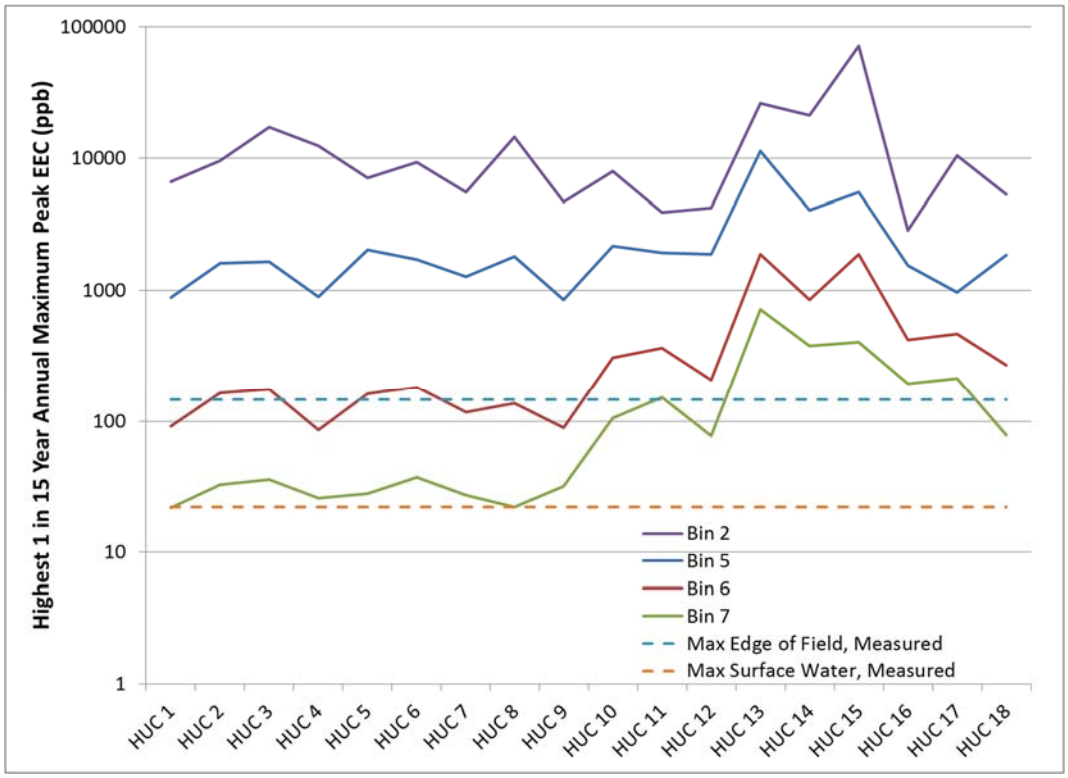

Figure 3-1 **Highest malathion EECs by HUC2 for 1 flowing and 3 static bins compared to edge of field and surface water concentrations.**

An additional analysis in Figure 3-2 below shows a comparison of malathion 1 in 15-year annual maximum peak EECs in Bin 2 compared to the 1 in 15-year annual maximum peak edge of field concentrations from the corresponding PRZM5 simulations. The VVWM peak concentrations from the Bin 2 scenarios were run with drift fractions set a negligible amount (0.0001) so that direct comparisons could be made between runoff-driven receiving water peak EECs and the edge-of-field concentrations from PRZM5. The two PRZM edge of field concentrations shown represent the total concentration (soluble and sorbed pesticide) in the runoff water as well as the dissolved concentration only. The dissolved edge of field concentrations are most comparable to the VVWM dissolved peak concentrations, because the sorbed pesticide load from PRZM instantaneously partitions to the benthic layer and remaining suspended sediment in VVWM. This comparison shows that the Bin 2 peak EECs are higher than edge of field concentration for nearly all 867 ESA Bin 2 scenarios shown. Often times, the difference is by greater than an order of magnitude.

It is physically impossible for pesticide concentrations in a receiving water to be higher than the edge of field concentrations entering the water body from a runoff event. The dilution that occurs in the receiving water body will always reduce the pesticide concentration to less than the edge of field concentration. The magnitude of that reduction will be dependent upon the volume of water in the receiving water body relative to the volume of runoff water carrying new pesticide, as well as the antecedent concentration of pesticide in the receiving water body; however, there will always be a reduction.

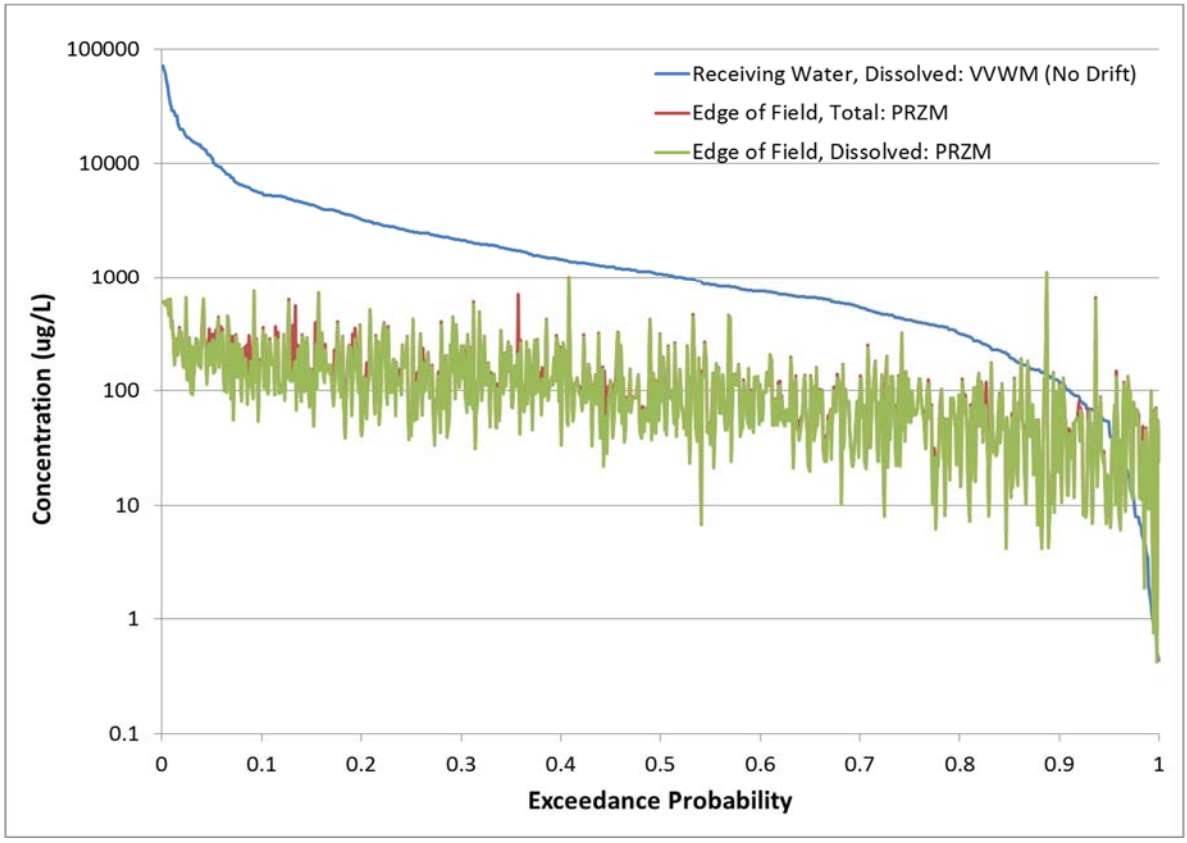

Figure 3-2 **Bin 2, 1 in 15-year annual max peak malathion EECs compared to 1 in 15-year annual maximum peak edge of field EECs, 867 ESA scenarios assuming no spray drift.**

The receiving water concentrations of malathion can be higher than the edge of field concentrations for the three habitat bins that were modeled as well. The ratio of the receiving water to edge of field 1 in 15-year annual maximum peak concentrations from the malathion BE Step 2 ESA scenarios, run assuming no drift, are shown in Figure 3-3 below. Ratios of 1 or greater indicate that receiving water peak concentrations are higher than edge of field concentration. While this is most common for Bin 2 and Bin 5, it also occurs for some scenarios for Bin 6 and Bin 7. All of the scenarios shown below were run with the PWC model with the assumption of negligible drift (drift fraction of 0.0001) to ensure a direct comparisons of runoff-driven EECs. For Bin 5, approximately 19% of scenarios have edge of field concentrations less than the receiving water EECs. For Bin 6 and Bin 7, this percentage drops to 11% and 2% of scenarios respectively. However, for water bodies the sizes of Bin 6 and especially Bin 7, we would expect the receiving water concentrations from runoff events to be substantially less than edge of field runoff concentrations

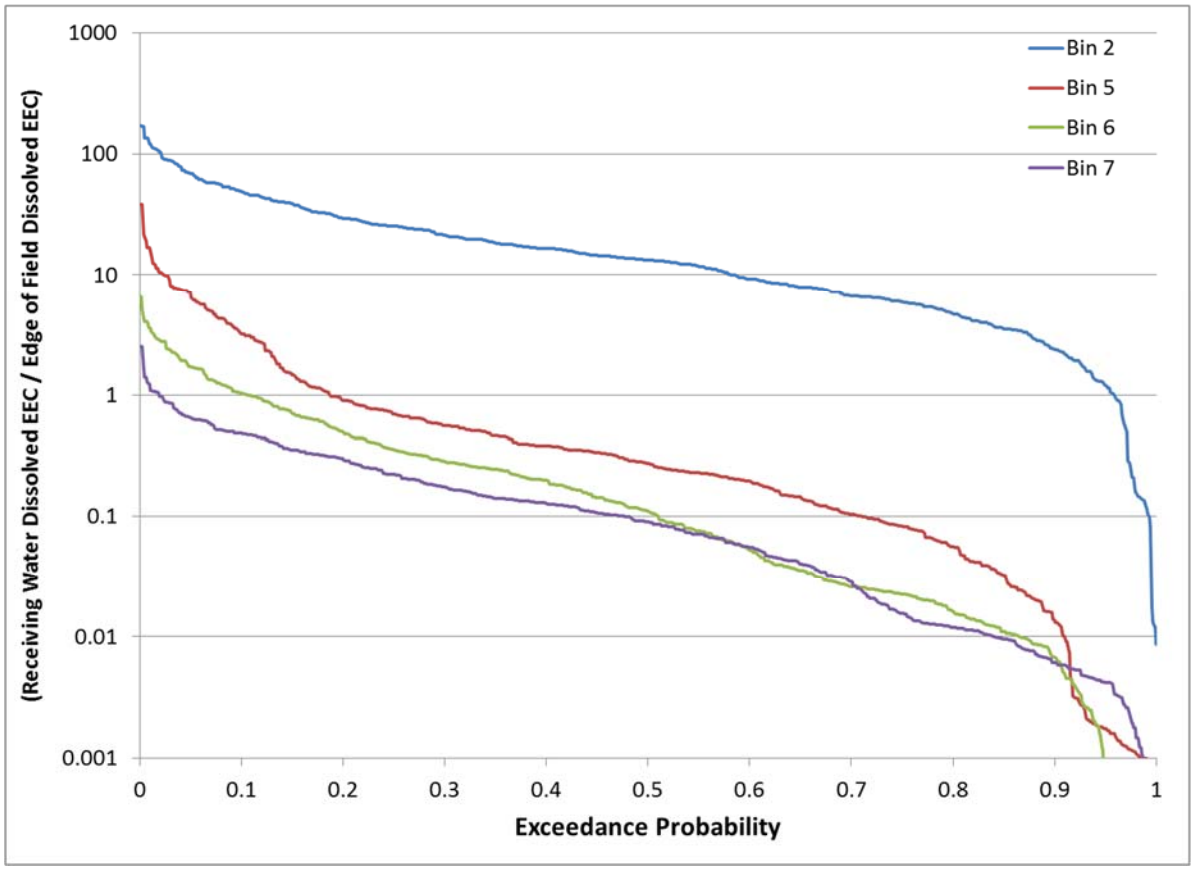

Figure 3-3 **Malathion 1 in 15 year annual maximum peak concentration ratios (receiving water/edge of field), 867 - 894 scenarios per bin, assuming no spray drift.**

The US EPA standard farm pond scenario, the receiving water body used by EPA for pesticide registration ecological risk assessment for the past two decades, has a watershed area of 10 ha and a receiving water volume of 20,000 m^3. This water body is analogous to Bin 7. A summary of dilution factors based on several different sized significant runoff events that would contribute to a peak annual exposure is shown in below. Given a negligible antecedent pond pesticide concentration, these dilution factors would approximate the ratio of receiving water concentrations to edge of field runoff concentrations. This suggests that, following the farm pond scenario conceptual model, we would expect Bin 7 ratios of receiving water to edge of field peak concentrations be general be below a value of around 0.05 to 0.2. Based on the BE parameterization of Bin 7, 27% of scenarios have ratios higher than 0.2 and 61% of scenarios have ratios of above 0.05. This dilution factor evaluation and comparison to the EPA farm pond scenario have been provided to show that, even for Bin 7, the receiving water concentrations are unrealistically high based on the magnitude of dilution that water bodies of that size would provide relative to edge of field concentrations.

Table 3-1 Receiving water EEC dilution factors for the EPA standard farm pond scenario based on several example runoff events.				
Runoff Depth (cm)	***Area (ha)***	***Runoff Volume (m3)***	***Pond Volume (m3)***	***Dilution Factor***
1	10	1000	20000	0.05
2	10	2000	20000	0.1
4	10	4000	20000	0.2

This review of ESA exposure scenarios has demonstrated that all of the Bin 2 flowing water peak EECs, and many of the Bin 5, 6, and 7 peak EECs are impossibly high due to flaws in the modeling approach and assumptions. It is physically impossible and does not comply with our conceptual understanding of the dilution process to have receiving water concentrations of pesticide several orders of magnitude higher than edge of field concentrations resulting from a runoff event. We would expect dissolved receiving water EECs to be lower than edge of field dissolved EECs. Deriving EECs for larger flowing water bodies (Bin 3 and Bin 4) from erroneous Bin 2 EECs propagates this error to a greater number of species that occupy the larger flowing water bodies and must be corrected. The impossibly high peak runoff exposure for static water bodies is most significant for Bin 5, with fewer scenario affected for Bin 6 and Bin 7. Nevertheless, for the scenarios that are affected in Bin 6 and Bin 7, the peak EECs are not physically possible and require correction. Approaches to parameterizing the static and flowing habitat bins in a screening level assessment are provided in Greer et al. (2016) and Teed et al. (2016).

Comment 4:
The errors in the screening level flowing water EECs can be addressed by taking the following steps:

- Replacing VVWM with a receiving water model designed to simulate pesticide fate and transport in a flowing channel. The Soil and Water Assessment Tool (SWAT) has this capability and has been shown to produce realistic peak exposure values for small, medium, and large flowing water bodies (refer to Greer et al., 2016 and Teed et al., 2016 for details).
- Incorporating a baseflow rate equal to the minimum of the flow range associated with each habitat bin.
- Constraining the watershed areas to that which can drain into a main channel with 1 day.
- Applying Percent Cropped Area (PCA) adjustments at a minimum to Bin 3 and Bin 4.

Comment 5:
The errors in the screening level static water EECs can be addressed by taking the following steps:

- Correcting the assumption that the entire watershed's pesticide mass generated in 1 day arrives at the receiving water body instantaneously.

- Constraining the watershed areas of the static water body habitats to areas based on typical bin-specific water body configurations on the landscape, as opposed to allowing climatologically driven water balance calculations to wholly determine the watershed area.
- The watershed areas should also be constrained to allow a limited amount of regional variability. The significant amount of watershed area variability in the BE static bin scenarios across the HUC2s has led to an artificially wide range in EECs, which cannot be justified based on monitoring data or our conceptual understanding of hydrology and aquatic exposure pathways. Constraining the watershed areas within a limited range regionally will allow for a clearer interpretation of the relative risk of pesticide use based on regional variability in precipitation, soils and slopes, and use patterns.

Comment 6:
Aquatic exposure modeling at Step 2 should move beyond simple screening level approaches that use a single conservative PRZM simulation to predict EECs in flowing water bodies draining heterogeneous watersheds. Step 2 aquatic exposure modeling approaches should include the following:

- Representation of the heterogeneous landscape through explicit simulation of the land uses and soils that comprise a given watershed.
- Spatial explicit predictions of EECs that can be associated with species habitat locations.
- An accounting for variability in pesticide application timing that occurs at the watershed scale.
- Incorporation of Percent Treated Area (PTA) that acknowledges that 100% of potential use sites do not get treated with a given pesticide.

Document: Chapter 3, Exposure Characterization for Malathion
Section 2.9: Aquatic Modeling Sensitivity Analysis

Comment 1:
The aquatic exposure sensitivity analysis was only conducted for environmental fate parameters and application dates only. Given that the flowing water scenarios and modeling approaches are brand new, a sensitivity analysis that included the following parameters would have been more useful:

- Water body dimensions
- Water body flow rates within the range of the Bin
- Watershed area
- Flow-through options

Document: Chapter 3, Exposure Characterization for Malathion
Section 2.10.2: Available Monitoring Data

Comment 1:
EPA recognized monitoring data as information that could be included, qualitatively, in the weight-of-evidence approach to support potential exposure pathways as well as exposure concentrations. However, monitoring data were not included as a line of evidence in the assessments. Monitoring data should be used more explicitly in the analysis. For example, a systematic analysis of the proximity of detections to species locations should be conducted and included as a line of evidence.

Comment 2:
EPA indicates that preliminary investigation of bias factors and SEAWAVEQ is underway. These tools would enhance the ability to use general monitoring data quantitatively. The following references should be considered in developing robust bias factor approaches (Mosquin, 2012 JEQ).

Comment 3:
Out of more than 70,000 samples, malathion was detected at levels of between 1 and 22 µg/L in 53 samples (0.08% of samples). Yet, the range of modeled EECs across the different HUC2s were:

- Bin 2: 1750 µg/L – 71800 µg/L
- Bin 5: 852 µg/L – 11300 µg/L
- Bin 6: 71 µg/L – 1890 µg/L
- Bin 7: 20.8 µg/L – 710 µg/L

The highest monitoring data concentrations reported were approximately 2 to nearly 4 orders of magnitude lower than the highest modeled aquatic EECs across the four bins. However, this was not accounted for to assess the realism of the new modeling scenarios and approaches. The significant discrepancy should have been reason for assigning high uncertainty to the modeled EECs and led to the development of alternative modeling approaches.

A recent targeted malathion monitoring study in the Dalles Oregon, aimed at high temporal resolution sampling of two flowing water bodies surrounded by cherry orchards with high intensity malathion applications, found maximum malathion concentrations of 1.03 µg/L in one stream (Mill Creek) and 0.48 µg/L in a second stream (Threemile Creek). This study (Gulka et al., 2016) featured 6-hour composite malathion concentrations throughout the entire application season, with hourly samples for the two weeks with the highest usage of malathion, ensuring that peak concentrations were sampled. The malathion concentrations observed in this study, representative of peak exposures in low flow water bodies resulting from spray drift, further demonstrate the large over-prediction of malathion EECs in many of aquatic exposure scenarios reported in the draft BE.

Another recent monitoring study (Bahr et al., 2016) completed in 2015 by the Washington State Department of Agriculture evaluated the effectiveness of riparian vegetation at intercepting drift from aerial malathion applications. They compared the measurements from locations with and without the presence of riparian buffer. The highest observed malathion concentration was around 7.8 ug/L for a static waterbody. In waterbodies where there was water movement, concentrations were less than 0.3 ug/L. This study further confirms the results from the Dalles, OR study and emphasizes the overestimation of EPA's modeled EECs across the different HUC2s.

Document: Chapter 3, Exposure Characterization for Malathion
Section 2.13.1 Uncertainties in Aquatic Modeling and Monitoring Estimates, Surface Water Aquatic Modeling

Comment 1:
One source of uncertainty described for static water modeling was the effects of increase or decreases in pond levels. Significant increases in levels occurred for many of the static bin scenarios as a result of the very large watersheds. This source of uncertainty should be quantified.

Comment 2:
For flowing water bodies, changes in volume were only associated with surface runoff, and did not account for shallow subsurface flow (interflow) or groundwater contributions (baseflow). These components of streamflow are account for more than 50% of total stream flow in many regions of the country and should be accounted for.

Comment 3:
Multiple conservative assumptions for spray drift modeling were described, including the constant 10 mph wind speed, always blowing from the treated field to the receiving water body, no interception of drift on vegetation or other barriers, or BMPs followed by applicators. While these conservative assumptions are appropriate for Step 1 modeling, Step 2 modeling should incorporate more realistic assumptions through a probabilistic analysis of the likelihood of a range of spray drift conditions.

Comment 4:
Malathion EECs were shown to be very sensitive to application dates. The uncertainty in application dates should be accounted for in Step 2 modeling in order to achieve a more comprehensive probability distribution of annual maximum EECs to compare against species end points.

Comment 5:
The BE acknowledged that to simulate EECs in the medium and larger flowing water habitat (Bin 3 and Bin 4), that a watershed scale model is needed to aggregate field-scale loadings to the larger flowing systems. It is recommended that the Soil and Water Assessment Tool (SWAT) be adopted in part or its entirety as the appropriate model for predicting flowing water habitat concentrations of pesticides for use in endangered species aquatic exposure risk

assessments (Gassman et al., 2011). The model has the ability to model both landscape and channel components of pesticide fate and transport and offers the advantages of:

- Comprehensive hydrology, including subsurface and groundwater contributions to streamflow
- Crop specific crop growth responses to climate and nutrients
- Representation of agronomic practices including irrigation, tillage, filter strips, grassed waterways, tile drainage
- Landscape erosion processes
- In-channel sediment transport
- In-channel pesticide fate, including degradation, volatilization, equilibrium partitioning, deposition, and resuspension

The SWAT model in its present form, with only minor modifications, can readily be adopted to serve the needs of the US EPA and the Services in estimating pesticide exposure in endangered species flowing water habitat.

Document: Attachment 3-1: Background Document: Aquatic Exposure Estimation for Endangered Species
Section 3.1.1: Aquatic Modeling-Surface Water Modeling-Traditional Approach

Comment 1:
In Table A 3-1.1, the “Model Component/Process” or “water body flow/dilution” for Endangered Species Assessment Refinements” states that, “Downstream dilution may be used from the edge of the use area, which consists of a percent use area adjustment. Concentrations are reduced by the use area adjustment factor until concentrations are below levels of concern.” This approach, which presumably accounts for “water body flow/dilution”, was not followed in the model predictions of EECs for use in the risk assessment for any of the aquatic bins for malathion or any of the other 2 OPs. This concept was only considered in the determination of the action area, but only for diazinon, because the action area was assumed to be the entire US for malathion and chlorpyrifos. The statement associated with “Current Aquatic Modeling for Ecological Assessments” of “Pesticide mass added instantaneously to fixed water body volume. No flow in Standard Pond (static)” accurately describes the ESA modeling approach for static water and, aside from the static volume, is more accurate for how the flowing water habitat was modeled. This part of Table A 3-1.1 should be clarified. Accounting for downstream flow dilution and percent use area should be explicitly accounted for in Step 2 modeling.

Document: Attachment 3-1: Background Document: Aquatic Exposure Estimation for Endangered Species
Section 3.1.2: Revised Conceptual Model and Approach for ESA

Comment 1:
The BE states that, “For the ESA Biological Evaluations, 1-in-15-year exposure concentrations are estimated using the daily time series of estimated concentrations from 30-year

PRZM5/VVWM simulations, instead of 1-in-10 year concentrations as in traditional ecological exposure assessments." The basis for this change in exposure endpoint from traditional ecological risk assessments is unclear. The registration review cycle for pesticides is independent of exposure likelihood due to use of the pesticide. Additional justification for this change from the traditional approach is needed.

Comment 2:
The conceptual model presented in Figure A 3-1-1 shows that aquatic habitat is only impacted by runoff if it is located within 30-meters of a treated field, beyond which only spray drift impacts the aquatic habitat. A few questions from this conceptual model arise:

- How was the 30-meter distance arrived at?
- The EECs modeled and the results reported were all based on runoff and spray drift contributions. How does the 30-meter runoff distance maximum come into play within the risk assessment?
- The conceptual model would suggest that Bin 9 (subtidal near shore) and Bin 10 (offshore marine) would only be impacted by spray drift, because they will be further than 30-meters from the shore. However, given the use of surrogate bins for the marine habitats (described later), this does not seem to have been the case, as runoff exposure was included in the surrogate bins. It would be helpful to provide some additional explanation for how this 30-meter factor pertains to the marine bins.

Comment 3:
The conceptual model presented in Figure A 3-1-1 depicts exposure to a very broad range of habitat, from very low volume static pools to the open ocean. This conceptual model has been largely derived from the traditional conceptual model for ecological risk assessments for pesticides, which is the standard farm pond. The standard farm pond has long served well as a conservative, high exposure scenario used for screening level pesticide risk assessments. The concept of a single treated field immediately adjacent to a receiving water body (over its entire length) is a much less appropriate representation of watershed scale processes affecting flowing water bodies, and even less so for any of the marine aquatic habitats. In the case of the flowing water habitat bins, this conceptual model's simplification of watershed scale processes may be appropriate at Step 1 of the endangered species risk assessment process, where the high conservativeness of the EEC predictions can be used as a coarse filter to screen out species of no concern. However, Step 2 of the endangered species risk assessment process requires more appropriate conceptual models for predicting exposure in flowing habitats and marine habitats, which are very different than small static ponds.

Document: Attachment 3-1: Background Document: Aquatic Exposure Estimation for Endangered Species
Section 3.1.3.1: Modeling Components, Input Scenarios

Comment 1:
The BE document described that, “There is a large suite of existing surface water scenarios available (123 total) for use in PRZM5/VVWM simulations, spanning a range of agricultural and non-agricultural pesticide use sites.” Figure A 3-1.2 is provided to provide the locations of those scenarios. However, there are far fewer than the 123 standard scenarios shown in this figure. It is unclear how the 123 scenarios mentioned were reduced to this smaller subset.

Comment 2:
In the discussion on the “Development of Representative Scenarios”, the BE document states that, “For those HUC2 region-crop group combinations where input scenarios are not available, a surrogate scenario (with the highest runoff potential) from a neighboring HUC2 region is selected.” These scenarios and surrogate scenarios are then presented in Table A 3-1.4. However, the details of the methodology for choosing a surrogate scenario was not provided. It is important to know, for example, how the MIbeans.std scenario was chosen to represent Corn in HUC2 01, as opposed to the existing corn scenario, PAcornSTD located close to HUC2 01. Providing the methodology for surrogate assignments, including if the method was professional judgement, is important for transparency of the process.

Comment 3:
In the discussion on the “Spatial Delineation, Weather Data”, it is said that, “For HUC2 regions 15, 16 and 20, a large disparity exists between the highest precipitation station and remaining stations in the HUC2. For these HUC2 regions, the highest precipitation weather station is selected along with the weather station with the median cumulative 30-year precipitation value for the remaining stations.” The discussion did not provide the criteria for determining what constitutes a “large disparity”. This criteria should be clarified. In addition, the choice of using a weather station within a HUC2 with the highest cumulative precipitation in these cases does not take into account whether or not this outlier station has any relevance to the scenarios that it is being assigned to. For example, is the highest rainfall station located high in the mountains, and if so, does that precipitation pattern have any relevance to agricultural areas located in the valleys? The choice of a weather station that has significantly different weather than the rest of the stations in the HUC2 can lead to the selection of an un-representative station, unless further analysis can show otherwise.

Document: Attachment 3-1: Background Document: Aquatic Exposure Estimation for Endangered Species
Section 3.1.3.2: Aquatic Habitat Bins

Comment 1 (page 17):
In the introduction to the aquatic habitat bins, the BE document states that, “The nine aquatic habitat bins are used in the BEs for both Step 1 and Step 2 and will be used for the Biological

Opinions in Step 3." The 9 aquatic habitat bins seem to have been developed to allow a means for efficient screening of pesticide exposure over a range of different types of aquatic environments. During Step 2 of the Biological Evaluation, and for the Biological Opinions in Step 3, more conceptually accurate, less generalized, and more species habitat specific representations of aquatic habitat are needed to assess many species, particularly for those in flowing and marine environments.

Comment 2:
The Bin 2 parameters do not represent the vast majority of low flow habitats. The Bin 2 description is as follows, "Some examples of low flow habitats include springs, seeps, brooks, small streams, floodplain habitats (oxbows, side channels, alcoves, etc.), dendritic channels that occur within exposed intertidal areas, and distributary channels in estuaries on the incoming and slack tides." The assumed velocity, of 1 foot per minute, is indicative of an extremely slow moving, essentially stagnant, waterbody, and does not reflect the vast majority of types of habitat listed that Bin 2 represents. Hydraulic calculations using Manning's equations and a very high roughness value of 0.125 results in a channel slope of 0.00001, which is not typical of headwater streams across the US. The typical range for natural streams with few trees, stones, or brush is (0.025 – 0.065) while for natural streams with heavy timber and brush, the range is 0.05 – 0.15 (Neitsch et al., 2011).

Comment 3:
Regrading the flowing Bin flow rates, the flow rate characteristics of each Bin were given a range (e.g., Bin 2 = 0.001 – 1 m^3/s). In each of the 3 flowing bins, the lowest flow rate was assumed when parameterizing the models. While this is conservative, given that the flow rates vary over 3 orders of magnitude within a single Bin, it would be good to take a look at the effects of higher flow rate assumptions when modeling in Step 2.

Comment 4:
In the discussion on Bin 2, it is mentioned that "The formula in the attached EPA link was used to estimate the speed for each flowing water habitat assuming the muddy substrate coefficient of 0.8 (http://water.epa.gov/type/rsl/monitoring/vms51.cfm)." This link does not contain a formula that is readily apparent. The assumed velocity of Bin 2 and the other two flowing Bins is very important in the resulting exposure duration. This velocity formula and the assumptions used to parameterize the calculations should be provided within the BE documents.

Comment 5:
Bin 5, the volume static habitat is described as having a volume of 0 – 100 m^3. As is stated in the BE document, all of the static aquatic bins were parameterized using the low end of the volume range. In the case of Bin 5, a volume of 0.1 m^3 was assumed. This 1 square meter in area, 10 cm deep water body, which can most accurately be described as a puddle, would not be identifiable as a feature on most agricultural landscapes. Their presence would occur immediately after rainfall events, and rapidly disappear due to infiltration and evaporation. The notion that malathion EECs in this sized water body have a direct bearing on the protection of listed species has not been defended in a rigorous enough scientific manner, particularly for use

in a Step 2 analysis. Questions to further address regarding the appropriateness of this habitat Bin and how it was parameterized include:

- Is there a biological or ecological significant to a water body of this size?
- Where and how often is a given species found in waters bodies this size compared to water bodies on the higher end of the volume range for the bin?
- A puddle this size can occur after rainfall on any land use, including agricultural fields treated with malathion or any other pesticide. Does protecting this sized water body preclude the use of any pesticides for crop protection?
- How can pesticide runoff to this sized water body be accurately modeled? How can watershed area for a puddle be estimated? Is the assumption of a static volume for this sized water body appropriate?

Comment 6:
The parameterization of all three static bins was based on the low end of the volume range that each habitat represents. The issues associated with modeling the low volume (Bin 5) habitat following this assumption was addressed in the previous comment. For the moderate and high volume bins, the conservative assumption of the low end of the volume ranges is sensible for developing screening level EECs in Step 1 or the endangered species risk assessment. At Step 2, the full range of aquatic habitat volumes associated with each bin should be considered in determining the likelihood of exposure.

Comment 7:
The BE states that, “Current pesticide models do not account for transport via tidal and wind generated currents in marine systems . As such, surrogate bins have been identified among the flowing and static bins to represent pesticide concentrations that may be expected in these environments”. It is agreed the standard pesticide exposure models used by EPA for ecological risk assessment do not account for processes required to estimate pesticide concentrations in the three generic marine habitats defined. The appropriateness of the surrogate bins to represent the marine habitats will be addressed in a later section.

Document: Attachment 3-1: Background Document: Aquatic Exposure Estimation for Endangered Species, Section 3.1.3.3: Watershed Sizes

Comment 1:
Watershed areas for the flowing bins were estimated using a regression of area versus average annual flow from the USGS NHDPlus dataset specific to each HUC2 watershed. In all HUC2s, the regressions were based on LN-transformed watershed area and flow data. While very good fits were achieved in some HUC2s, in others, the linear regression fit (as shown by the r-squared values) was quite low (e.g., region 11, region 14, region 18). In these cases, performing a linear regression of flow and drainage area without LN-transforming the data may have produced better results.

Comment 2:
The watershed sizes estimated, while accurately representative of the targeted flow rates of the three bins (0.001, 1, and 100 m^3/s), are not appropriately sized drainage areas for use with the VVWM model for the following reasons:

- VVWM receives runoff and pesticide loads from PRZM on a daily time step. The watershed areas derived based on the NHDPlus regression equations result in watershed areas that exceed a 1-day unit hydrograph base time for Bin 4 scenarios. Watershed areas need to be constrained to areas that can fully drain into a main channel within 1 day.
- The watershed areas for the flowing bins resulted in Drainage area to normal capacity ratios (DA/NC) of 1.) Bin 2: 334 – 7725, 2.) Bin 3: 604 – 11218, 3.) Bin 4: 739 – 35614. The EXAMS/VVWM model has long been used to simulate receiving waterbody pesticide concentrations from much smaller watersheds, with DA/NC of 5 (EPA farm pond). The use of VVWM for scenarios with such extremely different ratios of drainage area to water body size needs to be validated to ensure that results are appropriate for such a different hydrologic scenario.
- The instantaneous loading of pesticide runoff and erosion mass into the flowing receiving waters is one of the primary reasons for the anomalously high peak EECs obtained in the Step 2 aquatic exposure modeling reported in the malathion BE.

Comment 3:
The watershed sizes estimated for the static water bodies followed a water balance approach to approximate the drainage area required to maintain the volume of water designated for each generic habitat. Following this approach, climates with less runoff and precipitation and higher evaporation will require larger drainage areas to supply the target volume of water. The BE states that a median (across HUC2s) drainage area to normalized capacity ratio (DANC) of 5 – 15 m^2/m^3 was targeted. Justification is later provided that specified how the targeted median DANC varied by habitat bin: Bin 5 = 15 m^2/m^3, Bin 6 = 12 m^2/m^3, and Bin 7 = 5 m^2/m^3 (the same as the standard farm pond). The analysis was conducted such that these target median DANC values were achieved. However, the variability across the HUC2 regions is very high. This variability, reported in Table A 3-1.8, was as follows:

- Bin 5: 0.84 – 1255
- Bin 6: 0.65 – 204
- Bin 7: 0.25 – 78.8

The BE states that, “Using this approach, the static aquatic bin capacity is not exceeded, and the pesticide loading is conservative.” This statement is inaccurate. For the HUC2/bin scenarios with the higher DANC ratios (approximately greater than 20 m^2/m^3) a rainfall event of 3 inches in 24-hours (a 1 in 10-year storm that is exceeded in all but the arid portions of the western US (NOAA, 1961)) will result in runoff and direct precipitation volume that exceeds the volume of the entire water body. In these cases, even a completely empty pond’s capacity would be exceeded. The runoff volume in this calculation is based on the SCS Curve number equation

(NRCS, 1986) assuming a curve number value of 90 (the approximate value of the high vulnerability PRZM scenarios used in the BEs) and the 3-inch rainfall assumption. It should be noted that a 1 in 10-year 24-hour rainfall exceeds 5 inches in roughly half of the eastern United States.

The graph below shows the ratio of runoff volume from a 3-inch rainfall event to the volume of the three static habitat bins. These calculations used the DANC ratios by HUC2 and habitat bin provided in the BE Table A 3-1.8. Across all 3 bins, the volume of runoff and precipitation from a single large rainfall event will exceed the entire capacity of the pond. For Bin 5 and Bin 6, this occurs in more HUC2s, and the exceedance of the water body capacity is more significant. Even in the cases where the ratio shown is less than 1.0, the amount of runoff volume on top of any water in the pond before the runoff event would be enough to exceed the water body capacity in many HUC2s.

This analysis demonstrates an error in the stated assumption that the watershed areas derived for the static bins will not result in the water body volume being exceeded. While this may be true in some HUCs in the more humid eastern US, and for the larger water bodies (Bin 6 and Bin 7). In many western HUC2 watersheds the runoff volumes from large storm events will exceed the receiving water capacity many times over, which leads to significant overflow of water and pesticide, which does not fit the conceptual model adopted for the BEs of constant volume with no overflow. The originally targeted DANCs of 5 m^2/m^3 to 15 m^2/m^3 serve as reasonable upper bounds that should be applied across all HUC2 watersheds. The much higher DANC values derived in the parameterization of the static bin scenarios for some HUC2s and habitat bins are conceptually inaccurate because:

- The drainage areas result in significant water body overflow during large runoff events that drive the peak pesticide concentrations, which is inconsistent with the conceptual model.
- The wide disparity in drainage areas associated with each habitat bin across HUC2s leads to runoff-driven exposure values orders of magnitude higher in some HUC2s (the drier ones) compared to others, all because of the greater pesticide mass from a large drainage area instantaneously entering the same volume of water. This is conceptually wrong, as the edge of field pesticide concentration for the same pesticide use pattern, soil type, and rainfall event should be the same regardless of geographic location.

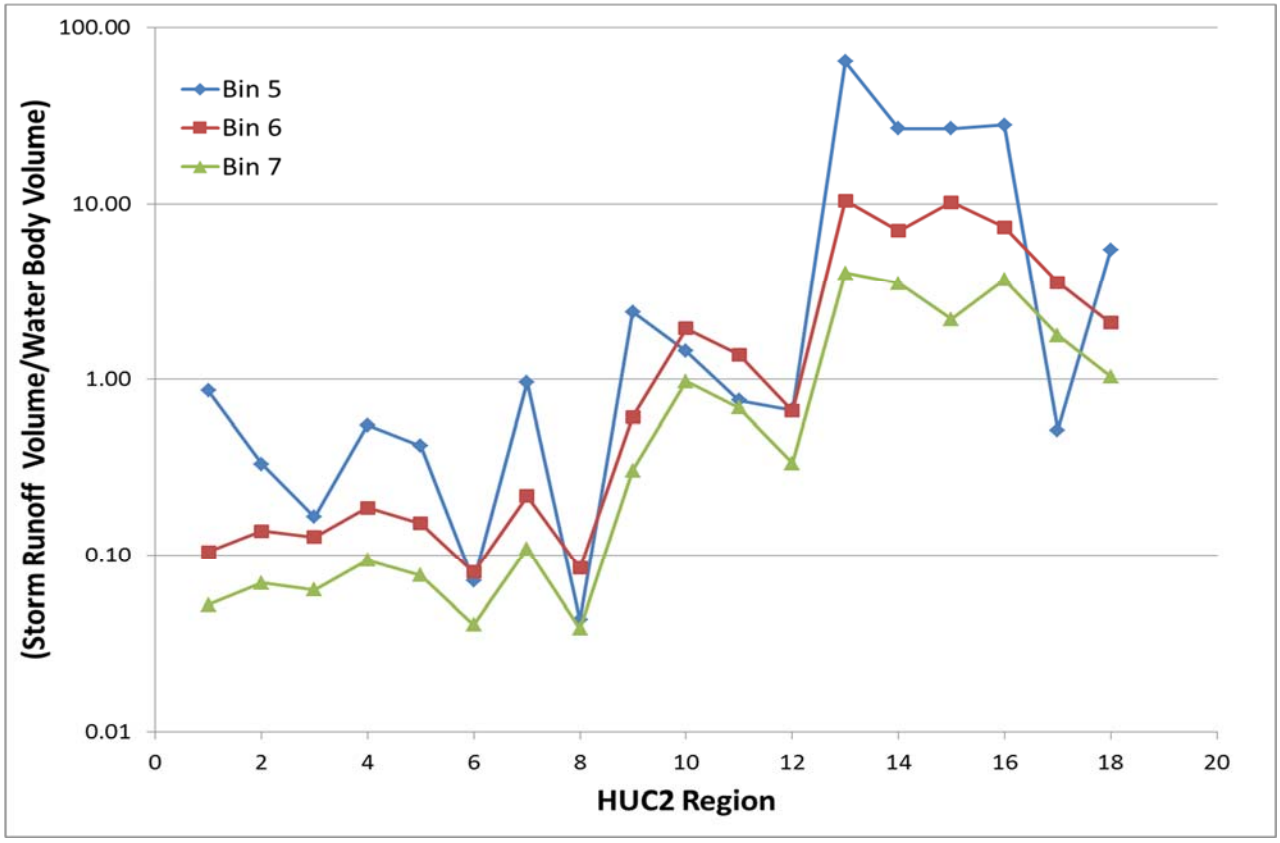

Figure 3-4 Storm runoff to water body ratio from an 3 inch storm by HUC2.

Comment 4:
The discussion on estuarine and marine bins indicates that the following surrogate freshwater bins will be used for the marine habitat bins:

- Bin 8 (Intertidal near shore): Bin 5, Bin 2, Bin 3
- Bin 9 (Subtidal near shore): Bin 4, Bin 7
- Bin 10 (Offshore marine): none specified

The use of these freshwater bin scenarios as surrogates to estimate malathion concentrations in marine aquatic environments is inappropriate. Both the potential transport mechanisms of malathion to these marine environments and hydrological characteristics of the marine systems are too dissimilar to draw any reasonable comparison to the freshwater scenarios. The following points support this position:

- Neither tidal pools nor nearshore/offshore marine environments can be modeled appropriately with existing habitat scenarios which represent homogenous watershed areas draining into pond-like receiving waters that are either static or allow for steady inflow/overflow. In addition, the EPA standard scenarios defining weather, crop, and soil

characteristics for each HUC2 are based on inland environmental conditions and, therefore, are not representative of coastal environments.

- Tidal pools by definition are filled with seawater by tidal flushing and wave activity during high wind/storm conditions. The ratio between tidal pool volume and watershed area is not determined by rainfall/runoff. Therefore, the watershed sizes from the surrogate habitats, determined through regression of flow rate with drainage area (flowing bins) or precipitation/evaporation/runoff balance to maintain a specific normal capacity (static bins), are not relevant for modeling pesticide exposure in tidal pools and are not related to tidal pool capacity. Tidal pools, which vary in volume and may be completely submerged during high tide, do not experience runoff loadings from a fixed-size watershed into a fixed-volume receiving body in the way that classical ponds and streams do. In addition to having inappropriate watershed areas for tide pools, the habitat surrogates have inappropriate flow regimes for tidal pools. The rate, magnitude, and frequency of tidal flushing cannot be approximated by the steady stream inflow/outflow rates used in the surrogates. Tides will have a leading order impact on dilution of any pesticide residues present in the pool via partial or total exchange of the tidal pool water with seawater every 12 hours. This is fundamentally different and requires a different modeling approach from the continual, constant baseflow assumed for flowing habitat bins. The watershed areas and flow rates are critical parameters influencing pesticide loadings and dilution in receiving waters. The values of these parameters determined for the static and flowing habitat bins that were used as surrogates for tidal pool habitats are not representative of tidal pools and require a different modeling approach. Both watershed area and diluting flow/exchange rates for tidal pools are not determined by the same factors used to define these values for the surrogates. In addition, for tidal pools these values are highly variable over the course of one day and these variations are not captured with the surrogates.
- Like tidal pools, the dimensions of subtidal nearshore and offshore marine environments and their drainage areas are not determined by regional variations in precipitation/evaporation/runoff balances or typical stream baseflow rates. Subtidal and offshore marine habitats are tidally influenced and, therefore, vary in volume due to twice daily mixing and flushing of the tides. Their volumes and the related dilution of any pesticide residues are determined more by their connection to bays, seas, and oceans (large reservoirs of residue-free seawater) than runoff from upland drainage areas. For offshore marine environments, the drainage areas contributing pesticide loadings will vary for each receiving water body based on local geography and elevation. There is not a generalized relationship between the volume of the marine environment and the contributing coastal watershed area. Specific offshore habitats of interest need to be modeled with spatially explicit characteristics by water models capable of simulating tidal effects.
- The immense volume, currents, and mixing that occurs in offshore habitat will lead to malathion EECs many orders of magnitude lower than the lowest EECs from any of the freshwater habitat bins. This fact should be demonstrated through scientifically defensible calculations of loads from freshwater sources and appropriate levels of mixing and dilution in these environments.

- Pesticide aquatic exposure scenarios implementing appropriate marine and estuary water quality models that account for tidal hydraulics and currents, as well as methods for predicting the pesticide loading from the landscape into these marine systems, need to be developed to allow reasonably conservative predictions of pesticide EECs to be made in these types of habitats.

Document: Attachment 3-1: Background Document: Aquatic Exposure Estimation for Endangered Species, Section 3.1.3.4: Application Date Selection

Comment 1:
The BE states that, "In selecting application dates for aquatic modeling, a number of factors are considered including label directions, timing of pest pressure, weather conditions, and pre-harvest restriction intervals." The discussion on the selection of application dates goes on to describe the selection of application dates as occurring during the wettest month for post-emergent applications. It is unclear how the factors other than the wettest month (such as pest pressure and agronomic conditions) factor into the ultimate selection of an application dates. Additional information on this would be helpful.

Document: Attachment 3-1: Background Document: Aquatic Exposure Estimation for Endangered Species, Section 3.1.3.5: Spray Drift Exposure

Comment 1:
The table of spray drift fractions shown in Table A 3-1.10 are default values and are not applicable to malathion due to the spray drift buffers for many malathion application methods and end-use product.

Document: Attachment 3-1: Background Document: Aquatic Exposure Estimation for Endangered Species, Section 3.1.4: Issues Modeling Medium- and High-Flowing Waterbodies

Comment 1:
The overview of this section in the BE states that, "Preliminary PRZM5/VVWM modeling of ESA aquatic Bins 3 (medium flow) and 4 (high flow) resulted in peak concentrations greater than several parts per million for all of the HUC2 regions and scenarios (e.g., for chlorpyrifos 44,000-121,000,000 µg/L, 30 to 86,000 times higher than the solubility limit of chlorpyrifos, 1,400 µg/L)." While these unrealistically high concentration predictions reported in Bin 3 and Bin 4 were for chlorpyrifos, the same issues of modeling the medium and high flow habitats apply to malathion. The overview section in the BE goes on to provide several reasons (factors – described below) for these extreme over-predictions for Bin 3 and Bin 4. However, it should be noted that many of these factors are not limited to Bin 3 and Bin 4 over-predictions.

- Factor 1, *The large ratio of the watershed drainage area to the water body capacity*: The DANC values for flowing bins were presented in Table A 3-1.7 for flowing and A 3-1.8 for status bins. These ratios for Bin 2 were as high or higher than the Bin 3 and Bin 4 values

in many of the HUC 2 regions. It is agreed that this factor was one that led to the over-prediction of EECs in Bin 3 and Bin 4, but it was also a problem with Bin 2. The effects of the large DANC values on the static water modeling was already commented on previously.

- Factor 2, *The entire watershed is treated with pesticide on the same application days (e.g., when a runoff event occurs, the pesticide loading is from the entire watershed)*. Given the sizes of some Bin 2 and Bin 7 watersheds, the assumption of all pesticide being applied on the same day in a watershed is an unrealistic assumption leading to over –prediction of EECs in these aquatic habitats as well.
- Factor 3, *Flow contributions from groundwater or sources upstream are not accounted for, and only runoff influences daily flow*: This applied to Bin 2 as well.
- Factor 4, *The instantaneous peak concentrations may not adequately reflect the pesticide concentrations in medium and high flowing water bodies (residence times << 1 day) within large watersheds*. The instantaneous peak concentration for both static and flowing waterbodies is calculated by taking the entire load of pesticide generated from the entire watershed in 1 day and adding it to the receiving water body without any change in water volume. This is a scientifically indefensible assumption for any size or type of water body. The only way that the pesticide load from a runoff and erosion event gets to a receiving water body is via transport with runoff water. This is the reason that the instantaneous peak concentrations are orders of magnitude higher than the edge-of-field runoff concentrations for all habitat bins.

Overall, these factor that the BE described as leading to model over-predictions for Bin 3 and Bin 4 are equally applicable to the Bin 2 model results, and for many of the static water scenarios.

Document: Attachment 3-1: Background Document: Aquatic Exposure Estimation for Endangered Species, Section 3.1.4.2.1: Issues Modeling Medium- and High-Flowing Waterbodies, Modifications Considered But Not Incorporated

Comment 1:
Incorporation of Base Flow: This section discussed approaches to incorporating baseflow into the model parameterization for Bin 3 and Bin 4. Several methods for estimating baseflow rates were presented. However, no conclusions were presented as to a preferred approach to estimate baseflow. Estimation of baseflow for Bin 2 was not mentioned in this discussion. Baseflow occurs for all sized streams across much of central, eastern, and more humid parts of the western US. If there were no baseflow, we would not have a flowing aquatic habitat, with the exception of storm events. For screening level flowing water modeling, a nominal baseflow rate should be set for each of the 3 flowing habitat bins. Given that the flow rates that define each bin span a range (e.g., 1 to 100 m^3/s for Bin 3), the lowest flow of the range should be assumed to represent the baseflow condition. Flows below this amount do not define the bin of the species that inhabit that class of waterbody. During storm events, the flow rates will increase to levels above the minimum defined for the bin.

Comment 2:
Percent Use Area and Percent Use Treatment Adjustment Factors: The concept of using Percent Crop Areas (PCA) Percent Use Areas (PUA) or Percent Treated Areas (PTA) was proposed. It was noted that these factors are used by HED in their risk assessments for human exposure. Both PCA and PTA should be incorporated for all 3 flowing bins. PCAs can be calculated based on aggregate use site footprints from multiple crop groups, and PTA, while based on state-level data, can be meaningfully included in the determination of EECs at Step 2. PCAs should be determined independently for each habitat bin and be based on appropriately sized watersheds (e.g., NHDPlus catchments for Bin 2, HUC10 to HUC12 for Bin 3). The use of a state-level PTA should transfer directly to the larger Bin 4 watersheds, which are at the same scale. State-level PTA can be used in several ways for Bin 2 and Bin 3, either as multiplicative factor to PCA, or, by splitting the EECs into percentages assuming worst case 100% PTA and the remainder assuming 0% PTA (no exposure) to then determine the likelihood of exposure at the level associated with use on all labeled crops. Because PCA and PTA are so important in determining exposure concentrations, these factors should be considered rigorously at Step 2, rather than waiting until Step 3.

Comment 3:
Adjustment of Water Body Length: It was suggested that increasing the water body length, accounting for a meandering stream, would increase the water body volume and thereby reduce the DA/NC ratios lowering the EECs to more realistic levels. Given the current use of VVWM, this effect would be true, but it is the underlying use of the VVWM to model a flowing channel that leads to the problems of unrealistically high EECs with large DA/NC ratios. The extreme EECs with higher DA/NC ratios do not occur with models designed to simulate channel flow, such as SWAT (see Winchell et al., 2016). We suggest that water body length is not the main issue to address for the flowing scenarios.

Comment 4:
Spreading Out Applications: Based on testing with diazinon, it was determined that spreading out applications across a window equivalent to a retreatment interval, did not have a significant effects on peak EECs. Over larger areas, it is likely that applications timing will vary over a wider window than only the application interval, and would have significant effect on peak EECs. In addition, the effect of spreading out application on peak EECs will depend on the environmental fate characteristics of the pesticide. For malathion, with a very short soil half-life, the difference between applying all the pesticide at once across a watersged as opposed to spreading applications over time will have a huge impact in EECs. Accounting for application date variability should be considered in Step 2 aquatic exposure modeling for all pesticides.

Document: Attachment 3-1: Background Document: Aquatic Exposure Estimation for Endangered Species, Section 3.1.4.2.2: Issues Modeling Medium- and High-Flowing Waterbodies, Modifications Explored and Incorporated into Modeling

Comment 1:
Curve Number Adjustment: An area weighted CN value at the HUC12 level was calculated and used to select a 90th percentile CN value for each HUC2 and used as the representative CN value for Bin 3 and Bin 4. This is a very sound approach to obtain appropriately conservative CN values. A suggestion would be that for Bin 4, a larger watershed (say HUC8 or HUC6) 90th percentile CN value be used to more appropriately represent that Bin. In addition, this approach should be applied for Bin 2 as well, using NHDPlus catchments as the watershed units from which to select a 90th percentile CN value for each HUC2.

Comment 2:
Daily Flow Averaging: The option of using daily flow averaging (instead of 30-year flow averaging) in VVWM was evaluated. It was mentioned that the SAM model was used for daily flows, but it is very unclear how this was done. It is assumed that simply the VVWM flow averaging period was simply changed from 30 years to 1 day to implement this option, and that SAM was not required. Nevertheless, the daily flow averaging option should be a requirement for modeling of all flowing bins (2, 3, and 4).

Comment 3:
Adjustment of Water Body Dimensions: This option used a “representative length” of 40 m to represent that water body length (as opposed to the full watershed length) and adjusted the volume accordingly. This “representative length” has been proposed for use in SAM. While this option may be effective at lowering EECs closer to realistic levels, it is conceptually inaccurate, and would not be necessary if an appropriate flowing water receiving model were used to route the flows (such as SWAT).

Comment 4:
Use of Daily Average EECs: The use of daily average EECs instead of peaks was proposed. One argument made by EPA was that typical toxicological studies have a 48-hour duration. For this reason, daily average or 48-hour average EECs should be used instead of peak EECs for all aquatic habitat bins. There are no toxicological tests that measure effects of instantaneous exposure. However, an analysis was conducted in Section 4.1.4 of this response document that demonstrates that LC50 values are much higher at shorter exposure durations for malathion, and for organophosphates and carbamates in general. This assumption was investigated using the results of thirteen fish and aquatic invertebrate toxicological studies that reported cumulative mortality and LC50s at various exposure time points.

Document: Attachment 3-1: Background Document: Aquatic Exposure Estimation for Endangered Species, Section 3.1.4.4: Modifications Evaluation, Pilot Chemicals, Final Approach

Comment 1:
The alternative modeling approaches for Bin 3 and Bin 4 modeling were evaluated for atrazine and compared against monitoring data. The proposed approaches resulted in the most refined EECs comparing relatively favorably with monitoring data, so were tested for the 3 OPs. Based on testing with the 3 OPs, the alternative modeling approaches produced similar results for some of the AIs, but not for others. Values higher than solubility were still noted and cases of Bin 4 and Bin 3 EECs higher than Bin 2 were also noted. The lack or improvement in EECs is being driven by the limitations in receiving water model (VVWM). Several of the alternative modeling approaches considered (CN, daily average EECs) are good ideas for refinement and should be pursued in conjunction with a more appropriate receiving water model.

Comment 2:
Final Approach to Calculating Bin 3 and Bin 4 EECs: Based on review of atrazine monitoring data, scaling factors were derived to estimate Bin 3 and Bin 4 EECs from the model-simulated Bin 2 EECs. While these values are more appropriate than the modeled values that had been calculated, there are limitations to this approach:

- There is no data presented to support that the relationship between atrazine from small headwater streams to larger rivers would apply directly to malathion. Atrazine's environmental fate properties differ from the malathion, including solubility, adsorption to sediment, and degradation rates, all of which impact the fate of the pesticide over time in a flowing water system.
- For the monitoring datasets assessed (AEMP and Heidelberg University), atrazine is used on crops with very high PCA (corn/sorghum) and is very widely used on those crops (a high PTA). These differences in use pattern between atrazine in the watersheds evaluated and the malathion make the transfer of conclusions on atrazine to malathion inappropriate.
- The Bin 2 EECs from which the Bin 3 and Bin 4 EECs were derived are several orders of magnitude higher than a realistic worst case scenario (see comments on peak EECs relative to edge of field concentrations). Therefore, scaling the Bin 3 and Bin 4 EECs from these erroneously high Bin 2 EECs propagates the errors to the other flowing water habitats.

Document: Appendix 3-3: Spray Drift Considerations for Malathion
Table B 3-3.1 Spray Drift Estimates for Aquatic Bins and Various Aquatic Buffer Combinations

Comment 1:
The text on Page 2 described the spray drift analysis as having been conducted using AgDRIFT Tier 1 model. However, the footnotes in the Table B 3-3.1 describe inputs for the Aerial ULV

application that are not available in the AgDRIFT Tier 1 model. If other higher tier tools were used for this modeling, than that should be noted appropriately, along with all the necessary inputs for the higher Tier model.

Comment 2:
The drift fractions for Aerial Non-ULV application method presented in table B 3-3.1 are based upon an ASAE fine to medium droplet size of 255μm. The malathion ULV labels supported by Cheminova require a droplet size of medium (300 μm) or coarser. Medium to coarse droplet size is also a specific requirement of the 2006 RED. Therefore, the AgDRIFT Tier 1 aerial assumption of an ASAE medium to coarse droplet size should have been selected for spray drift modeling. This will affect the spray drift fractions for the Aerial non-ULV and the Boll Weevil Eradication Program.

Comment 3:
The drift fractions for the Aerial Non-ULV application method presented in table B 3-3.1 cannot be reproduced based on the inputs indicated in the footnotes of the table. The AgDRIFT Tier 1 model generates different results than those reported for all six habitat bins.

Comment 4:
The drift fractions for ground spay, with no buffer, assumed a very fine to fine droplet spectrum. Instructions for malathion ground spray on labels supported by Cheminova require a medium (300 μm) or coarser droplet size. Spray drift modeling with the AgDRIFT Tier 1 ground model should have used the Fine to Medium\Coarse spray option.

Comment 5:
The malathion ULV product labels supported by Cheminova require a droplet size of medium (300 μm) or coarser. The assumptions for ULV aerial spray shown in Table B3-3.1 indicate that a droplet size of 60 μm from a release height of 25 feet was assumed in calculating the ULV drift fractions for the aquatic modeling. The malathion ULV label supported by Cheminova requires a droplet size of 300 μm or greater with spray height less than 10 feet above the crop canopy. The droplet size and release height requirements as specified on the malathion ULV product labels should have been followed in this modeling.

Comment 6:
The AgDisp8.26 aerial modeling for mosquito adulticide is based on uncalibrated ULV deposition. ULV deposition is designed to drift for further distances and may have a very different drift deposition curve than the aerial drift deposition curve predicted by the current parameterized Air Tractor At-401 scenario.

Comment 7:
EPA states that in 2013, a comparison analysis was conducted in ground and aerial deposition of adulticides using literature information and “other modeling”. Conclusions from this study indicate that deposition was similar between ground and aerial methods. No specific sources were listed nor the data included in the report. The drift deposition fractions assigned to the

aquatic bins were derived from the end result of these analyses. Consequently, these drift fractions are not reproducible and the quality of the literature data values cannot be assessed.

Document: Appendix 3-5: Downstream Dilution Tool Results

Comment 1:
Downstream dilution was not conducted for malathion "Because of the widespread use of malathion and the uncertainty with where the adulticide, wide area, and non-agricultural uses could occur, the entire United States is considered the action area for malathion for Step I." (Appendix 3-5) The same rationale was applied for Step 2. However, the tables in Appendix 1.6 and I .7 indicate that developed uses (which include mosquito control) are separate from agricultural uses and there are certain agricultural use sites that are not applicable across all HUCs (e.g., soybeans). If applications are excluded for soybeans, it would not be valid to assume use on these sites for any purposes, including mosquito control. Some agricultural crop groups, where use is restricted, should not be assumed to allow a non-Ag, wide area use in Action Area delineation and co-occurrence analysis.

Document: Chapter 4 Effects Determinations
Section 4: Effects Determinations of NLAA - No overlap

Comment 1:
A NLAA is determined in Step 2 for all species and/or designated critical habitats with less than 1% co-occurrence with use sites (including off-site transport). There is no information provided for the basis of the 1% cut-off. It is unclear whether the 1% co-occurrence represents a level of co-occurrence determined to be ecologically significant, or if these is other justification in the selection of 1% as opposed to a different nominal threshold (e.g., 0.5%, 2%, 5%). In addition, this implementation of a co-occurrence threshold does not account for uncertainty in the CDL pixel classifications. Recent approaches for developing probabilistic crop footprints using the CDL would help quantify the likelihood of co-occurrence while accounting for dataset uncertainty.

3.3 Summary of Concerns Regarding the Aquatic Exposure Analysis

The aquatic exposure modeling presented in the draft BEs included astronomically high malathion EECs that are impossible to achieve under real world use and environmental conditions in both agricultural and urban settings. This included malathion concentrations as high as 71,800 µg/L in low flow stream habitat, 11,800 µg/L in low volume statics water habitat, and 1,890 µg/L in medium volume static water habitat. These predicted concentrations compare to a maximum surface water concentration ever recorded in the United States of 22 µg/L out of over 70,000 samples taken across sites that include high intensity agriculture with significant malathion use crops as well as urban locations (EPA, 2016a). These implausibly high EECs are also significantly higher than maximum measured edge of filed runoff concentrations of 146 µg/L (EPA, 2016a). A confirmation that many EECs predicted in EPA's malathion BE are impossible

high can be made by comparing the simulated receiving water concentration to the simulated edge-of-field runoff concentrations.

A comparison of 1 in 15 year annual maximum peak receiving water EECs to their corresponding 1 in 15 year annual maximum edge-of-field EECs was made for over 850 of EPA's PRZM/VVWM endangered species scenarios that were reported on the BE. These simulations were made assuming a negligible contribution of spray drift to receiving waters. This analysis showed that the receiving water EECs were higher than the edge of field concentrations for nearly every scenario, and often 10 to 100 times higher than edge of field concentration for many of the ESA scenarios for the low flow (Bin 2) aquatic habitat. Aquatic modeling scenarios with receiving water concentrations higher than the edge of field concentrations occur for all three of the static water scenarios as well. Due to the physical dilution process, it is impossible for receiving water concentrations to be higher than edge of field concentrations, and in fact, we expect receiving water concentration to be substantially less than edge of field concentrations for the larger volume water bodies (Bin 6 and Bin 7) and flowing water bodies, including Bin 2. This points to fundamental errors in the modeling that impacts results for all of the aquatic habitat bins, including the medium flow (Bin 3) and high flow (Bin 4) habitats that were estimated form the Bin 2 EECs. The most important step that can be taken to correct the erroneously high EECs for all aquatic habitat bins is correcting the assumption that the entire watershed's pesticide mass generated in 1 day arrives at the receiving water body instantaneously, without any dilution form the runoff water that transported the pesticide to the water body. Additional steps that should be taken for improving flowing water body habitat simulations include:

- Replacing VVWM with a receiving water model designed to simulate pesticide fate and transport in a flowing channel. The Soil and Water Assessment Tool (SWAT) has this capability and has been shown to produce realistic peak exposure values for small, medium, and large flowing water bodies (refer to Greer et al., 2016 and Teed et al., 2016 for details).
- Incorporating a baseflow rate equal to at least the minimum of the flow range associated with each habitat bin.
- Constraining the watershed areas to that which can drain into a main channel with 1 day.
- Applying Percent Cropped Area (PCA) adjustments at a minimum to Bin 3 and Bin 4.

In addition to correcting the errors that have been noted in the screening level modeling, the overall aquatic exposure modeling at Step 2 needs to move beyond simple screening level approaches that use a single conservative PRZM simulation to predict EECs in static or flowing water bodies. Step 2 aquatic exposure modeling approaches must include the following:

- Spatially explicit predictions of EECs that can be associated with species habitat locations.
- Representation of the heterogeneous landscape through explicit simulation of the land uses and soils that are relevant to aquatic habitat for a given species.
- Accounting for weather and climate variability throughout a species range.

- An accounting for variability in pesticide application timing that occurs at both the field and watershed scales.
- Use of actual Percent Crop Area characteristics for both static and flowing water body habitat based on best available spatial dataset.
- Incorporation of Percent Treated Area (PTA) that acknowledges that 100% of potential use sites do not get treated with malathion.
- Use of probabilistic approaches that account for environmental and agronomic variability and model input and assumption uncertainty to arrive at robust probability distributions to evaluate the likelihood of exposure exceeding effects end points.

4 EFFECTS ENDPOINTS AND DERIVATION OF THRESHOLDS

This section is organized by general comments (Section 4.1), Cheminova's recommended threshold values contrasted with EPA's threshold values (Section 4.2), and a comparison of the threshold values presumably used by EPA in their effects determinations with those actually used in their predictive tools (Section 4-3).

4.1 General comments

4.1.1 SETAC Pellston Workshop on Improving the Usability of Ecotoxicology in Regulatory Decision-making

Recently a SETAC Pellston Workshop was held from the 30th of August to the 4th of September 2015 at the U.S. Fish and Wildlife Service National Conservation Training Center in Shepherdstown, West Virginia, with the objective of "Improving the Usability of Ecotoxicology in Regulatory Decision-making." SETAC Pellston Workshops provide a platform for discussions and collaborations between academia, business, government and NGOs. The workshop was attended by 36 experts (Anita Pease from EPA and Roger Breton from Intrinsik participated in this workshop) from four continents. These workshops give stakeholders the opportunity to meet and find solutions to common problems, try new ideas, and promote understanding of the various views.

The goal of the workshop was to develop guidance on steps that can be taken to improve the use of all ecotoxicity data in risk assessments, whether the data are derived from guideline studies conducted under Good Laboratory Practices (GLP) or from research studies published in the peer-reviewed literature. The workshop addressed the following processes:

1. Improve the reliability and reproducibility of ecotoxicity studies;
2. Improve the use of peer-reviewed studies in regulatory risk assessment of chemicals; and,
3. Improve the methods used in risk assessments when evaluating single pieces of evidence or lines of evidence.

Recommendations on good practices for study design, establishment of minimum requirements for reporting the methodology, performance and results, and proposals for improving consistent use of the information during the regulatory processes were discussed. Other issues of importance were regulators' view of academic research, the role of scientific journals in promoting reliability and reproducibility of studies, actions industry can take to increase the transparency of studies, methods for enabling transfer of knowledge between stakeholders, and tools for improved risk assessment.

A summary of the workshop was presented at the SETAC North America 36th Annual Meeting from November 1–5, 2015 in Salt Lake City, Utah. Some of the main outcomes of the workshop follow.

Experts at the Pellston Workshop acknowledged that there has been a steady convergence in peer reviewed journals of data transparency and reporting aspects (Stavely et al., 2016; McCarty et al., 2012; Ågerstrand et al., 2011), but that improvements are still needed. Indeed, improvements are needed as will be shown by an evaluation of the data quality of many of the key studies EPA used to derive threshold values. Many important study details on design and experimental parameters were often not reported in these papers. Very importantly, chemical identification and characterization were often not provided in these peer-reviewed journals. These points will be further discussed on a case-by-case basis in the sections that follow.

The mandate of one of the working groups was to provide guidance on the type of information that should be presented in a peer-reviewed paper for it to be considered for regulatory decision-making. This group was formed because it was recognized that improvements in the quality of peer-reviewed journals are needed for them to be accepted as best available data for risk assessment. As a result, most peer-reviewed journal articles today are not sufficiently technically sound to support risk assessment decisions. Some of the key points discussed during the workshop that support this assertion include:

- The peer-review process provides credibility to the information contained in a journal article. However, this does not necessarily mean a study is high quality or appropriate for application in regulatory decision-making (Hall et al., 2012). The process of peer review is not consistent between journals, or even within a field (McCarty et al., 2012). Reviews of scientific articles vary in quality and in one study it was shown that reviewers disagree considerably in regards to basic issues such as clarity, organization, methods and discussion (Onitilo et al., 2013).
- Peer reviewers who evaluate papers before journals accept them for publication most often do not attempt to spot check for mistakes (The Economist, 2013). There is often no data quality verification of the results and calculations. The Economist (2013) noted that reviewers do not typically re-analyze data and accept that the authors' analysis is properly conceived. Academic research is seldom conducted for the specific purpose of risk assessment in a regulatory setting (Beronius et al., 2014a).
- The information reported in peer-reviewed articles reflects what the researcher considers important in relation to the hypothesis tested. In the eyes of the researcher, the information reported in the article may be adequate to meet the research objective, but may still be insufficient for risk assessment. This is often the case when evaluating the quality and relevance of peer-reviewed journals, particularly "older" studies. Reasons for this include unawareness of information needed for regulatory use, and lack of space available in scientific journals (Beronius et al., 2014a). These weaknesses have been confirmed by several sources (SCENIHR, 2012; Beronius et al., 2014a,b). Experiences from the German Federal Environment Agency (UBA) (Küster et al., 2009), and regulatory organization, and from Ågerstrand et al. (2011) also show that studies published in the open scientific literature often are insufficiently reported and non-transparent to fulfill the requirements that are necessary for regulatory environmental risk assessments.

- Peer-reviewed journal articles are typically selected for publication due to their stimulation of scientific debate (McCarty et al., 2012). With this in mind, it is expected that insignificant or negative results (e.g., no effects) are less likely to be submitted and published in peer reviewed journals, and this bias may be growing (Harris et al., 2014; The Economist, 2013). This is one of the major problems in today's research. The publication of insignificant or no effects data would be extremely valuable, particularly now that over 1,500 listed species need to be assessed.
- Repeatability of toxicological results is central to good science, particularly when effects are observed at extremely low effect concentrations (Stavely et al., 2015; Harris et al., 2014). As pointed out by Miller (2014), toxicology is not toxicology without reproducibility. It is practically impossible for other scientists to repeat experiments because of the lack of experimental details provided in peer-reviewed papers. It is also very much recognized that there is increasing pressure in academia to chase funds, to publish new or novel results in as many papers as possible. Repeating previously published work to contradict or confirm results are less likely to be accepted for publication in scientific journals (Stavely et al., 2016; Harris et al., 2014; Nature, 2013). Repeatability of toxicological results is most often not achievable when analyzing a peer-reviewed paper. As will be shown by study evaluations conducted as part of this response, data gaps would prevent other scientists from repeating testing results used to derive threshold values.
- Studies in the peer-reviewed scientific literature seldom follow GLP, and may or may not follow an acceptable guideline.

4.1.2 Data Selection and Evaluation Process

The process by which toxicity data are identified, evaluated, and selected as best available data for use in risk assessment plays a critical role in the eventual outcome of any effects determination. While EPA has produced several guidance documents to aid in the internal evaluation of toxicity studies (EPA, 2002, 2003, 2004a,b, 2011b), it is questionable as to whether or not these criteria are consistently followed by reviewers. In their most recent guidance, EPA (2011b) prescribes that studies used for risk assessment be evaluated for quality, and that best available data only be used for risk assessment. As it will be presented in this response document, EPA (2016a) has not adequately described or documented the study evaluation and data selection process applied in the malathion BE. We will show that most studies used by EPA to make risk decisions have not been properly quality assured. This is very concerning for Cheminova.

For example, it is noted in the BE (Section 1.4.2.2.b.1 of Chapter 1) that registrant-submitted studies were reviewed using "the agency's Standard Evaluation Procedures (SEP)". However, these evaluation procedures are not described in the BE or any associated attachment or appendix, nor is an external link to a publicly available description of these procedures provided. Additionally, neither the study evaluations nor the final study classifications for these registrant-submitted studies are provided anywhere in the BE. For clarity and transparency, EPA should provide the study Data Evaluation Records (DERs) for all registrant-submitted studies

considered in the BE (EPA, 2016a). Cheminova has prepared a list of all of the registrant submitted studies referred to in the BE (EPA, 2016a) with respect to ecological effects, environmental fate, and residue chemistry and has indicated whether or not Cheminova had received the DER for this study as of June 3, 2016. Of the 164 studies in the list, Cheminova had received DERs for only 73 of these studies despite multiple requests for EPA to provide them (see Appendix C of this response document).

Likewise, for open literature, the BE notes that reviews were carried out “largely using the approach outlined in the agency’s guidance for evaluating ecological toxicity data in the open literature” with modifications from that guidance detailed in Attachment 1-8. A reference (hyperlink) is provided to the agency’s guidance on page 60 of Chapter 1 of the BE. However, this link redirects to a general EPA pesticide website and no study evaluation guidance can be immediately identified on that page. It is possible that the link is meant to refer to existing EPA evaluation guidelines for ecological toxicity data in the open literature (EPA, 2011b), but this cannot be confirmed on the basis of the information present in the BE (EPA, 2016a). EPA appears to have modified their 2011 study evaluation guidance (EPA, 2011b) for reviewing open literature studies specific for this BE. However, it is inappropriate to evaluate toxicological studies differently in this assessment than in other assessments. Furthermore, there is no justification for the inconsistent standards EPA follows for the review of registrant-submitted studies compared to those published in open literature, with a much less stringent review process required for the latter. A single, thorough set of data quality guidelines should be set and followed by EPA for each study that is considered for use in every risk assessment.

The study evaluation ‘modifications’ described in Attachment 1-8 of the BE are also ambiguous. This attachment specifies that open literature toxicity data identified from the EPA Ecotoxicity Database (ECOTOX) were reviewed according to a strategy outlined in Figure 4-1 below (copied from Figure 1-8.1 in Attachment 1-8 of EPA (2016a)). According to this screening process, the information provided from ECOTOX was used to categorize open literature data as i) limited to use in an effect array, ii) limited to qualitative consideration in the effects characterization, or iii) in need of further review to determine whether study endpoints are suitable for use to develop a threshold or generate an SSD.

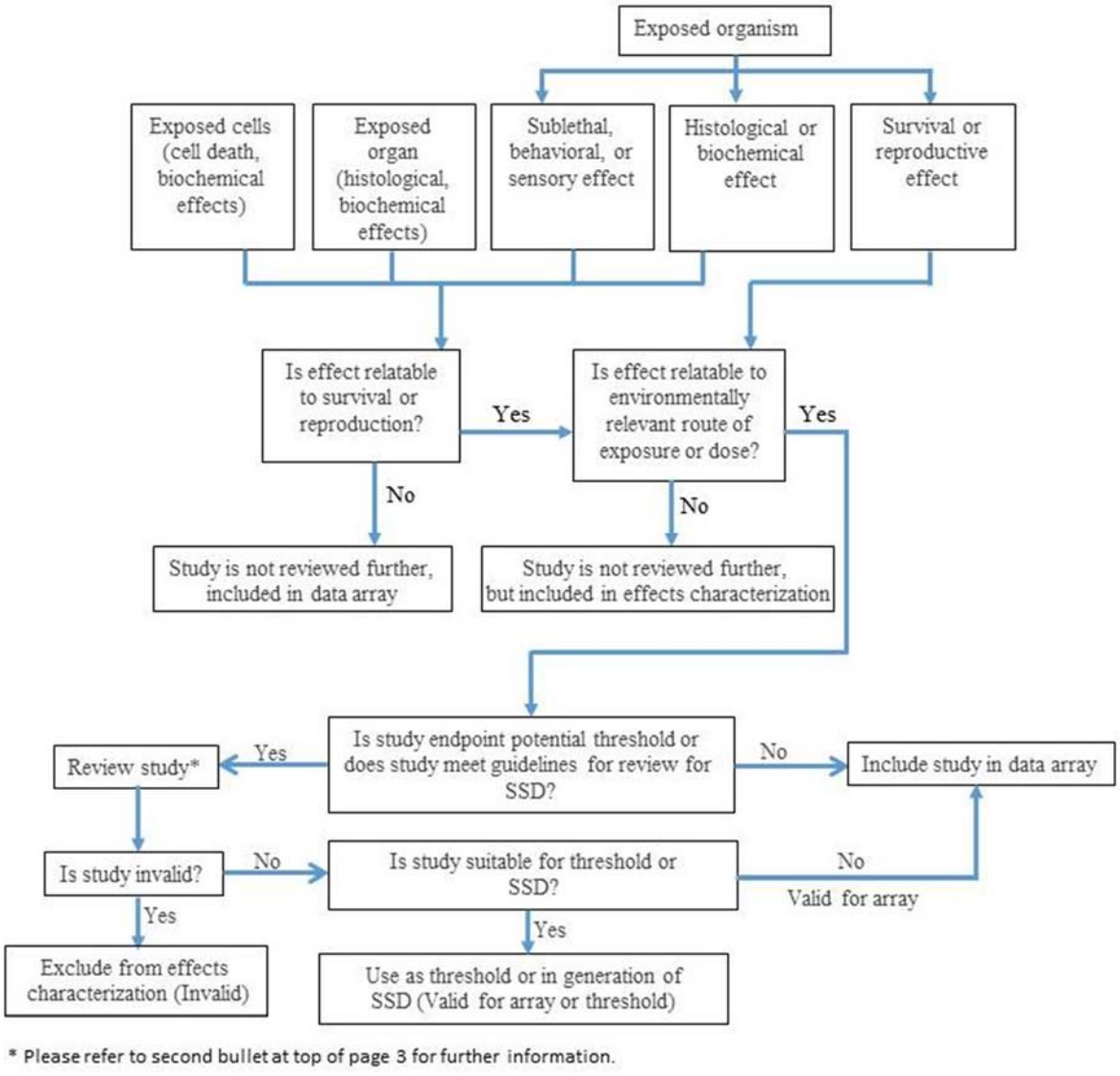

Figure 4-1 **Flow diagram showing stepwise process for consideration and review of ECOTOX studies (Copied from Figure 1-8.1 from Attachment 1-8 of EPA (2016a))**

However, the screening process diagram (Figure 4-1, above) also contains a caveat regarding which studies should receive a full review (see asterisk (*) at the 'Review Study' stage). The bullet points referred to by this asterisk indicate that EFED only conducted full study reviews for:

- The lowest reported concentration/dose/rate for mortality or sublethal effects.
- A subset of studies used to assemble a species sensitivity distribution (SSD) for a given taxa (e.g., fish). Specifically, those studies that represent the tails and central area of the species sensitivity distribution (SSD) (i.e., values near the 5th, 50th and 95th percentiles and the median) were reviewed unless previously reviewed for prior assessments. If

these studies were considered scientifically valid, they were considered appropriate for use in the SSD.

This caveat leaves an unresolved gap in the study evaluation process for open literature studies as it does not specify how the remaining endpoints that "meet guidelines for review for SSD" were evaluated. Additionally, as will be shown in the following sections for each assessed taxon, it does not appear that EPA actually followed these bullets in deciding which studies to assess. There are several examples of studies that contained the "lowest reported concentration/dose/rate for mortality or sublethal effects" with no study evaluation reported in Appendix 2-3 of the BE, which provides "Open Literature Review Summaries for Malathion".

Furthermore, even if the study evaluation approach described in the above bullets was followed, this would mean that studies were included in SSDs based solely on information provided in ECOTOX without any review of the original study. For example, the SSD for fish and aquatic-phase amphibians prepared by EPA (see Section 2.4.1 of the BE) was derived using endpoints from 39 open literature studies and 13 registrant submitted studies. However, study reviews are reported in Appendix 2-3 of the BE for only four of the open literature study sources relied on in that SSD. Given the importance of using the best possible data for evaluating ecological effects, the inclusion of endpoints that have not been fully evaluated in an SSD used to derive an effect threshold is inappropriate. EPA should provide full open literature review summaries (OLRS) for all open literature studies considered in the BE (EPA, 2016a).

Overall, there is a significant lack of transparency with respect to both study evaluation and data selection in the BE (EPA, 2016a). Without full DERs and OLRS, it is difficult to understand how EPA classified their key toxicological studies for data reliability and data relevance. Additionally, in the case of open literature, it appears that EPA has relied on studies that were not evaluated at all, other than the initial screen required prior to entry into ECOTOX. This is not a suitable data quality screen as the information provided in ECOTOX is occasionally incorrect and often does not contain sufficient information to confirm its reliability for risk assessment.

EPA's evaluation schemes are not clear and lack transparency. EPA (2011b) has recently acknowledged that, "there are inconsistencies among OPP risk assessors regarding the use of open literature data to address an existing data gap, the classification of open literature studies, and the use of these studies in an ecological risk assessment." EPA has also noted that OLRS are often not completed and rarely submitted for storage and tracking. This is the case for the BE. Moreover, EPA is inconsistent in how they evaluate open literature versus registrant-submitted studies (EPA, 2004a; 2011b). They do not always follow their own evaluation criteria, and their criteria are not stringent enough with respect to the relevance of the test substance to Cheminova products currently registered in the US (see Breton et al., 2014a [MRID 49333901] for further discussion). It is imperative that EPA provide a clear and transparent process for the classification of toxicological studies and selection of effects metrics.

Many of the effects metrics identified in the BE are based on old peer-reviewed literature or a compilation of data that provide little documentation to evaluate the reliability and relevance of

the data. This fact also supports the findings of the Pellston Workshop. Moreover, it is not possible to be able to reproduce any of the results due to poor experimental documentation. For the most part, EPA's recommendations fail to identify registrant-submitted studies as effects metrics, studies that have been conducted under GLP, in a transparent manner, and have been properly documented for reproducibility (cornerstone of sound science) and data quality evaluation (Borgert et al., 2016).

Consistent with the outcome of the Pellston Workshop, all studies should be evaluated using a common scheme to identify best available data for risk assessment, and not focus on most sensitive data irrespective of data quality. The evaluation scheme should be transparent, and the criteria and guidance need to be made clear to be able to reproduce the evaluations. Most studies used by EPA as threshold values are classified as unacceptable for risk assessment based on Cheminova data quality criteria (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]). The fundamental question of data quality and use of "best available data" does not appear to have been addressed in the BE.

In its recent review and guidance document entitled: "Assessing Risks to Endangered and Threatened Species from Pesticides", the National Academy of Sciences (NRC) examined scientific and technical issues related to the approach that government agencies (e.g., EPA) use to determine risks posed to listed species by pesticides (NRC, 2013). This report states that toxicological data should be evaluated prior to use in risk assessment and that test material must be determined to be relevant to the US. NRC (2013) noted the lack of a formal, consistent approach in defining "best data available" among EPA, US Fish and Wildlife Service and the National Marine Fisheries Service. It was suggested that the strengths and weaknesses of key studies be properly documented to ensure the relevance of each study and transparency of methods. NRC (2013) advised, with respect to the quality of data used in ecological risk assessment, that enough information should be provided by the author(s) for adequate scientific evaluation and reproducibility of the results. Cheminova is in agreement with this recommendation. If adequate information on study quality is not provided in a source document, the quality of the study is unknown (NRC, 2013). Data of lower quality should not be used to drive risk assessments when higher quality studies are available (NRC, 2013). Accordingly, all relevant data should be evaluated for quality before it is used in risk assessment.

As a result, Cheminova has derived a set of study evaluation criteria. The details of this evaluation scheme were provided to EPA (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]). The criteria and guidance needed to correctly address each criteria is clearly presented. As suggested in Breton et al. (2014a [MRID 49333901]), Cheminova recommends that EPA consider these criteria for evaluating the quality of toxicity studies for use in risk assessment. These criteria pertain to toxicity studies for several taxa: aquatic invertebrates, aquatic plants, fish, aquatic-phase amphibians, birds, mammals, terrestrial arthropods, and terrestrial plants. Following Cheminova's criteria, studies can be categorized as acceptable, supplemental, or unacceptable. Each study rating is based on an evaluation of study design and execution, adherence to toxicity testing protocols, statistical analyses, and other key aspects of the study. The criteria are impartially applied by Cheminova to registrant-submitted studies,

publicly available peer-reviewed open literature and other sources. The use of this approach ensures that only high quality toxicity data are considered in ecological risk assessments.

In brief, for a study to be deemed supplemental or acceptable (i.e., not unacceptable) it must meet at least the following criteria:

- single chemical exposure;
- conducted according to a recognized international standard (e.g., OPPTS, OECD, ASTM, ISO, etc.), or provided sufficient information on procedures in the report to confirm that laboratory practices were acceptable;
- purity, impurity profile and source of the test substance reported or otherwise known;
- ecologically relevant effects measured;
- appropriate exposure duration;
- appropriate control results reported; and
- statistical procedures reported and appropriate.

For a study to be deemed acceptable, in addition to the above criteria, the following must also be provided:

- details of test concentrations or doses, measured and maintained where appropriate;
- characteristics and acclimation of test species; and
- test conditions.

The purity criterion is of particular importance for malathion. This criterion can be met only if the test substance is known and comparable to current US registered products. Technical malathion has been manufactured over the years by a number of companies with an active ingredient content ranging from 91 to 99% and varying levels of impurities. In 1992, Cheminova submitted an updated confidential statement of formula (CSF) with higher malathion purity and reduced impurities. The reduction in impurities is an important feature of technical malathion manufactured by Cheminova as it has a significant impact on toxicity (Hillwalker and Reiss, 2014 [MRID 49316501]). In their evaluation of the Sweilum (2006) study conducted on Nile tilapia (*Oreochromis niloticus L.*) in Appendix 2-3 of the BE, EPA acknowledged that the non-reporting of the purity of malathion was a limitation to the study. Cheminova agrees that this is indeed a limitation of a study, and that purity and impurity profiles play a key role for malathion in determining the relevance of the tested material to a risk assessment.

In Section 1.4.2.2.f of the BE, EPA included a discussion on impurities of malathion and their potential effect on ecotoxicity levels. EPA acknowledges possible difference in impurity levels between Cheminova's product and other malathion products, but concludes from a comparison of ecotoxicity data that tests with Cheminova's product yields similar results to tests reported in the open literature. This section reviews EPA's impurity discussion and conclusions about similar toxicity results for tests with Cheminova products and those reported in the open literature.

EPA cites a document from the California Department of Food and Agriculture from 1981 that "reported 15 impurities" "in an ultra-low volume malathion formulation." EPA lists the impurities and their levels in Table 1-11 of the BE. However, many of the impurity levels shown in the table match the upper-limit of the Cheminova malathion specification. Therefore, it is very likely that the levels in the 1981 California document are simply specification upper limits (as they were in 1981) and not actual levels. In many cases, typical levels in technical malathion are much lower. Refer to Hillwalker and Reiss (2014) for a presentation of analysis results for impurities for a range of malathion batches.

EPA notes that some data suggest that malathion becomes significantly more toxic in storage (e.g., Umetsu et al., 1977). The increase in malathion toxicity associated with storage has focused on increased isomalathion levels with increased storage temperature. As discussed by Hillwalker and Reiss (2014), it is important to note that Cheminova's product contains significantly lower levels of isomalathion than other commercial products that have been on the market and with commercial standards used in testing. The Cheminova upper-limit for isomalathion is 0.2%. The previous lead registrant, American Cyanamid, had an upper limit specification of 0.9%. Another registrant (Griffin) that sold product during the height of the Boll Weevil Eradication Program, had an upper certified limit of 0.41%. Many academic researchers use Chem Service to obtain standard reference material for malathion (e.g., the recent NOAA study by Laetz et al., 2013). As detailed in Hillwalker and Reiss (2014), Cheminova had a batch of Chem Service's product tested and found it contained 0.72% isomalathion, which is more than 3-fold higher than the Cheminova limit. Thus, while it is true that isomalathion levels increase with storage, Cheminova's product contains significantly less isomalathion than other products previously or currently available. Therefore, it is possible that historical tests included higher isomalathion levels than are possible with Cheminova's product.

Another important impurity in malathion is malaoxon. Hillwalker and Reiss (2014) show that Cheminova's product has significantly lower levels of malaoxon compared to the American Cyanamid and Griffin products.

EPA also provides a discussion about the potential effect of other malathion impurities stating that "it appears that some malathion (and other organophosphate) impurities can increase malathion toxicity and also are toxic alone." However, isomalathion is the only impurity that meaningfully increases with storage temperature (Hillwalker and Reiss, 2014), so even if other impurities are more toxic than malathion itself, their effect is already included in standard toxicity testing. What is unknown is the level of these impurities in tests conducted with materials other than supplied by Cheminova. However, one example of an impurity cited by EPA as more toxic than malathion is diethyl fumarate. EPA cites Bender (1969) as the source for a 3-fold increased toxicity of diethyl fumarate to fathead minnows compared to malathion. However, Wilson (1966) found that malathion was more than 2-fold more toxic to fathead minnows than diethyl fumarate.

EPA also alludes to one impurity (O,O,S-trimethyl phosphorothioate) being significantly more persistent than malathion. Presumably, the concern here is that malathion may become more toxic in the environment as it degrades with the more toxic impurities degrading more slowly

than the parent. However, Wilson (1966) tested malathion toxicity with fathead minnows in long-term studies and found that the “toxicity of malathion decreases with time.” This is expected because, as noted in Section 1.2.3 of the BE, malathion rapidly degrades in both soil and water and the major metabolites are malathion carboxylic acid (MCA) and malathion dicarboxylic acid (DCA). MCA and DCA are significantly less toxic than malathion. Given that most of the mass starts as parent malathion and it converts to significantly less toxic constituents in the environment, even if a few impurities degrade more slowly than the parent, the overall toxicity will decline over time as confirmed by Wilson (1966).

In Tables 12 and 13 of the problem formulation of the BE, EPA provides comparisons of toxicity data derived by Cheminova with other reported data, mostly from the open literature. EPA concludes that the Cheminova and literature toxicity levels are “well within one order [of magnitude] of each other.” While Cheminova does not disagree with this conclusion, there are certainly some differences between the toxicity levels for certain species and an order of magnitude is not a trivial difference in some risk assessments. The information presented above shows significantly lower levels of some key impurities in the Cheminova product compared to historical products and with a recently purchased reference standard from a commonly used source. Therefore, where differences occur in toxicity levels (even small ones), EPA should default to the Cheminova-derived data as it is most representative of the currently used product. More broadly, each situation where Cheminova and literature data are available for the same species should be approached on a case-by-case basis to understand the potential for differences in toxicity levels caused by different impurity levels and measurement protocols.

Cheminova is the only producer of technical malathion sold in the US, and because all end-use products are derived from Cheminova’s technical malathion, it is our contention that only studies conducted using Cheminova’s technical malathion are relevant to risk assessments in the US. Technical malathion sold in the US must have a purity of at least 95%. For a study to be classified as acceptable for use in risk assessment in the US, the purity of malathion must meet this specification, composition of impurities must be known, and all other generic and acceptable criteria must be met. If a study meets all generic and acceptable criteria except for purity, but that study was conducted by American Cyanamid, then that study is deemed supplemental. The reason for this is that Cheminova has knowledge of the composition of the impurities of American Cyanamid products since we purchased the malathion business from them.

The toxicity of a formulation can be influenced by the inerts in that formulation. Toxicity data conducted on end-use products that are currently registered in the US are generally considered to be relevant to the US risk assessments. However, many of the older formulations contained inerts that are no longer approved for use in the US today. In addition, many registrants have proactively replaced older inerts with less harmful solvents, emulsifiers, etc. Thus, toxicity data that were submitted years ago on a currently registered formulation may no longer be representative of currently registered end-use products today. On two occasions, EPA reviewers acknowledged, as a major limitation of the quality of a study, that it was unknown whether or not tested formulations were representative of current formulations or would be

expected to have an increased toxicity relative to the technical grade active ingredients (Sauter and Steele (1972 [E38642, MRID 00015279]) for both malathion and diazinon). For diazinon, one reviewer noted as a major limitation that “The relationship of the tested formulation to US registrations is unknown (Natal-Da-Luz, 2012 [E160446]). It is, therefore, apparent, that EPA acknowledges that the testing of current registered formulations is a major data quality criterion for assigning relevance and identifying best available data for risk assessment. Thus, this further reinforces the fact that tested formulations relevant in risk assessment need to be representative of currently registered formulations in the US.

Because the inert profiles of end-use products are considered to be “confidential business information”, it is not possible for Cheminova to evaluate the relevance of toxicity data that may be available for products registered in the US by other companies. Because Cheminova is familiar with the inert profiles of the end-use products tested in Cheminova and American Cyanamid studies, they are generally considered acceptable for use in risk assessment if they meet our study evaluation criteria and, in particular, the identification and relevance of the test material. Studies conducted with formulations should not be used in risk assessment unless EPA has sufficient information on the test material and its inerts to establish relevance to currently registered end-use products. Test materials must be comparable to products currently registered for use in the US.

Using the study evaluation criteria summarized above (and explained in detail in Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]), Cheminova has previously completed full study evaluations for many of the studies used to develop ‘effects thresholds’ in the malathion BE (see full evaluations in Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]). As will be discussed in the following taxon-specific sections, many of the studies relied on by EPA (2016a) were rated as ‘unacceptable’ for risk assessment by Cheminova. Additionally, for this response document, Cheminova has assessed the reliability of additional studies used in the BE as effects thresholds that were not previously reviewed by Cheminova (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]). These additional study reviews are provided in Appendix D of this response document. It was not possible to review all previously unreviewed studies that EPA (2016a) included in their SSDs due to time limitations. However, it is expected that many of these studies would also fail a stringent quality screen. As noted above, the SSDs generated by EPA cannot be considered reliable in the absence of thorough study reviews for all studies included in the SSD. Moreover, many of the studies used to construct SSDs had a chemical characterization identified as “unknown” in the BE. How can EPA use toxicity data from studies that have not properly characterized the tested chemical for relevance? This is scientifically unsound.

Cheminova also disagrees with EPA’s procedure for evaluating chronic risk to aquatic and terrestrial species. Chronic guideline studies typically use continuous pesticide exposures ranging from 21 days for aquatic invertebrates to greater than 10 weeks for birds and mammals. However, such exposures are unrealistic because malathion would, in reality, degrade rapidly between applications, particularly in marine environments, making pulse exposures far more relevant than maintained chronic exposures. For example, in a targeted monitoring study

conducted by Gulka et al. (2016), malathion concentrations in two Oregon streams were measured at least every six hours over a two-month period of intensive malathion use on cherries. Using the data from Gulka et al. (2015), 21-day rolling averages of malathion concentrations were calculated for Mill Creek and Threemile Creek. Samples with non-detectable levels of malathion were estimated to be equal to half the LOD of 0.010 µg/L. The 21-day average concentrations ranged from 0.0218 to 0.0561 µg/L and from 0.0154 to 0.0364 µg/L for Mill Creek and Threemile Creek, respectively, and are both below the most sensitive chronic NOEL for *Daphnia magna* (0.06 µg/L; Blakemore and Burgess, 1990 [MRID 41718401]).

Lastly, NOELs are the effects thresholds driving most, if not all of the risk designations, and in turn the species and critical habitat calls in the pilot BEs (Section 5.2 of this response document). The use of NOELs in ecological risk assessment has long been criticized (Hoekstra and Van Ewijk, 1993; Moore and Caux, 1997; Landis and Chapman, 2011; Jager, 2012; Murado and Prieto, 2013). This criticism stems from the inherent deficiencies of the metrics as a relative measure of toxicity, which include an absolute dependence on the selected treatment levels and sample size, and related issues of low statistical power. As a result, regulatory risk assessors are moving away from the use of NOELs in favour of ECx values (e.g., OECD, 1998; CCME, 2007). Given the criticism of using NOELs in ecological risk assessment in the peer-reviewed scientific literature, it is surprising that the Agency would consistently use these metrics in an evaluation that is purported to be based on best available scientific information. In the Interagency Interim Approaches, the Agencies (2013) stated that ECx values would be considered in the interim approach. However, it seems that in most cases the EPA opted to circumvent data analyses and simply use the author-reported NOELs from toxicity studies. In some instances the use of NOELs may be practical, for instance when sample size is large it may make sense to use a NOEL as a cursory screening-level metric, and/or when the data are not conducive to generating a meaningful dose-response. However, in a succeeding analysis, such as Step 2, the Agency should be giving precedence to more refined metrics (e.g., dose-response curves) when possible.

In Sections 4.2.1 through 4.2.8 of this response document, Cheminova has provided taxon-specific discussions of the data relied on by EPA (2016a) to generate effects thresholds. Additionally, where appropriate, Cheminova has recommended alternate effects metrics for each taxon based on their previous study reviews (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]).

4.1.3 Consideration of Endpoints of Uncertain Ecological Relevance

The Interagency Interim Approaches (Agencies, 2013) states that “Establishing “may effect” thresholds for given taxa may also, when supported by professional judgment, be based on toxicity studies that are conducted at the sub-organism level (*e.g.*, on organs or cells), provided they can be linked to environmentally relevant exposures that can influence survival, growth, or reproduction”. In addition, in Attachment 1-4 of the BE, EPA (2016a) describes the process for determining effects thresholds. For sublethal effects to animals, it is specified that endpoints will generally be developed from *in vivo* studies that are conducted with whole organisms, are

representative of environmentally relevant exposures routes, and are able to be quantitatively or qualitatively linked to effects on survival, growth or reproduction.

However, in the same attachment (Attachment 1-4 of the BE), EPA notes that “Establishing “may affect” thresholds for given ESA-listed taxa may also be based on toxicity studies that are conducted at the suborganismal level (e.g., on organs or cells), provided data are consistent with other criteria for use.” It is not explained further how such suborganismal data could be used in the BEs to establish thresholds, especially given the difficulty of relating such endpoints to effects on survival, growth or reproduction.

As noted by NRC (2013), to properly incorporate sublethal effects into an ecological risk assessment, it is necessary to provide an explicit relationship between the sublethal effect in question and apical endpoints (i.e., survival, growth, and/or reproduction). In many cases, where EPA (2016a) has presented sublethal endpoints (e.g., the inclusion of biochemical, cellular, and behavioral effects in many of the ‘data arrays’), there is no discussion as to the ecological relevance of these endpoints. Without establishing this relationship, it is unclear how these effects can be considered in a weight of evidence approach. This comment applies to all of the ‘data arrays’ presented in the BE.

Furthermore, beyond the inclusion of non-apical endpoints in many of the data arrays, for some taxa, EPA (2016a) is also using sublethal non-apical threshold values without providing evidence of any qualitative or quantitative link between these endpoints and survival, growth or reproduction. For example, in Table 3-1 of Chapter 2 of the BE, EPA (2016a) relies on a NOEC and LOEC for ‘capture net abnormalities/ AChE’ for the caddisfly *Hydropsyche slossonae* from Tessier et al. (2000 [E65789]). However, no discussion is provided regarding whether the levels of capture net abnormalities and/or AChE inhibition observed in the study are sufficient to impair survival, growth, or reproduction in the caddisfly. Similarly, for birds, EPA (2016a) included sublethal effects based on AChE inhibition in their thresholds tables (Tables 6-1 and 6-2 of Chapter 2 of the BE, incorrectly labeled as Table 0-1 and 0-2). The suitability of this effect to derive a threshold value is questionable given that studies have shown that AChE inhibition in birds returns to control levels within 24 hours of removal of sublethal doses of malathion (Pym et al., 1984; Mehrotra et al., 1967). Likewise, in Tables 9-1 and 9-2 of the BE, EPA (2016a) has indicated sublethal thresholds for mammals based on inhibition of red blood cell (RBC) acetyl cholinesterase (AChE) in rats as reported in Daly (1996 [MRID 43975201]). This appears to be a referencing error as Daly (1996 [MRID 43975201]) is actually a toxicity study of the effects of malaoxon on rats rather than malathion. Regardless, as was noted for birds, the suitability of AChE inhibition to derive a sublethal threshold value is questionable given that an explicit relationship between AChE inhibition and effects to survival, growth, or reproduction has not been demonstrated by EPA (2016a). As noted by Russom et al. (2014), there are major data gaps associated with the application of the adverse outcome pathway for organophosphates to different types of risk scenarios. For instance, it is uncertain whether using measures of enzyme activity at the cellular/tissue level (or from in vitro studies) can effectively estimate, in a quantitative manner, adverse outcomes in the whole organism.

For comparison to known effects on mortality and/or reproduction, consider the two generation rat study carried out by Schroeder (1990 [MRID 41583401]). The most sensitive NOEL reported in this study, which was relied on by EPA (2010b) as their selected chronic effects metric, was a NOEL for pup growth at 1,700 mg a.i./kg diet (132 mg a.i./kg bw/d for males, 153 mg a.i./kg bw/d for females). Additionally, as noted by Cheminova (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]), this pup growth NOEL may be a direct effect of pups feeding on treated diet and not a true developmental effect caused by exposure to the fetuses *in utero* or to the pups via the milk. If this endpoint is rejected, the next lowest NOEL from this two generation study is that for male parental body weight at 5,000 mg a.i./kg diet (394 mg a.i./kg bw/d) (Schroeder, 1990 [MRID 41583401]). Overall, the results of the Schroeder study (1990 [MRID 41583401]) demonstrate that the LOEL of 1 mg a.i./kg bw for AChE inhibition in rats (from an unknown study) selected as a sublethal effect threshold by EPA (2016a) is orders of magnitude lower than the chronic exposure dose required to cause significant impairment to either mortality or reproduction in rats. Therefore, the LOEL of 1 mg a.i./kg bw selected by EPA (Table 9-1 and 9-2 of Chapter 2 of the BE) should be rejected as an effect threshold.

Overall, these examples indicate the limitations of relying on effects other than mortality, growth, or reproduction in risk assessment without further evidence of a link between the effect and one of the apical endpoints (NRC, 2013; Agencies, 2013). Endpoints without a direct link to specific apical adverse effects are not considered to be biologically significant. EPA (2016a) should not rely on such endpoints unless sufficient evidence can be presented for the direct link between the endpoint and a known ecologically relevant response.

4.1.4 Mismatch of Exposure Duration Between Toxicity Endpoints and Estimated Environmental Concentrations (EECs)

EPA (2016a) predicted acute risk to aquatic organisms by comparing instantaneous aquatic peak EECs to threshold values derived from toxicity tests wherein organisms were exposed to constant concentrations of malathion for much longer exposure durations. For example, EPA (2016a) relied on 96-hour toxicity tests to derive acute effects thresholds for fish and 48-hour or 96-hour toxicity tests to derive acute effects thresholds for aquatic invertebrates. However, malathion degrades very quickly in aqueous systems, with estimated half-lives ranging from 0.3 to 3.3 days (Blumhorst, 1991 [MRID 42271601]; Knoch, 2001b [MRID 46769502]; Hiler and Mannella, 2012 [MRID 48906401]). It is highly unlikely that aquatic organisms would be exposed to a ‘peak’ concentration of malathion for a 48- or 96-hour period under realistic conditions. Therefore, the EPA (2016a) risk assessment approach conservatively assumes that exposure to a peak malathion concentration followed by rapid dissipation/ degradation will result in the same effects on fish and aquatic invertebrates as exposure to a constant concentration of malathion for a 48- or 96-hour duration.

The following analysis demonstrates that LC50 values are much higher at shorter exposure durations for malathion, and for organophosphates and carbamates in general. This assumption is investigated using the results of thirteen fish and aquatic invertebrate toxicological studies that reported cumulative mortality and LC50s at various exposure time points. All of these

studies were evaluated as acceptable or supplemental based on Cheminova's study evaluation criteria (Breton et al. 2014a [MRID 49333901], 2015 [MRID 49692301]).

Freshwater fish

Seven freshwater fish studies were available that reported mortalities and calculated LC50 values at exposure durations ranging from two to 96 hours (Claude et al., 2013a, [MRID 48752901, 49252802]; Gries and Purghart, 2001a,b,c,d [MRID 49051202, 47540302, 49051203 & 47540308, 47540304]; Gries et al., 2002a [MRID 48998004]). These studies maintained constant exposure concentrations using flow-through conditions. The results of these studies are summarized in Table 4-1 through Table 4-7. LC50 values were higher for shorter exposure durations. Additionally, in all studies except for Gries and Pughart (2001c [MRID 49051203 & 47540308]), there was a treatment concentration that exceeded the 96-hour LC50 that caused no mortality at four hours. This demonstrates the inadequacy of using the 96-hour LC50 to predict mortality at much shorter exposure periods. In another example, Claude et al. (2013a) predicted a 96 h LC50 of 27,000 µg a.i./L, yet observed no mortality at 40,000 µg a.i./L after 4 hours of exposure (Table 4-1). The 96-hour LC50 predicted by Gries and Pughart (2001c) (300 µg a.i./L) also falls short of predicting survival after four hours of exposure given that only 14% mortality was observed at 320 µg a.i./L (Table 4-5). The 2h-LC50 value of 920 µg a.i./L is more than three-fold greater than the 96h-LC50 of 300 µg a.i./L.

Table 4-1 Cumulative mortality during 96-hour exposure of fathead minnow (*P. promelas*) to technical malathion[a]

Concentration (µg test substance /L)	*Fraction Dead*	*% Cumulative Mortality*				
	96 h	*4 h*	*24 h*	*48 h*	*72 h*	*96 h*
LC50 (µg a.i./L)[b]		>40,000	28,000	27,000	27,000	27,000
0	0/20	No mortalities				
2,500	0/20	No mortalities				
5,000	0/20	No mortalities				
10,000	0/20	No mortalities				
20,000	1/20	0%	0%	5%	5%	5%
40,000	20/20	0%	100%	100%	100%	100%
80,000	20/20	100%	100%	100%	100%	100%

[a] Claude et al. 2013a [MRID 49252801]. The test material used in this study contained 0.8% iso-malathion, which is currently higher than the allowable level for malathion products registered in the US. However, the data presented above demonstrate the change in toxicity results when organisms are exposed for varying durations. These data are not intended to be considered for quantitative use in the risk assessment of malathion.

[b] Test concentrations reported by authors in µg a.i./L.

[c] NA = Not available

Table 4-2 Cumulative mortality during 96-hour exposure of fathead minnow (*P. promelas*) to technical malathion[a]

Concentration (µg test substance/L)	*Fraction Dead*	*% Cumulative Mortality*				
	96 h	*4 h*	*24 h*	*48 h*	*72 h*	*96 h*
LC50 (µg a.i./L)[b]		>40,000	38,000	35,000	28,000	28,000
0	0/20	No mortalities				
2,500	0/20	No mortalities				
5,000	0/20	No mortalities				
10,000	0/20	No mortalities				
20,000	0/20	No mortalities				
40,000	20/20	0%	55%	65%	100%	100%
80,000	20/20	100%	100%	100%	100%	100%

[a] Claude et al. 2013b [MRID 49252802]
[b] Test concentrations reported by authors in µg a.i./L.
[c] NA = Not available

Table 4-3 Cumulative mortality during 96-hour exposure of bluegill sunfish (*L. macrochirus*) to malathion formulation[a]

Concentration (µg a.i./L)[b]	*Fraction Dead*	*% Cumulative Mortality*					
	96 h	*2 h*	*4 h*	*24 h*	*48 h*	*72 h*	*96 h*
LC50 (µg a.i./L)[b]		>104	>104	>104	74	67	53
0	0/7	No mortalities					
6.5	0/7	No mortalities					
13	0/7	No mortalities					
26	1/7	0%	0%	0%	0%	0%	14%
52	2/7	0%	0%	0%	14%	14%	29%
103.9	7/7	0%	0%	43%	86%	100%	100%

[a] Gries and Purghart, 2001a [MRID 49051202]
[b] Test concentrations reported by authors in µg a.i./L.

Table 4-4 Cumulative mortality during 96-hour exposure of rainbow trout (*O. mykiss*) to technical malathion[a]

Concentration (µg test substance/L)[b]	*Fraction Dead*	*% Cumulative Mortality*					
	96 h	*2 h*	*4 h*	*24 h*	*48 h*	*72 h*	*96 h*
LC50 (µg test subtance/L)[b]		1,100	690	410	370	270	180
0	0/6	No mortalities					
100	0/6	No mortalities					
200	4/7	0%	0%	14%	14%	14%	57%
400	7/7	0%	0%	43%	57%	100%	100%
800	7/7	0%	86%	100%	100%	100%	100%
1,600	7/7	100%	100%	100%	100%	100%	100%

[a] Gries and Purghart, 2001b [MRID 47540302]
[b] Test concentrations reported by authors in µg test substance/L.

Table 4-5 Cumulative mortality during 96-hour exposure of rainbow trout (*O. mykiss*) to malathion formulation[a]

Concentration (µg a.i./L)[b]	*Fraction Dead*	*% Cumulative Mortality*					
	96 h	*2 h*	*4 h*	*24 h*	*48 h*	*72 h*	*96 h*
LC50 (µg a.i./L)[b]		920	550	360	330	330	300
0	0/6	No mortalities					
80	0/6	No mortalities					
160	2/7	0%	0%	14%	14%	14%	29%
320	2/7	0%	14%	14%	29%	29%	29%
650	7/7	14%	57%	100%	100%	100%	100%
1,300	7/7	86%	100%	100%	100%	100%	100%

[a] Gries and Purghart, 2001c [MRID 49051203 & 47540308]
[b] Test concentrations reported by authors in µg a.i./L.

Table 4-6 Cumulative mortality during 96-hour exposure of bluegill sunfish (*L. macrochirus*) to technical malathion[a]

Concentration (µg test substance/L)[b]	*Fraction Dead*	*% Cumulative Mortality*					
	96 h	*2 h*	*4 h*	*24 h*	*48 h*	*72 h*	*96 h*
LC50 (µg test substance/L)[b]		>128	>128	120	120	76	54
0	0/7	No mortalities					
8	0/7	No mortalities					
16	0/7	No mortalities					
32	0/7	No mortalities					
64	5/7	0%	0%	0%	0%	43%	71%
128	7/7	0%	0%	57%	57%	71%	100%

[a] Gries and Purghart, 2001d [MRID 47540304]
[b] Test concentrations reported by authors in µg test substance/L.
[c] NA = Not available

Table 4-7 Cumulative mortality during 96-hour exposure of fathead minnow (*P. promelas*) to technical malathion[a]

Concentration (µg a.i./L)[b]	*Fraction Dead*	*% Cumulative Mortality*					
	96 h	*2 h*	*4 h*	*24 h*	*48 h*	*72 h*	*96 h*
LC50 (µg a.i./L)[b]		>7,980	>7,980	>7,980	>7,980	>7,980	>7,980
0	0/7	No mortalities					
469	0/7	No mortalities					
946	0/7	No mortalities					

Table 4-7 Cumulative mortality during 96-hour exposure of fathead minnow (*P. promelas*) to technical malathion[a]							
Concentration (µg a.i./L)[b]	***Fraction Dead***	***% Cumulative Mortality***					
	96 h	***2 h***	***4 h***	***24 h***	***48 h***	***72 h***	***96 h***
LC50 (µg a.i./L)[b]		>7,980	>7,980	>7,980	>7,980	>7,980	>7,980
7,980	2/7	0%	0%	14%	14%	29%	29%

[a] Gries et al. 2002a [MRID 48998004]
[b] Test concentrations reported by authors in µg a.i./L.

Marine/estuarine fish

Four marine/estuarine fish studies were available that reported mortalities and calculated LC50 values at exposure durations ranging from two to 96 hours (Burke, 2011 [MRID 49055701], Gries et al. 2002b [MRID 48998006], Bowman, 1989a,b [MRID 41174301, 41252101]). These studies maintained constant exposure concentrations using flow-through conditions. The results of these studies are summarized in Table 4-8 through Table 4-11. As was observed for freshwater fish, LC50 values were higher for shorter exposure durations. For example, the 24h-LC50 value for sheepshead minnow observed by Burke (2011) of 181.8 µg a.i./L was nearly four-fold greater than the 96h-LC50 of 47.4 µg a.i./L (Table 4-8). Likewise, the suitability of the 96-hour LC50 to predict mortality at shorter exposure durations was generally poor. For example, Burke (2011) predicted a 96-hour LC50 of 47.4 µg a.i./L, but observed no mortality after 3.5 hours for treatment concentrations as higher as 200 µg a.i./L (Table 4-8). In another example, the 2h-LC50 is greater than the highest tested concentration, a value that is more than three-fold greater than the 96h-LC50 of 21.7 µg test substance/L (Gries et al. 2002b [MRID 48998006])(Table 4-9). Similar examples are found in Table 4-10 and Table 4-11.

Table 4-8 Cumulative mortality during 96-hour exposure of sheepshead minnow (*C. variegatus*) to technical malathion[a]						
Concentration (µg a.i./L)[b]	***Fraction Dead***	***% Cumulative Mortality***				
	96 h	***3.5 h***	***24 h***	***48 h***	***72 h***	***96 h***
LC50 (µg a.i./L)[b]		>200	181.8	122.0	113.5	47.4
0	0/7	No mortalities				
44.6	3/7	0%	0%	0%	0%	43%
60.2	3/7	0%	0%	14%	14%	43%
81.3	2/7	0%	0%	14%	14%	29%
110.0	7/7	0%	14%	43%	43%	100%
148.0	6/7	0%	0%	43%	57%	86%
200	7/7	0%	57%	71%	71%	100%

[a] Burke, 2011 [MRID 49055701]
[b] Test concentrations reported by authors in µg a.i./L.
[c] NA = Not available

Table 4-9 Cumulative mortality during 96-hour exposure of three-spined stickleback (*G. aculeatus*) to technical malathion[a]

Concentration (µg test substance/L)[b]	*Fraction Dead*	*% Cumulative Mortality*					
	96 h	*2 h*	*4 h*	*24 h*	*48 h*	*72 h*	*96 h*
LC50 (µg test substance/L)[b]		>81.4	>52.7	21.7	21.7	21.7	21.7
0	0/7	No mortalities					
1.90	0/7	No mortalities					
5.01	0/7	No mortalities					
11.1	2/7	0%	0%	29%	29%	29%	29%
19.8	3/7	0%	0%	43%	43%	43%	43%
41.8	5/7	0%	29%	71%	71%	71%	71%
81.4	7/7	14%	86%	100%	100%	100%	100%

[a] Gries et al. 2002a [MRID 48998004]
[b] Test concentrations reported by authors in µg test substance/L.

Table 4-10 Cumulative mortality during 96-hour exposure of sheepshead minnow (*C. variegatus*) to technical malathion[a]

Concentration (µg a.i./L)[b]	*Fraction Dead*	*% Cumulative Mortality*			
	96 h	*24 h*	*48 h*	*72 h*	*96 h*
LC50 (µg a.i./L)[b]		43	43	40	40
0	0/20	No mortalities			
18	0/20	No mortalities			
35	7/20	25%	25%	35%	35%
74	20/20	100%	100%	100%	100%
110	20/20	100%	100%	100%	100%
320	20/20	100%	100%	100%	100%

[a] Bowman, 1989a [MRID 41174301]
[b] Test concentrations reported by authors in µg a.i./L.

Table 4-11 Cumulative mortality during 96-hour exposure of sheepshead minnow (*C. variegatus*) to malathion formulation[a]

Concentration (µg a.i./L)[b]	*Fraction Dead*	*% Cumulative Mortality*			
	96 h	*24 h*	*48 h*	*72 h*	*96 h*
LC50 (µg a.i./L)[b]		>62.7	53.6	44.5	31.3
0	1/20	0%	0%	5%	5%
3.6	0/20	No mortalities			
8.0	0/20	No mortalities			
16.5	1/20	0%	0%	5%	5%

Table 4-11 Cumulative mortality during 96-hour exposure of sheepshead minnow (*C. variegatus*) to malathion formulation[a]					
Concentration (µg a.i./L)[b]	***Fraction Dead***	***% Cumulative Mortality***			
	96 h	***24 h***	***48 h***	***72 h***	***96 h***
LC50 (µg a.i./L)[b]		>62.7	53.6	44.5	31.3
26.8	6/20	5%	5%	15%	30%
62.7	20/20	20%	65%	75%	100%

[a] Bowman, 1989b [MRID 41252101]
[b] Test concentrations and LC50 values reported by authors as µg/L and converted by Intrinsik to µg a.i./L using purity of 57%.

Freshwater aquatic invertebrates

One study that reported LC50 values and rates of mortality at varying test durations (24 or 48 hours) for freshwater aquatic invertebrates was available (Burgess, [MRID 41029701], see summary in Table 4-12). This study also used flow-through conditions to maintain a constant exposure concentration. As for fish, the shorter exposure duration resulted in a higher LC50 value than the longer exposure duration. Additionally, the 48-h LC50 (1.2 µg a.i./L) was inadequate for predicting survival after 24 h exposure as only 7.5% mortality was observed at 2.7 µg a.i./L after 24 hours (Table 4-12).

Table 4-12 Cumulative mortality during 48-hour exposure of water flea (*D. magna*) to malathion formulation[a]			
Concentration (µg a.i./L)[b]	***Fraction Dead***	***% Cumulative Mortality***	
	48 h	***24 h***	***48 h***
LC50 (µg a.i./L)[b]		>2.7	1.2
0	0/40	No mortalities	
0.11	0/40	No mortalities	
0.20	0/40	No mortalities	
0.52	4/40	7.5%	10%
1.0	7/40	7.5%	17.5%
2.7	40/40	7.5%	100%

NA = Not available
[a] Burgess, 1989
[b] Test concentrations and LC50 values reported by authors as µg/L and converted by Intrinsik to µg a.i./L using purity of 57%.

Marine/estuarine aquatic invertebrates

One study was identified that compared mortality rates and LC50 values at varying exposure durations (24 to 96 hours) for marine/estuarine aquatic invertebrates (Forbis, 1990 [MRID 41474501])(Table 4-13). Again, shorter exposure durations resulted in higher LC50 values. Specifically, the reported 24-hour LC50 value of >6.8 µg/L was more than three-fold higher than the reported 96-hour LC50 of 2.2 µg/L (Table 4-13). Additionally, the 96-hour LC50 (2.2 µg a.i./L)

was inadequate for predicting survival after 24 hours exposure as only 20% mortality was observed at 6.8 µg a.i./L after 24 hours.

Table 4-13 Cumulative mortality during 96-hour exposure of mysid (*A. bahia*) to technical malathion[a]

Concentration (µg/L)[b]	*Fraction Dead*	*% Cumulative Mortality*			
	96 h	*24 h*	*48 h*	*72 h*	*96 h*
LC50 (µg/L)[b]		>6.8	3.6	2.3	2.2
0	0/20	No mortalities			
0.31	0/20	No mortalities			
0.85	0/20	No mortalities			
1.5	0/20	No mortalities			
2.6	15/20	0%	15%	70%	75%
6.8	20/20	20%	100%	100%	100%

[a] Forbis, 1990 [MRID 41474501]
[b] It was unclear whether concentrations were of test substance or active ingredient.

When assessing risk of chronic effects, the rapid degradation and dissipation of malathion in aquatic systems indicates that sustained exposures are highly unlikely. Chronic guideline studies typically use continuous pesticide exposures ranging from 21 days for aquatic invertebrates to 96 days for fish life cycle studies. However, such exposures are unrealistic because malathion would, in reality, degrade and dissipate rapidly between applications, particularly in marine environments, making pulse exposures far more relevant than maintained chronic exposures. A series of studies on pulse versus constant chronic exposures were conducted with waterflea exposed to omethoate, a degradate product of the organophosphate dimethoate. These studies were conducted in the same laboratory within a few years of one another. In the pulse exposure studies, organisms were exposed to the test item once or twice for 24 hours, and subsequently transferred to control media (with 74 hours between exposures for the double pulse test). The 21-day single and double pulse studies both reported no reproductive effects at the highest concentrations tested (≥ 6.55 and ≥ 6.67 µg a.i./L respectively), whereas the standard 21-day study reported a NOEC and LOEC of 0.247 and 0.494 µg a.i./L for time to first brood (Schafers, 2013a,b [MRIDs 49299709, 49299708; Simon, 2011 [48821413]). The results of these studies indicate that under conditions with greater environmental relevance, where pulse exposures simulate pesticide application events, exposure to aquatic ecosystems is greatly reduced and thus so are any potential adverse effects. This should be taken into consideration when assessing potential chronic risk to aquatic organisms from malathion exposure.

Conclusion

An examination of the cumulative mortalities from these studies shows that the malathion LC50s for fish and aquatic invertebrates are typically higher for shorter exposure periods and that using 96- or 48-hour LC50 values to predict mortality for much shorter exposure periods (e.g., four hours) is likely to overestimate risk. Similar results have been reported in the literature for

malathion and other organophosphate pesticides. For example, toxicity tests for organophosphorus and carbamate compounds using the test species *Chironomus riparius* found that two 1-hour pulses (with at least 2 to 6 hour of clean water between doses) caused significantly fewer symptoms of intoxication than 2 hours of continuous exposure (Kallander et al., 1997). Similarly, Bogen and Reiss (2012) observed that the LC50 for chlorpyrifos in fathead minnows was more than three times as high when minnows were exposed to the chemical for 24 hours followed by 72 hours of recovery than when minnows were exposed to a constant concentration of the chemical for 96 hours. Further increases in the LC50 were observed for even shorter durations (Jarvinen et al., 1988). Therefore, the EPA (2016a) approach of comparing peak exposure concentration to effects thresholds derived based on 48- and 96-hour toxicity tests for fish and aquatic invertebrates is likely to overestimate risk. Likewise, chronic exposure is likely overestimated due to the rapid degradation and dissipation of malathion in water bodies. Pulse exposure studies on omethoate show that compared to standard 21-day exposure, observed effects were greatly reduced (i.e., no effects observed at concentrations 13-fold greater than the standard chronic LOEL) (Schafers, 2013a,b [MRIDs 49299709, 49299708; Simon, 2011 [48821413]). Cheminova recommends comparing the existing acute effects data with time weighted average exposure concentrations that are compatible with the toxicity test durations used for risk assessment. Cheminova also suggests EPA consider the overestimation of chronic exposure when assessing risk of chronic effects to aquatic organisms.

4.1.5 Degradates of Concern

EPA (2016a) identified malaoxon as a “significant concern for ecological risk” and indicated that their assessment would include a qualitative discussion of the potential risks associated with malaoxon. However, Cheminova suggests that even a qualitative assessment for malaoxon is unnecessary. Based on the available fate and toxicity data for malaoxon, it is unlikely to contribute significantly to ecological risk of organisms compared to the parent compound. Specifically, although malaoxon has been demonstrated to be slightly more toxic than malathion to some aquatic species, the fate and behavior of malaoxon suggests that it is likely not produced in the aquatic environment. Moreover, malaoxon degrades rapidly in water, sediment and soil samples. On the rare occasions when it is detected, malaoxon is found only at small percentages of applied malathion. As such, malaoxon is unlikely to be transported at environmentally relevant concentrations in which exposure would cause significant effects on growth, reproduction and survival. In the terrestrial environment, malaoxon has half-lives shorter then one day, indicating that malaoxon degrades quickly on arthropods and vegetation, which are important feed items for terrestrial organisms. Given the above, risk associated with malaoxon exposure is likely negligible for birds and mammals. For further discussion, see Appendix E.

4.1.6 Incident Reporting

EPA (2016a) summarized their approaches for evaluating incident data in BE Section 1.4.2.2.b of the problem formulation and in Attachment 1-1. Section 1.4.2.2.b states that available incident data were evaluated for relevance using EFED's current incident guidance (Guidance for Using Incident Data in Evaluating Listed and Non-listed Species under Registration Review. Environmental Fate and Effects Division, Office of Pesticide Programs, US Environmental Protection Agency, Washington, DC. Oct. 13, 2011).

EPA (2015a) used the OPP Incident Data System (IDS), the Ecological Incident Information System (EIIS), and the Avian Incident Monitoring Systems (AIMS) to find data on ecological incidents pertaining to the OPs. Attachment 1-1 describes the IDS, EIIS, and AIMS, as well as the certainty index and legality of use classifications for ecological incidents.

Incident reports were summarized in the effects characterization chapter and separated by taxon. For the most part, EPA (2016a) followed their written guidance (EPA, 2011b). Notable differences between the EPA BE (2016a) and their guidance (EPA, 2011b) included the following:

- Incidents in the "unlikely" and "unrelated" certainty categories were not evaluated for accuracy per EPA guidance.
- EPA did not evaluate the results for applicability to currently registered uses and products.
- EPA did not determine if mitigation measures have been put in place since the incident to prevent similar incidents from re-occurring.
- No summary of relevance or interpretation of results was provided.

Malathion incident reports for fish and aquatic-phase amphibians are reported in Section 2.7 of Chapter 2 of the BE. In Table 2-8, EPA (2016a) reported a total of 31 incidents (29 if those categorized as 'unlikely' are excluded). The number of aquatic animal incidents was further decreased to 23 if both unlikely and misuse incidents were excluded. These 23 incidents were summarized in BE Table 2-9 with a more detailed description of selected incidents provided in BE Table 2-10 (mislabeled as Table 2-9 in the table title). However, it is unclear where the details in Table 2-10 were drawn from. Some incidents match the incident numbers from EIIS, but others do not or the incident number was not reported. Nowhere does EPA explain the use of Table 2-10 or characterize the results.

Malathion incident reports for aquatic invertebrates are summarized in Section 3.7 of Chapter 2 of the BE. In this section, one incident involving blue crabs (from EIIS), one crayfish mortality incident from the USDA/APHIS 1995 report, and four incidents from the Aggregate Incident Reports database are briefly mentioned. Additionally, EPA (2016a) provides a brief discussion of a severe mortality event for the American lobster in Long Island Sound that some have suggested may have been influenced by widespread spraying of malathion and other products for mosquito control in response to a West Nile Virus outbreak. The full reference for the lobster incident was not provided by EPA (2016a). The reference given only states "Pearce and Balcom,

2005" and no full reference that matches this reference is provided in either Appendix 2-2 or Appendix 2-4 of the BE. Cheminova was able to track down the reference. The reduction of lobsters in Long Island Sound was evaluated through two independent modeling exercises (Miller et al., 2005; Wilson et al., 2005). In both cases, estimated exposure concentrations of malathion in the Sound were lower than the lowest toxicity value available for adult lobsters. Moreover, recent studies have failed to demonstrate a link between malathion exposure and the decline in lobster populations. Those populations have continued to decline in the absence of detectable concentrations of pesticides which bolsters the belief that warming temperatures is the main culprit (CDEEP, 2012; Collins, 2016; Middletown Press, 2016). Finally, all available lobster toxicity studies were rated as unacceptable by Cheminova because they did not follow standard testing guidelines and did not provide enough details to fully replicate the studies. Overall, EPA (2016a) does not attempt to determine if later investigations attributed the cause of the incident to malathion exposure. Most incident reports provide no causative link between pesticide exposure and the observed incident.

In Section 4.7 of Chapter 2, EPA (2016a) notes that no incidents concerning aquatic plants were reported in EIIS. EPA (2016a) does state in this section that terrestrial plant incident reports are available in EIIS as well as "a total of 231 aggregate plant incidents reported to the agency". However, no discussion is provided to explain whether terrestrial plant incidents have any bearing on aquatic plant incidents. Additionally, it is recognized that the aggregate plant incidents are of little utility given that details about these incidents other than product information and year were not provided.

No errors or discrepancies were noted in the incident report sections for birds, mammals and terrestrial plants. For terrestrial invertebrates, some minor errors were noted. Specifically, on p. 2-209 of Chapter 2, EPA reports that there were 12 terrestrial invertebrate incident reports in the EIIS with a certainty index of 'unlikely', 'possible', 'probable', or 'highly probable', but only 11 incidents are listed in the summary table (Table 10-8 in Chapter 2). Additionally, incidents I014341-043 and I014341-044 were mislabeled in the EPA BE as "possible" certainty, but were reported as "highly probable" in EIIS.

4.2 Taxon-specific Review and Critique of Effects Characterizations Presented in Chapter 2 of EPA (2016a)

In response to the NRC (2013) recommendations, EPA, FWS, NMFS and USDA developed their Interim Approaches for National-Level Pesticide Endangered Species Act Assessments Based on the Recommendations of the National Academy of Sciences April 2013 Report (Agencies, 2013). The Interagency Interim Approachesrecommends that the following endpoints be used to assess the potential for direct and indirect effects to endangered species (see Table 4-14).

Table 4-14 Prescribed endpoints as per the Interagency Interim Approaches (Agencies, 2013)

Taxon	*Mortality*		*Sublethal Effects*	
	Direct Effects	*Indirect Effects*	*Direct Effects*	*Indirect Effects*
Fish Aquatic Invertebrates Aquatic-phase amphibians	Concentration that would result in a 1 in a million chance of causing mortality to an individual[a]	Concentration that would result in a 10% decrease of individuals[a]	The lowest available NOEC or other scientifically defensible effect threshold (ECx)[b]	Lowest available LOEC for growth or reproduction
Aquatic plants - vascular Aquatic plants - non-vascular	None	None	Concentration equal to the lowest value among the available NOAEC and EC05 values.	Concentrations equal to the lowest available LOAEC and EC25 values
Birds Mammals Reptiles Terrestrial-phase amphibians Terrestrial invertebrates	Dose that would result in a 1 in a million chance of causing mortality to an individual[a]	Dose that would result in a 10% decrease of individuals[a]	The lowest available NOEL or other scientifically defensible effect threshold (EDx)[b]	Lowest available LOEL for growth or reproduction
Terrestrial plants - monocots Terrestrial plants - dicots Terrestrial plants - non-angiosperm	None	None	Concentration equal to the lowest value among the NOAEC and EC05 values from the available seedling emergence and vegetative vigor studies	Concentrations equal to the lowest available LOAEC and EC25 values from the available seedling emergence and vegetative vigor studies

[a] Calculated using the HC5 of an SSD of LC50/EC50 values and a representative slope. If an SSD cannot be derived, the most sensitive LC50 or EC50 is used.

[b] Endpoints will generally be a) from *in vivo* studies that are conducted with whole organisms and b) linked to environmentally relevant exposures.

As stated in CLA (2016), EPA provides no justification that the EC05 values used as direct effects thresholds for aquatic and terrestrial plants are meaningful toxicity endpoints on a statistical or biological basis. Rather, a 5% effect was found by Staveley et al. (2015; 2016a,b) to be within the expected control variance in standard toxicity tests conducted for aquatic and terrestrial plants and, thus, could not be statistically distinguished from control results. Although we followed the Interagency Interim Approaches (Agencies, 2013) in selecting effects metric for our refined malathion ESA, Cheminova fundamentally disagrees with the use of EC05 values as effects thresholds in EPA's BE (EPA, 2016a).

Following this guidance, Cheminova derived screening-level threshold values that should be used for the BE. The most sensitive and relevant measures of effect were selected for each taxon in Step 1 of Cheminova's National Endangered Species Assessment (NESA) (Teed et al., 2016). Evaluation of available studies was conducted to ensure that the best available data were used (Breton et al., 2014a [MRID 49333901], 2015 [MRID 49692301]). Toxicity study reports were screened using study evaluation criteria designed to ensure that only high quality data were used. Each study rating was based on an evaluation of study design and execution, adherence to toxicity testing protocols, statistical analyses, and other key aspects of the study

(Breton et al., 2014a [MRID 49333901], 2015 [MRID 49692301]). Recommendations of threshold values for Step 1 for each taxonomic group follows.

4.2.1 Fish and Aquatic-phase Amphibians

In Section 2.2 of Chapter 2, EPA (2016a) summarizes the threshold values for fish and aquatic-phase amphibians.

Cheminova's Acute Threshold Values

Sufficient acceptable toxicity data were available to derive an acute SSD for freshwater and estuarine/marine fish. Toxicity studies were screened using criteria designed to ensure that only the best available scientific data were used in deriving the SSD (Breton et al., 2014a [MRID 49333901], 2015 [MRID 49692301]). To develop the fish SSD, acute freshwater and estuarine/marine fish LC50s from nine acceptable studies conducted with technical malathion were used (Table 4-15). The exposure period for all endpoints was 96 hours. The sensitivity ranges of freshwater and estuarine/marine fish species overlaps (Breton et al. (2014a [MRID 49333901], 2015 [MRID 49692301]). The range of acceptable and supplemental LC50 data for freshwater species is 52 to 38,000 µg a.i./L, whereas the range for estuarine/marine species is 20.8 to 181.8 µg a.i./L. Therefore, it is reasonable to combine freshwater and marine fish species to derive the acute SSD. Study evaluations for all data included in the acute fish SSD can be found in Breton et al. (2014a [MRID 49333901], 2015 [MRID 49692301]).

Table 4-15 Acceptable 96-hour LC50 data for technical malathion used to develop the acute SSD for fish

Freshwater or Estuarine/ Marine Fish	*Common Name*	*Scientific Name*	*96-h LC50 (µg a.i./L)*	*Probit Slope*[a]	*Reference [MRID]*
Freshwater	Bluegill sunfish	*Lepomis macrochirus*	52	26.0	Gries and Purghart, 2001a [47540304]
Freshwater	Coho salmon	*Oncorhynchus kisutch*	720	27.2[b]	Brougher et al., 2014a [49479003]
Freshwater	Fathead minnow	*Pimephales promelas*	28,000	64.8[c]	Claude et al., 2013a [49252802]
Freshwater	Green sunfish	*Lepomis cyanellus*	130	7.60	Brougher et al., 2014b [49364101]
Freshwater	Medaka	*Oryzias latipes*	1500	4.24	Brougher et al., 2014c [49364102]
Freshwater	Rainbow trout	*Oncorhynchus mykiss*	174	21.9	Gries and Purghart, 2001b [47540302]
Freshwater	Western mosquitofish	*Gambusia affinis*	2900	5.00	Brougher et al., 2014d [49422801]
Estuarine/Marine	Sheepshead minnow	*Cyprinodon variegatus*	47.4	2.81	Burke, 2011 [49055701]
Estuarine/Marine	Three-spined stickleback	*Gasterosteus aculeatus*	20.8	3.04	Gries et al., 2002a [48998006]

[a] Probit slopes calculated in SAS Version 9.3, SAS/STAT 12.1 unless otherwise indicated.
[b] Partial mortality was only observed for one test concentration in the coho salmon dataset. European commission guidance recommends that at least two partial mortalities be observed in order to use Probit analysis for the calculation of an EC/LC50 (EC, 2003).
[c] No partial mortalities were observed in the fathead minnow dataset. European commission guidance recommends that at least two partial mortalities be observed in order to use Probit analysis for the calculation of an EC/LC50 (EC, 2003).

The acute SSD was generated using SSD Master v3.0 (Rodney et al., 2013), an Excel-based tool that fits five different cumulative distribution functions (normal, logistic, extreme value, Gumbel) in both log and arithmetic space. This software allows for testing of model fit. The Gumbel model using log LC50 and EC50 values was the best-fitting model according to the Anderson-Darling (AD) goodness-of-fit test statistic (A^2 = 0.170) and various graphical plots of model residuals (e.g., p-p and q-q plots). The Gumbel model equation is shown below.

$$f(x) = e^{-e^{\frac{(L-x)}{s}}}$$ **Equation 4-1**

Where,

x is the concentration metameter;
f(*x*) is the proportion of taxa affected; and
L and *s* are the location and scale parameters of the model.

The fitted model parameters were: *L* = 2.068 and *s* = 0.890 (Figure 4-2) for acute toxicity data reported in µg a.i./L. The HC5 (concentration that will affect 5% of species in the SSD) from the acute SSD is 12.3 µg a.i./L.

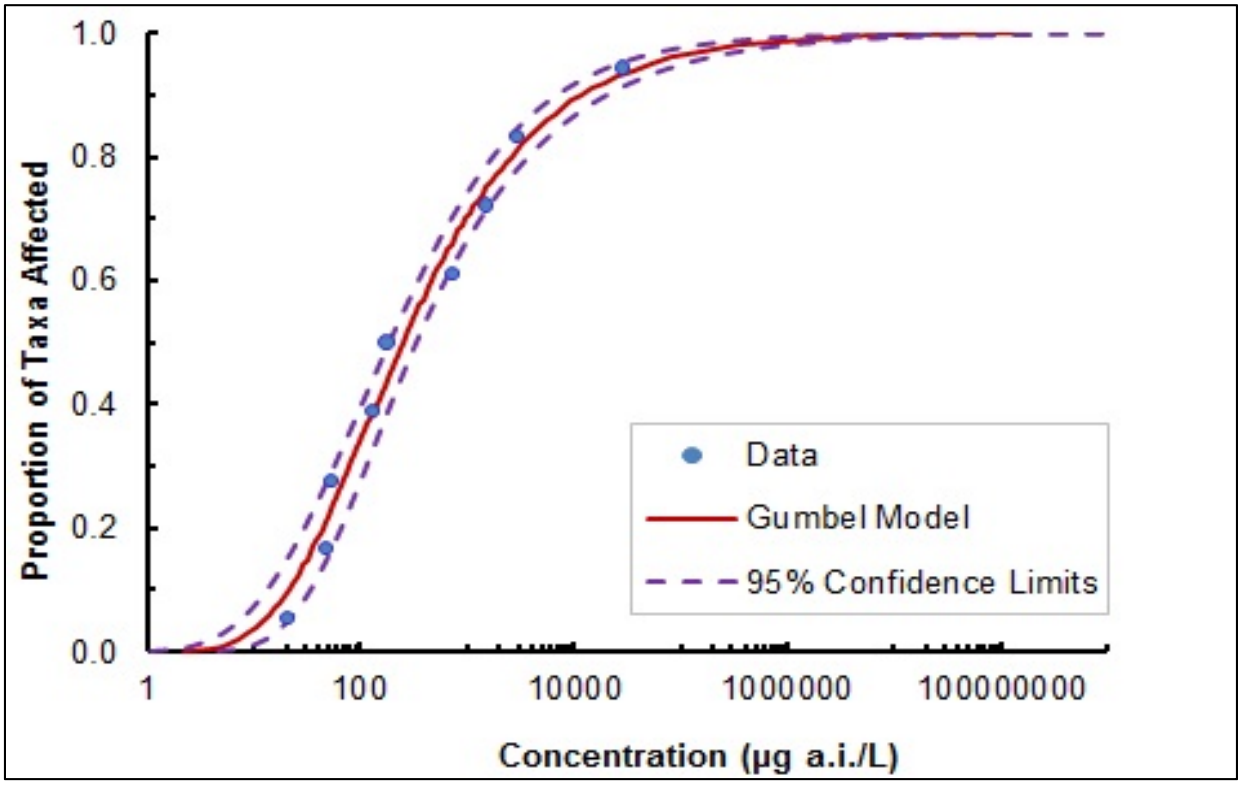

Figure 4-2 **Acute SSD with approximate 95% confidence limits for fish species exposed to malathion**

Probit slopes for all 96-hour LC50s included in the fish SSD were calculated in SAS Software® (SAS Version 9.3, SAS/STAT 12.1) and are presented in Table 4-15 Using the most conservative slope of 2.81 for the sheepshead minnow and the HC5 of 12.3 µg a.i./L, the concentration that would result in a chance of one in a million (0.0001%) of causing mortality to fish was estimated to be 0.250 µg a.i./L. The concentration resulting in indirect effects causing 10% mortality to fish was estimated to be 4.3 µg a.i./L (Table 4-16).

Cheminova recommends that the 96-hour LC50 of 4,700 µg a.i./L for the African clawed frog (*Xenopus laevis*) (Palmer et al., 2011a [MRID 48409302]) be used as the effect concentration for assessing acute risk to aquatic-phase amphibians (Table 4-16). This is a GLP study conducted with technical grade malathion and is the only acute study for aquatic-phase amphibians that was rated acceptable based on Cheminova's study evaluation criteria. Using a probit slope of 6.78, the concentration that would result in a chance of one in a million (0.0001%) of causing mortality to aquatic-phase amphibians was estimated to be 935 µg a.i./L (Table 4-16). The indirect effects thresholds representing 10% probability of effect was 3,040 µg a.i./L.

Cheminova's Chronic Threshold Values

Cohle (1989 [MRID 41422401]) is a GLP study and was rated as supplemental based on Cheminova's study evaluation criteria because this study used technical malathion produced by the American Cyanamid Company. Cheminova is aware of the purity and impurity profile of the malathion used in American Cyanamid studies (see Hillwalker and Reiss, 2014 [MRID 49316501]). The rainbow trout 97-day early life stage NOEL for direct effects to fry survival of 21 µg a.i./L for rainbow trout (*Oncorhynchus mykiss*) reported by Cohle (1989 [MRID 41422401]) is the only NOEL from an acceptable or supplemental early life stage toxicity test in freshwater fish and should, therefore, be used as the screening-level threshold value to assess chronic risk to fish in freshwater environments (Table 4-16). The LOEL for indirect effects is 44 µg a.i./L.

The 34-day NOEL (fry survival) of 8.2 µg a.i./L for sheepshead minnow (*Cyprinodon variegatus*) (Hurd and Sharpe, 2011 [MRID 48705301]) was the most sensitive NOEL for marine/estuarine fish and should, therefore, be used as the screening-level threshold value for assessing direct chronic risk to fish in marine/estuarine environments (Table 4-16). The LOEL for indirect effects is 16 µg a.i./L.

Cheminova recommends that the 21-day NOEL (growth, survival and development) of ≥320 µg a.i./L for the African clawed frog (*Xenopus laevis*) (Palmer et al., 2011a [MRID 48617501]) be used as the screening-level threshold value for assessing direct chronic risk to aquatic-phase amphibians (Table 4-16). Furthermore, the 21-day NOEL of ≥320 µg a.i./L is the most sensitive endpoint from all chronic studies for aquatic-phase amphibians regardless of study rating. The LOEL for indirect effects is > 320 µg a.i./L. This is a GLP study conducted with Cheminova's technical grade malathion and is the only chronic toxicity study rated acceptable based on Cheminova's study evaluation criteria for aquatic-phase amphibians (Table 4-16).

Table 4-16 Mortality and chronic (sublethal) threshold values recommended by Cheminova for fish and aquatic-phase amphibians contrasted with those selected by EPA (2016a)

Taxon	Recommended by Cheminova				EPA (2016a)			
	Species; Reference(s); Cheminova Study Rating	Effect Concentration from Original Study or SSD (µg a.i./L)	Direct Effect Threshold (µg a.i./L)[a]	Indirect Effect Threshold (µg a.i./L)[b]	Species; Reference(s); Cheminova Study Rating	Effect Concentration from Original Study or SSD (µg a.i./L)	Direct Effect Threshold (µg a.i./L)	Indirect Effect Threshold (µg a.i./L)
Acute (Mortality) Effects								
Aquatic-phase amphibians	African clawed frog (*Xenopus laevis*); Palmer et al., 2011a [MRID 48409302]; **Acceptable**	Mortality LC50 = 4,700 Slope = 6.78	935	3,040	Fish and aquatic-phase amphibians SSD; Multiple studies; **See footnote**[c]	Mortality HC5 = 43.3 Slope = 4.5	3.8	22.5
Freshwater fish	Fish SSD; Multiple studies;	Mortality HC5 = 12.3 Slope = 2.81	0.25	4.3				
Estuarine/ marine fish								
Chronic (Sublethal) Effects								
Aquatic-phase amphibians	African clawed frog (*Xenopus laevis*); Palmer et al., 2011b [MRID 48617501]; **Acceptable**	Growth, Survival, and Development NOEC= ≥320 LOEC= >320	320	320	Sheepshead minnow (*Cyprinodon variegates*); Hansen and Parrish (1977 [E5074]) and Parrish et al. 1977 **Unacceptable**	Mortality (Partial life-cycle test) NOEC = 4 LOEC = 9	4	9
Freshwater fish	Rainbow trout (*Oncorhynchus mykiss*); Cohle, 1989 [MRID 41422401]; **Supplemental**	Fry Survival NOEC= 21 LOEC= 44	21	44				
Estuarine/ marine fish	Sheepshead minnow (*Cyprinodon variegates*); Hurd and Sharpe, 2011 [MRID 48705301]; **Acceptable**	Fry Survival NOEC= 8.2 LOEC= 16	8.2	16				

[a] For animals, direct effect thresholds for acute (mortality) effects represent one-in-one million mortality for either the most sensitive species or 5th centile species (HC5) from a species sensitivity distribution (SSD) and direct effect thresholds for chronic (sublethal) effects represent the most sensitive chronic NOEL.

[b] For animals, indirect effect thresholds for acute (mortality) effects represent 10% probability of effect for either the most sensitive species or 5th centile species (HC5) from a species sensitivity distribution (SSD) and indirect effect thresholds for chronic (sublethal) effects represent the most sensitive chronic LOEL or defensible low effect dose/concentration affecting x% of the test population (ED/ECx).

[c] SSD generated using LC50 values from 52 studies. Cheminova rated 29 of these studies as unacceptable and 12 as acceptable (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]). The remaining 11 studies were not evaluated by Cheminova and EPA (2016a) provided no evaluation. See details in Table 4-1 of this response document.

EPA's Acute Threshold Values

Although fish and aquatic-phase amphibians have very different sensitivities to malathion, these two taxonomic groups were combined for the selection of threshold values by EPA (2016a). Cheminova disagrees with this approach. If suitable toxicity data are available for aquatic-phase amphibians, these taxon-specific data should be applied to assess risk. Similarly, existing EPA guidance indicates that data for under-represented taxa are preferred over surrogate species data, whether the endpoints are more or less sensitive (EPA, 2011b). As indicated above, Cheminova has previously identified acute and chronic malathion toxicity data for the African clawed frog (*Xenopus laevis*) from Palmer et al. (2011a,b [MRID 48409302, 48617501], as reviewed by Breton et al., 2014a [MRID 49333901]). Cheminova suggests that the endpoints from these taxon-specific studies should be applied to assess risk to aquatic-phase amphibians rather than grouping them with fish (see Table 4-16).

For the mortality based threshold, EPA states that they derived an SSD using acute (96 hour) LC50 values for fish and aquatic-phase amphibians from studies that exposed animals to malathion as technical grade active ingredient (TGAI) (see Section 2.4.1 of Chapter 2 of the BE). Per Table 2-3 of Chapter 2 of the BE, this SSD was constructed using 115 endpoints from 52 studies (38 open literature studies identified with an ECOTOX ID, 13 registrant submitted studies identified with an MRID, and one study that was identified with both an MRID and an ECOTOX ID). As noted in Section 4.1.2 of this response document, study evaluations for these studies were largely unavailable in the BE or any of the associated appendices or attachments. None of the registrant submitted studies were reviewed and only four of the 38 open literature studies (representing only 13 of the 115 endpoints in the SSD) were reviewed in Appendix 2-3 of the BE, "Open Literature Review Summaries for Malathion". Furthermore, although EPA states that only studies using TGAI were included in the SSD, 21 of the 115 endpoints used in the SSD are from studies for which the test substance is indicated as 'unknown' in Table 2-3 of Chapter 2 of the BE. Given the importance of study evaluation as outlined in Section 4.1.2 of this response document (including the importance of test material purity), EPA (2016a) does not present sufficient information to suggest that the data relied on in the SSD are of sufficient quality to be used in risk assessment per guidance provided by EPA (2011b), NRC (2013), and Cheminova (Breton et al., 2014a [MRID 49333901]).

The lack of adequate study review (and quality) is also apparent when the studies included in Table 2-3 of the BE are cross-referenced with previous study reviews that have been completed by Cheminova and submitted to EPA (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]). Of the 52 studies relied on in EPA's SSD, there were 29 that Cheminova rated as unacceptable, and 12 rated as acceptable (summarized in Table 4-17 of this response document). Notably, all four of the studies that were reviewed by EPA in Appendix 2-3 of the BE and received a quantitative rating were rated as unacceptable by Cheminova (see Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301] and Table 4-17 of this response document). Due to time constraints, Cheminova was not able to complete data quality evaluations for all 11 studies previously unevaluated by Cheminova. Many of these studies would have failed the

Cheminova study evaluation criteria given that six of the 11 studies were those for which the test material was characterized as 'unknown' by EPA (2016a) (Table 4-17).

Table 4-17 Study classifications assigned by Cheminova (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]) for studies used to derive fish and aquatic-phase amphibian SSD by EPA (2016a)

Rated Acceptable by Cheminova	*Not Evaluated by Cheminova*	*Rated Unacceptable by Cheminova*		
MRID 41174301	E106641	E104561	E14861	E66506
MRID 41252101	E13456	E10763	E15472[a]	E74220
MRID 47540302	E14673	E10764	E16946	E77525
MRID 47540304	E162438	E112921	E17539	E88437
MRID 48998004	E17200	E11334	E20087	E89099
MRID 48998005	E17207	E11888	E20475	E92498
MRID 48998006	E3296	E12047	E4022	E995[a]
MRID 49055701	E5370	E12182	E5074[ab]	MRID 40089001; E6797
MRID 49364101	E89754	E12859	E563	MRID 48078003
MRID 49364102	E93401	E13270	E628[a]	
MRID 49422801	E94525			
MRID 49479003				

[a] Rated Quantitative by EPA

[b] EPA's study review for this study included additional supplemental data from a separate study by the same authors (Parrish, P., E. Dyar, M. Lindberg, C. Shanika, and J. Enos. 1977. Chronic toxicity of methoxychlor, malathion, and carbofuran to sheepshead minnows (*Cyprinodon variegatus*). Ecological Research Series, U.S. EPA, 600/3-77-059 36 p.). This additional material has now been reviewed by Cheminova (see Appendix D) and the study was still found to be of unacceptable quality.

[c] The references for the listed MRIDs and ECOTOX numbers were not provided in the Biological Evaluations and due to time constraints are not listed in our reference section.

EPA's Chronic (Sublethal) Threshold Values

The sublethal threshold values selected for fish and aquatic-phase amphibians relied on a study by Hansen and Parrish (1977 [E5074]), which was supplemented by data from Parrish et al. (1977) (see Table 2-1 of Chapter 2 of EPA (2016a). Cheminova previously evaluated this study and rated it as unacceptable due to lack of information about the test substance and absence of control result reporting (Breton et al., 2014a [MRID 49333901]). Even with the supplemented data provided in Parrish et al. (1977), Cheminova still finds the study to be of unacceptable quality because the test chemical characterization was insufficient and the conditions of the test system were not fully reported (see Appendix D).

In light of the poor quality data relied on by EPA (2016a) to derive effects thresholds for fish and aquatic-phase amphibians, Cheminova has developed alternative effects thresholds based on the highest quality data identified in Breton et al. (2014a [MRID 49333901]; 2015 [MRID 49692301]). Endpoints were derived following the Interagency Interim Approaches provided by the Federal Agencies (2013). Cheminova's recommended endpoints are contrasted with the endpoints recommended by EPA (2016a) in Table 4-15 of this response document.

In addition to the threshold values summarized in Table 4-15 of this response document, EPA (2016a) also provided a table of most sensitive toxicity values for different effect types for fish and aquatic-phase amphibians for potential use as a refinement in Table 2-2 of Chapter 2 of the

BE. In this table, rather than grouping all fish and aquatic-phase amphibians together, separate endpoints were provided for freshwater fish, estuarine/marine fish, all fish, and aquatic-phase amphibians. The SSDs referenced in this table were drawn from the same dataset used to derive the overall fish and aquatic-phase amphibians SSD and are, therefore, of limited data quality, as discussed above. The studies relied on as 'most sensitive endpoints' are listed in Table 4-18 of this response document and included four open literature studies and one registrant submitted study. Three of the open literature studies were reviewed by EPA in Appendix 2-3 of the BE and were all rated as 'Quantitative' (Table 4-18). An evaluation of data quality for the fourth study (Beauvais et al., 2000) and the registrant-submitted study (Palmer et al., 2011 [MRID 48617506]) were not provided in the BE. Cheminova conducted study evaluations for two of the open literature studies (Hermanutz 1978 [MRID 48078002]; Hansen and Parrish, 1977 [E5074]) and found them unacceptable (Breton et al., 2014a [MRID 49333901]; Appendix D). Hermanutz (1978 [MRID 48078002]) (incorrectly reported as 1977 in Chapter 2 of the BE) did not provide the source of the test material. Moreover, the impurity profile for the test substance is not known. For these reasons, Hermanutz (1978 [MRID 48078002]) was rated unacceptable based on Cheminova's study evaluation criteria and would also be considered invalid based on OPP's evaluation guidelines (EPA, 2011b). Although a formal evaluation was not conducted by Cheminova for Brewer et al. (2001 [E65887]) and Beauvais et al. (2000), these studies reported a behavioural based endpoint (swimming) and, therefore, would not pass Cheminova's study evaluation criteria due to a lack of proven relationship between the reported endpoint and survival, growth, or reproduction (see Section 4.1.3). Moreover, the Beauvais et al. (2000) study is listed in the rejected ECOTOX list in appendix 2-5 of the BE.

Table 4-18 Study classifications assigned by Cheminova for studies relied on as 'most sensitive endpoints' for fish and aquatic-phase amphibians for potential use as a refinement in Table 2-2 of Chapter 2 of EPA (2016a)

Rated Acceptable by Intrinsik	***Not Evaluated by Intrinsik***	***Rated Unacceptable by Intrinsik***
MRID 48617506	E65887[a,b]	E995/ MRID 48078002[b]
	Beauvais et al. 2000[a]	E5074[b,c]

[a] Although not formally accessed for data quality by Cheminova, these studies would be rated unacceptable because the reported behavioural based endpoint (swimming) could not be linked to survival, growth, or reproduction.
[b] Rated Quantitative by EPA
[c] EPA's study review for this study included additional supplemental data from a separate study conducted by the same authors (Parrish, P., E. Dyar, M. Lindberg, C. Shanika, and J. Enos. 1977. Chronic toxicity of methoxychlor, malathion, and carbofuran to sheepshead minnows (*Cyprinodon variegatus*). Ecological Research Series, U.S. EPA, 600/3-77-059 36 p.).

4.2.2 Aquatic Invertebrates

Cheminova's Acute Threshold Values

The number of acute toxicity studies available for freshwater invertebrates was limited. As a result, acute toxicity studies for estuarine/marine invertebrates (i.e., 48- or 96-hour EC50s for eastern oyster, grass shrimp and mysid shrimp) were included in the acute SSD dataset. In the case of malathion, there is no reason to believe that freshwater invertebrates are more sensitive than estuarine/marine invertebrates as the sensitivity ranges of freshwater and estuarine/marine

invertebrate species overlaps. The LC/EC50 range for freshwater species from acceptable studies conducted with technical malathion is 0.70 µg a.i./L to >35,000 µg a.i./L, whereas the range for estuarine/marine species is 7.8 to >7500 µg a.i./L. Therefore, it is reasonable to combine freshwater and estuarine/marine aquatic invertebrate species to derive the acute SSD. Study evaluations for all data included in the acute aquatic invertebrate SSD can be found in Breton et al. (2014a [MRID 49333901]; 2015 [MRID 49692301]).

The acceptable acute toxicity data used to derive the acute SSD are presented in Table 4-19.

Table 4-19 Acceptable toxicity data for technical malathion used to develop the acute SSD for aquatic invertebrates

Freshwater or Estuarine/ Marine Invertebrate	*Common Name*	*Scientific Name*	*Endpoint*	*Value (µg a.i./L)*	*Probit Slope*[a]	*Reference [MRID]*
Freshwater	Amphipod	*Gammarus pseudolimnaeus*	48-h LC50	4.8	21.1[b]	Brougher et al., 2014e [49389402]
Freshwater	Crayfish	*Procambarus clarkia*	48-h LC50	>35,000	NC	Brougher et al., 2014f [49534901]
Freshwater	Grass shrimp	*Palaemonetes pugio*	48-h LC50	>162	3.56	Brougher et al., 2014g [49534902]
Freshwater	Mayfly	*Centroptilum triangulifer*	48-h LC50	23	10[c]	Brougher et al., 2014h [49479001]
Freshwater	Midge	*Chironomus dilutus*	48-h LC50	3.5	8.4[c]	Brougher et al., 2014i [49479002]
Freshwater	Water flea	*Daphnia magna*	48-h EC50 (immobilization)	0.70	2.99	Gries and Purghart, 2001c [47540303]
Estuarine/ Marine	Eastern oyster	*Crassostrea virginica*	96-h LC50	>7500[d]	NC	Brougher et al., 2014j [49389403]
Estuarine/ Marine	Mysid	*Americamysis bahia*	48-h LC50	7.8	40.5[b]	Brougher et al., 2014k [49389401]

NC: not calculated.

[a] Probit slopes calculated in SAS Version 9.3, SAS/STAT 12.1 unless otherwise indicated.

[b] Partial mortality was only observed for one test concentration in the amphipod and mysid datasets. European commission guidance recommends that at least two partial mortalities be observed in order to use Probit analysis for the calculation of an EC/LC50 (EC, 2003).

[c] Author-reported probit slope.

[d] No effects on mortality were observed at the highest test concentration of 7500 µg a.i./L in the eastern oyster shell deposition study (Brougher et al., 2014k [MRID 49389403]).

The acute SSD was generated using SSD Master v3.0 (Rodney et al., 2013). The crayfish was the most tolerant species tested and had a qualified 48-hour LC50 of > 35,000 µg a.i./L (Brougher et al., 2014f [MRID 49534901]). Furthermore, only 30% mortality was observed at the highest concentration tested. The eastern oyster was the second most tolerant species (Brougher et al., 2014j [MRID 49389403]) and a different endpoint (shell deposition) was examined in this study than in all other acceptable aquatic invertebrate studies. There is uncertainty in the LC50 estimates for the crayfish and eastern oyster, therefore, the truncation option in SSD Master was used to exclude the crayfish and eastern oyster endpoints from the model fit. This option allows for the exclusion of tolerant taxa when fitting the models, while the observed proportion of taxa affected remains true to the full dataset (Rodney et al., 2013). A qualified 48-hour LC50 of >162 µg a.i./L was also obtained for the grass shrimp (*Palaemonetes pugio*) (Brougher et al., 2014g [MRID 49534902]). However, 50% mortality was observed at the

highest test concentration. The probit analysis performed by Brougher et al. (2014g [MRID 49534902]) on the grass shrimp data was replicated by Intrinsik and an LC50 of 174 µg a.i./L was obtained. However, since this value is extrapolated beyond the tested concentrations, the more conservative 48-hour LC50 reported by Brougher et al. (2014g [MRID 49534902]) was included in the SSD.

The Gumbel model using log LC50 and EC50 values was the best-fitting model according to the Anderson-Darling (AD) goodness-of-fit test statistic (A^2 = 0.340) and various graphical plots of model residuals (e.g., p-p and q-q plots). The gumbel model equation is shown above (Equation 4-1). The fitted model parameters were: *s* = 1.09 and *L* = 0.877 (Figure 4-3) for acute toxicity data reported in µg/L. The HC5 (concentration that will affect 5% of species in the SSD) from the acute SSD is 0.476 µg/L.

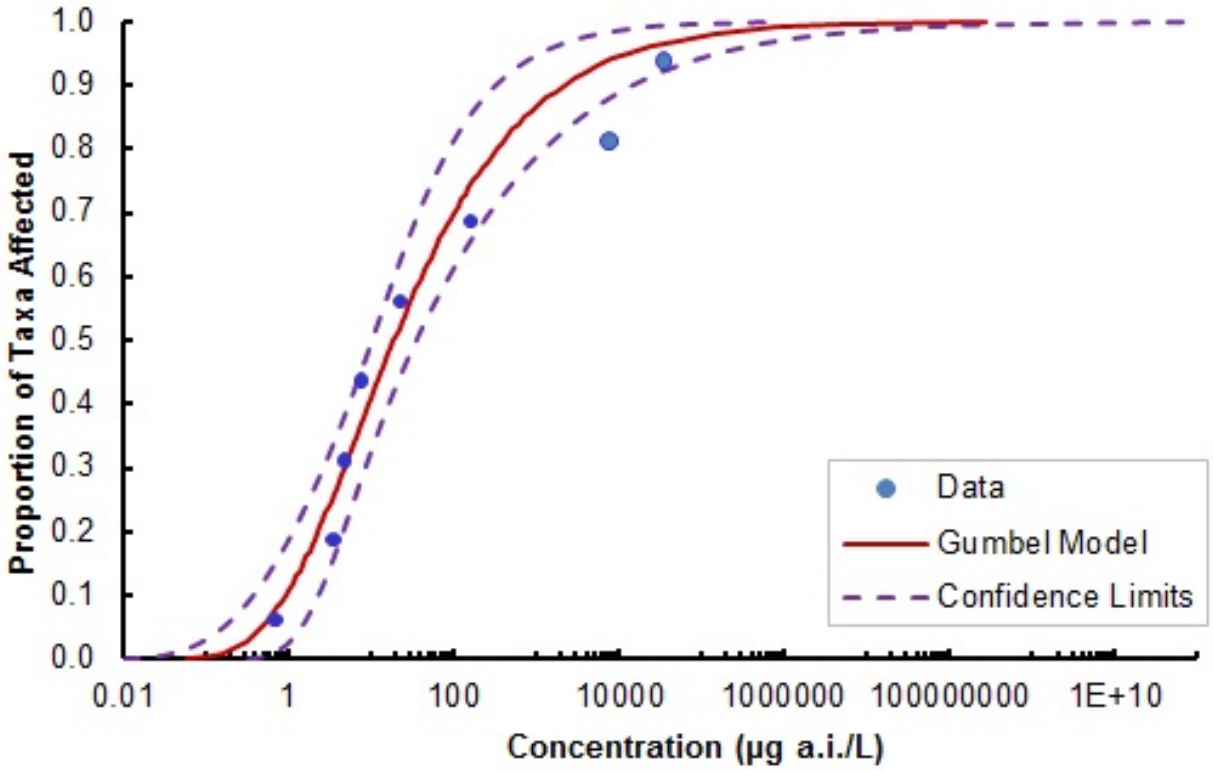

Figure 4-3 Acute SSD with approximate 95% confidence limits for aquatic invertebrate species exposed to malathion

Probit slopes for the 48-hour EC50/LC50s included in the aquatic invertebrate SSD are presented in Table 4-19. Brougher et al. (2014h,i [MRID 49479001, 49479002]) reported probit slopes for 48-hour mayfly and midge toxicity data reported in units of µg a.i./L. All other probit slopes were calculated using SAS Software® (SAS Version 9.3, SAS/STAT 12.1). Probit slopes were not calculated for the crayfish and Eastern oyster because these endpoints were truncated from the SSD. Using the HC5 of 0.476 µg a.i./L and the most conservative probit slope of 2.99 for *Daphnia magna*, the concentration that would result in a chance of one in a million

(0.0001%) of causing direct mortality to aquatic invertebrates was estimated to be 0.0122 µg a.i./L. The concentration resulting in indirect effects causing 10% mortality to freshwater invertebrates was estimated to be 0.177 µg a.i./L (Table 4-20).

Cheminova's Chronic Threshold Values

As per the Interagency Interim Approaches (Agencies, 2013), a NOEL and LOEL for growth and reproduction should be used as the chronic threshold values for direct and indirect effects, respectively. Since acceptable chronic studies were not available for freshwater invertebrates, the 21-day NOEL and LOEL (survival, growth and reproduction) of 0.06 and 0.10 µg a.i./L for the water flea (*Daphnia magna*), respectively, reported by Blakemore and Burgess (1990 [MRID 41718401]) should be used as the screening-level threshold values for assessing chronic risk to freshwater invertebrates in Step 1 (Table 4-20).

The 39-day NOEL and LOEL (reproduction) of 0.29 and 0.58 µg a.i./L for mysid (*Americamysis bahia*) (Claude et al., 2012 [MRID 48752901]), respectively, were the most sensitive chronic endpoints for marine/estuarine invertebrates and should, therefore, be used as the screening-level threshold values for assessing chronic risk to marine/estuarine invertebrates (Table 4-20). This study was found to be acceptable based on Cheminova's study evaluation criteria.

Table 4-20 Mortality and sublethal threshold values recommended by Cheminova for aquatic invertebrates contrasted with those selected by EPA (2016a)

Taxon	*Recommended by Cheminova*				*EPA (2016a)*			
	Species; Reference(s); Cheminova Study Rating	***Effect Concentration from Original Study or SSD (µg a.i./L)***	***Direct Effect Threshold (µg a.i./L)[a]***	***Indirect Effect Threshold (µg a.i./L)[b]***	***Species; Reference(s); Cheminova Study Rating***	***Effect Concentration from Original Study or SSD (µg a.i./L)***	***Direct Effect Threshold (µg a.i./L)***	***Indirect Effect Threshold (µg a.i./L)***
Acute (Mortality) Effects								
Freshwater invertebrates Estuarine/ marine invertebrates	Aquatic invertebrate SSD; Multiple studies; **See footnote[c]**	Mortality HC5 = 0.476 Slope = 2.99	0.0122	0.177	Aquatic invertebrate SSD; Multiple studies; **See footnote[c]**	Mortality HC5 = 0.39 Slope = 5.14	0.046	0.22
Chronic (Sublethal) Effects								
Freshwater invertebrates	Water flea (*Daphnia magna*); Blakemore and Burgess, 1990 [41718401] **Supplemental**	Survival, Growth, and Reproduction NOEC= 0.06 LOEC= 0.10	0.06	0.10	Caddisfly (*Hydropsyche slossonae);* Tessier et al. (2000, [E65789]) **Unacceptable**	Capture Net Abnormalities/ AChE NOEC= 0.048 LOEC= 0.097	0.048	0.097
Estuarine/ marine invertebrates	Mysid shrimp (*Americamysis bahia*); Claude et al., 2012 [48752901] **Acceptable**	Reproduction NOEC= 0.29 LOEC= 0.58	0.29	0.58				

[a] Effect concentrations for acute exposures represent one-in-one million probabilities of effects. Effect concentrations for chronic exposures represent lowest NOECs.

[b] Effect concentrations for acute exposures represent 10% probability of effect. Effect concentrations for chronic exposures represent lowest LOECs.

[c] SSD generated using LC50 values from 60 studies. Cheminova has rated 26 of these studies as unacceptable, 1 was rated as supplemental, and only 5 as acceptable (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]). The remaining 28 studies have not been evaluated by Cheminova and no evaluation was provided by EPA (2016a). See details in Table 4-4 of this response document.

EPA's Acute Threshold Values

In Section 3.2 of Chapter 2, EPA (2016a) summarizes the threshold values for aquatic invertebrates. For the mortality based threshold, EPA states that they derived an SSD using acute (48 and 96 hours) LC50 values from studies using TGAI only. Per Table 3-3 of Chapter 2 of EPA (2016a), this SSD was constructed using 161 endpoints from 60[2] studies (53 with an ECOTOX ID number indicative of an open literature source, 6 with MRIDs indicative of registrant submitted studies, and 1 study with both an MRID and ECOTOX reference number). As noted in Section 4.1.2 of this response document, study evaluations for these studies were largely not available in the BE nor were they available in any of the associated appendices or attachments. None of the registrant submitted studies were reviewed and only two of the 53 open literature studies were reviewed in Appendix 2-3 of the BE, "Open Literature Review Summaries for Malathion". As was previously noted for fish and aquatic-phase amphibians, EPA (2016a) does not present sufficient information to suggest that the data relied on in the SSD are of sufficient quality to be used in risk assessment per guidance provided by EPA (2011b), NRC (2013), and Cheminova (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]).

The lack of adequate study review (and quality) is also apparent when the studies included in Table 2-3 of the BE are cross-referenced with previous study reviews that have been completed by Cheminova and submitted to EPA (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]). Of the 60 studies referenced, one was rated as supplemental, 26 were rated as unacceptable, and only five were rated as acceptable (Table 4-21 of this response document). Due to time constraints, Cheminova was not able to complete data quality evaluations for 28 studies. A preliminary comparison between the Cheminova evaluations and the EPA (2016a) endpoints indicates that some data entry errors were made in the compilation of the EPA (2016a) data table. For example, in Table 3-3, EPA (2016a) reports an EC/LC50 of 67,000 µg/L for *Palaemonetes pugio* from MRID 49534902. However, the original study (and the applicable Cheminova study evaluation for that study) indicate that the correct EC/LC50 for this species was 67 µg a.i./L.

[2] The table actually lists 61 studies, but several labeled as MRID 40089001; E6797 appear to be using the incorrect MRID. Based on the ECOTOX ID, this study was mislabeled and should have been identified as MRID 40098001, which is the source of one other endpoint in the table.

Table 4-21 Study classifications assigned by Cheminova (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]) for studies used to derive aquatic invertebrate SSD by EPA (2016a)

Rated Acceptable by Cheminova	*Not Yet Evaluated by Cheminova*		*Rated Unacceptable by Cheminova*		*Rated Supplemental by Cheminova*
MRID 49389401	E126	E94536	E528	E18945	MRID 41474501
MRID 49479001	E5194	E101101	E887	E19281	
MRID 49479002	E5370	E104559	E2280	E20475	
MRID 49534901	E6502	E104624	E2667	E51439	
MRID 49534902	E6793	E118362	E4139	E56989	
	E7119	E120900[a]	E5182	E59962	
	E16752	E121216	E6449	E73331	
	E20421	E151495	E6665	E92616	
	E67777	E152279	E7775	E95923	
	E71060	E153560	E7917	E96171	
	E73317	E153561	E13513	E104603	
	E80724	E158065	E14346	E156795	
	E89498	E160217	E17860[a]	MRID 40089001; E6797	
	E89575	E162408			

[a] Rated Quantitative by EPA

[b] The references for the listed MRIDs and ECOTOX numbers were not provided in the Biological Evaluations and due to time constraints are not listed in our reference section.

EPA's Chronic (Sublethal) Threshold Values

The sublethal threshold values selected for aquatic invertebrates relied on a study by Tessier et al. (2000, [E65789]). EPA (2016a) provided a review of this study in Appendix 2-3 of the BE and ranked it as qualitative for some endpoints but quantitative for the endpoint (capture net abnormalities/ AChE) considered as a threshold value. Cheminova's evaluation found this study to be of unacceptable quality because no explicit link was demonstrated between the reported endpoint (capture net abnormalities/ AChE) and standard risk assessment endpoints (i.e., survival, growth, and/or reproduction; see discussion in Section 4.1.33 of this response document) (Appendix D). The level of inhibition must be associated with a clinical sign of toxicity that could affect growth, reproduction or survival. Endpoints used in risk assessment without demonstrating this link are not considered to be biologically significant.

In light of the poor quality data relied on by EPA (2016a) to derive effects thresholds for aquatic invertebrates, Cheminova has developed alternative effects thresholds based on the highest quality data identified in Breton et al. (2014a [MRID 49333901]; 2015 [MRID 49692301]). Endpoints were derived following the Interagency Interim Approaches provided by the Federal Agencies (2013). Cheminova's recommended endpoints are contrasted with the endpoints recommended by EPA (2016a in Table 4-20 of this response document.

In addition to the threshold values summarized in Table 4-20 of this response document, EPA (2016a) also provided a table of most sensitive toxicity values for aquatic invertebrates for potential use as a refinement in Table 3-2 of Chapter 2 of the BE. The SSDs referenced in this table were drawn from the same dataset used to derive the SSD for threshold values and are, therefore, of limited data quality, as discussed above. The studies relied on as 'most sensitive

endpoints' are listed in Table 4-22 of this response document and included four open literature studies and two registrant submitted studies. EPA (2016a) did not provide a study evaluation for the two registrant-submitted studies (Blakemore and Burgess, 1990 [MRID 41718401]; Claude et al. [MRID 48752901] (incorrectly cites as MRID 4875290). Cheminova categorized these studies as supplemental and acceptable, respectively, for risk assessment (Breton et al., 2014a [MRID 49333901]). EPA (2016a) did not evaluate the quality of Akarte et al. (1986 [E14269]). Cheminova found this study to be unacceptable for lack of proper chemical identification and unreported testing conditions (Breton et al., 2014a [MRID 49333901]). The last three studies, Cothran et al. (2010 [E120900]) (incorrectly cited as published in 2009), Tessier et al. (2000 [E65789]) and Wendel and Smee (2009 [E119266]), although classified as either qualitative or quantitative by EPA, were all classified as unacceptable by Cheminova for reasons elaborated in Appendix D and Breton et al. (2014a [MRID 49333901]).

Table 4-22 Study classifications assigned by Cheminova for studies relied on as 'most sensitive endpoints' for aquatic invertebrates for potential use as a refinement in Table 3-2 of Chapter 2 of EPA (2016a)

Rated Acceptable by Intrinsik	*Not Evaluated by Intrinsik*	*Rated Unacceptable by Intrinsik*
MRID 41718401	-	E120900[a]
MRID 48752901	-	E65789[a]
	-	E119266[a]
	-	E14269

[a] Rated Quantitative or Qualitative by EPA

4.2.3 Aquatic Plants

Cheminova's Threshold Values

For vascular plants, Cheminova recommends using the EC05 (biomass, yield) of 8,134 µg a.i./L for direct effects on Duckweed (*Lemna gibba*) (Dobbins et al., 2012a [MRID 48998003]), a GLP study rated acceptable based on Cheminova's study evaluation criteria. The LOEC for indirect effects from this study is 17,270 µg a.i./L (Table 4-23).

The recommended NOEC and LOEC (biomass, cell density, yield) for direct and indirect effects to freshwater non-vascular plants (*Pseudokirchneriella subcapitata*) is 635 µg a.i./L (Dobbins et al., 2012b [MRID 48963311]). This is a GLP study conducted with Cheminova's technical grade malathion and was rated acceptable based on Cheminova's study evaluation criteria. The study conducted on the Marine diatom (*Skeletonema costatum*) is recommended to assess marine non-vascular plants (Dobbins et al., 2012c [MRID 48998002]). Because the test concentrations were not maintained throughout the test and the test endpoints were calculated using the nominal test concentrations, this study was rated as supplemental based on Cheminova's study evaluation criteria. The EC05 (cell yield) for direct effects is 273 µg a.i./L, while for indirect effects the EC25 of 1,549 µg a.i./L is recommended (Table 4-23).

EPA's Threshold Values

EPA (2016a) relied on a single study by Yeh and Chen (2006 [MRID 48078001, E85816]) to derive threshold values for aquatic plants. EPA (2016a) provided a review of this study in Appendix 2-3 of the BE and rated it as quantitative. However, based on the OLRS for this study, it appears that EPA's review was conducted in April of 2010, which would have been prior to the release of EPA's 2011 guidelines for evaluating study quality (EPA, 2011b). Cheminova has previously reviewed this study and ranked it as unacceptable due to a lack of information about the methods, test item purity, and control performance (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]).

In light of the poor quality data relied on by EPA (2016a) to derive effects thresholds for aquatic plants, Cheminova has developed alternative effects thresholds based on the best available scientific data identified in Breton et al. (2014a [MRID 49333901]; 2015 [MRID 49692301]). Endpoints were derived following the Interagency Interim Approaches provided by the Federal Agencies (2013). Cheminova's recommended endpoints are contrasted with the endpoints recommended by EPA (2016a) in Table 4-23 of this response document.

EPA (2016a) has selected the study conducted by Dobbins et al. (2012 [MRID 48998003]) as a sensitive toxicity value for potential use as a refinement in Table 4-2 of Chapter 2 of the BE. Although EPA did not conduct a data quality evaluation of this study, Cheminova agrees with the use of this study for risk assessment and rated this study acceptable based on Cheminova data quality criteria (Breton et al., 2014a [MRID 49333901]). This is a GLP study conducted with Cheminova's technical grade malathion and is the only freshwater vascular plant study rated acceptable based on Cheminova's study evaluation criteria. As described above, this study should be used as a threshold value to differentiate between the sensitivity of vascular and non-vascular plants.

Table 4-23 Threshold values recommended by Cheminova for aquatic plants contrasted with those selected by EPA (2016a)

Taxon	*Recommended by Cheminova*				*EPA (2016a)*			
	Species; Reference(s); Cheminova Study Rating	***Effect Concentration from Original Study or SSD (µg a.i./L)***	***Direct Effect Threshold (µg a.i./L)[a]***	***Indirect Effect Threshold (µg a.i./L)[b]***	***Species; Reference(s); Cheminova Study Rating***	***Effect Concentration from Original Study or SSD (µg a.i./L)***	***Direct Effect Threshold (µg a.i./L)***	***Indirect Effect Threshold (µg a.i./L)***
Vascular aquatic plants	Duckweed (*Lemna gibba*); Dobbins et al., 2012a [MRID 48998003] **Acceptable**	Biomass Yield[c,d] EC05=8,134 LOEC=17,270	8,134	17,270	Green algae (*Pseudokirchneriella subcapitata*); Yeh and Chen (2006 [MRID 48078001, E85816]) **Unacceptable**	Oxygen Production NOEC=500 LOEC=1200	500	1200
Freshwater non-vascular aquatic plants	Freshwater algae (*Pseudokirchneriella subcapitata*); Dobbins et al., 2012b [MRID 48963311] **Acceptable**	Biomass, Cell Density, Yield[c] NOEC=<635 LOEC=635	635	635				
Marine non-vascular aquatic plants	Marine diatom (*Skeletonema costatum*); Dobbins et al., 2012c [MRID 48998002] **Supplemental**	Cell Yield[d] EC05=273 EC25=1,549	273	1,549				

[a] For aquatic or terrestrial plants, direct effect thresholds represent the most sensitive NOEL or EC05 (whichever is lower).
[b] For aquatic or terrestrial plants, indirect effect thresholds represent the most sensitive LOEL or EC25 (whichever is lower).
[c] NOEC and LOECs for aquatic plants and algae were based on mean measured concentrations, rather than the day 0 measured concentrations reported by Dobbins et al. (2012a,b [MRIDs 48998003 and 48963311]). For details, see Appendix F - Aquatic Plant Effects Metric Calculations of this response document.
[d] EC05 and EC25 values were calculated as described in Appendix F - Aquatic Plant Effects Metric Calculations of this response document.

4.2.4 Aquatic Communities

Section 5 of Chapter 2 of EPA (2016a) discusses effects on aquatic community (e.g., mesocosm studies that evaluate effects on aquatic invertebrates, aquatic plants, and aquatic-phase amphibians). In this section, EPA (2016a) provides summaries of 11 distinct aquatic community studies. However, full references are available in Appendix 2-2 of the BE for only five of the 11 studies (Table 2-24).

Table 4-24 Available field and mesocosm studies for malathion

Reference Citation in Section 5 of Chapter 2	*Full Reference, if provided*
Groner and Relyea, 2011, (E159029)[a]	Groner, M.L. and R.A. Relyea. 2011. A Tale of Two Pesticides: How Common Insecticides Affect Aquatic Communities. Freshw. Biol. 56(11):2391-2404.
Relyea and Diecks, 2008 (E118292)	Relyea, R.A. and N. Diecks. 2008. An Unforeseen Chain of Events: Lethal Effects of Pesticides on Frogs at Sublethal Concentrations Ecol. Appl. 18(7): 1728-1742.
Relyea 2009 (E114296)	Relyea, R.A. 2009. A Cocktail of Contaminants: How Mixtures of Pesticides at Low Concentrations Affect Aquatic Communities Oceanologia (Wroc.) 159:363-376.
Nataraj and Krishnamurthy, 2012: E158899	Nataraj, M.B. and S.V. Krishnamurthy. 2012. Effects of Combinations of Malathion and Cypermethrin on Survivability and Time of Metamorphosis of Tadpoles of Indian Cricket Frog (Fejervarya limnocharis) J. Environ. Sci. Health Part B: Pestic. Food Contam. Agric. Wastes 47(2): 67-73.
Sweilum, 2006 (E92183)[a]	Sweilum, M.A. 2006. Effect of Sublethal Toxicity of some Pesticides on Growth Parameters, Haematological Properties and Total Production of Nile Tilapia (Oreochromis niloticus L.) and Water Quality of Ponds Aquac. Res. 37(11): 1079-1089.
Conte and Parker (Texas A&M University, 1975)	Not provided
Proctor, Corliss, and Lightner, 1966	Not provided
Tagatz (1974)	Not provided
(Kennedy and Walsh, USFWS, 1970	Not provided
Finlayson, B.J., G. Faggella, H. Jong, E. Littrell, and T. Lew, 1981	Not provided
Kuhajda, B.R. et al, Dept. Of Biological Sciences, University of Alabama, 1996	Not provided

[a] Study not previously identified by Cheminova, but now included in Appendix G of this response document and in the summary below.

Cheminova was able to identify two of the missing references, namely:

- Tagatz, M.E., P.W. Borthwick, G.H. Cook and D.L. Coppage. 1974. Effects of ground applications of malathion on salt-marsh environments in northwestern Florida. Mosquito News 34(30):309-315.
- Kuhajda, B.R., C.C. Blanco, M.M. Green, C.G. Haynes, M.B. Hicks, D.B. Jones III, R.L. Mayden, A.M. Miller, G.A. Nichols and H.E. Smith-Somerville. 1996. Impact of Malathion on Fish and Aquatic Invertebrate Communities and on Acetylcholinesterase Activity in Fishes within Stewart Creek, Fayette County, Alabama. Unpublished study performed by

the Department of Biological Sciences, University of Alabama, for the US Department of Agriculture. [MRID 47587601].

EPA has overlooked many field and mesocosm studies, studies that were identified in Breton et al. 2015 ([MRID 49692301]). These studies are identified below:

Balcom, N.C. and A.O. Clemetson. 2006. Lobster resource failure in Long Island Sound, Fisheries Extension, and Litigation. Fisheries. 31(6):276-284.

Brogan, W.R. III, and R.A. Relyea. 2015. Submerged macrophytes mitigate direct and indirect insecticide effects in freshwater communities. PLoS ONE 10(5): e0126677.

Crane, M., P. Delany, S. Watson, P. Parker, C. Walker. 1995. The effect of malathion 60 on *Gammarus pulex* (L.) below watercress beds. Environ Toxicol Chem 14(7):1181-1188.

Ebke, K.P. 2002. Evaluation of Direct and Indirect Effects of a 440 g/L EW Formulation of Malathion on Aquatic Organisms in Outdoor Ponds. Unpublished study performed by Covance Laboratories GmbH, Münster, Germany, Report No. 1754-0676-019, for Cheminova A/S. CHA Doc No. 379 FYF. [MRID 46525901].

Giles, R.H. Jr. 1970. The ecology of a small forested watershed treated with the insecticide malathion: S35. Wildlife Monogr 24:3-81. [MRID 00058820].

Jensen, T., S.P. Lawler and D.A. Dritz. 1999. Effects of Ultra-low Volume Pyrethrin, Malathion, and Permethrin on Nontarget Invertebrates, Sentinel Mosquitoes, and Mosquitofish in Seasonally Impounded Wetlands. J Am Mosquito Contr 15(3):330-338.

Halstead, N.T., T.A. McMahon, S.A. Johnson, T.R. Raffel, J.M. Romansic, P.W. Crumrine, and J.R. Rohr. 2014. Community ecology theory predicts the effects of agrochemical mixtures on aquatic biodiversity and ecosystem properties. Ecology Letters 17(8):932-941.

Hua, J., and R.A. Relyea. 2012. East Coast vs West Coast: effects of an insecticide in communities containing different amphibian assemblages. Freshwater Science. 31(3):787-799.

Hua, J., and R. Relyea. 2014. Chemical cocktails in aquatic systems: Pesticide effects on the response and recovery of >20 animal taxa. Environ Poll 189:18-26.

Miller, R.E.L., J.R. Wands, K.N. Chytalo, and R.A. D'Amico. 2005. Application of water quality modeling technology to investigate the mortality of lobsters (*Homarus americanus*) in Western Long Island Sound during the summer of 1999. Journal of Shellfish Research. 24(3): 859-864.

Pearce, J. and N. Balcom. 2005. The 1999 Long Island Sound lobster mortality event: Findings of the comprehensive research initiative. Journal of Shellfish Research. 24(3): 691-697.

Relyea, R.A. 2005. The Impact of Insecticides and Herbicides on the Biodiversity and Productivity of Aquatic Communities. Ecological application 15(2):618-627.

Shrestha S.B., S. Jha, and S.K. Wagle. 1987. The Effects of Organophosphate Insecticides in Nursery Ponds. http://www.fao.org/docrep/field/003/AC199E/AC199E00.htm. September 1987. Accessed November 25, 2014.

Wilson, R.E., H. Crowley, B. Brownawell and R.L. Swanson. 2005. Simulations of transient pesticide concentrations in Long Island Sound for late summer 1999 with a high resolution coastal circulation model. 1. Shellfish Res 24(3):865-875.

A summary of all available studies is provided below. These studies indicate that malathion do not cause significant risk to aquatic populations, and populations recover quickly when exposure has ceased.

Field Study Summary

Five field studies evaluated the effects of malathion applied at rates ranging from 0.051 to 1 lb/A on aquatic ecosystems, particularly wetlands and watercress beds (Giles, 1970 [MRID 00058820]; Jensen et al., 1999; Tagatz et al., 1974; Kuhajda et al., 1996 [MRID 47587601]; Crane et al., 1995). These studies reported peak concentrations ranging from 0.34 to 8500 µg/L following malathion spraying (concentrations exceeding the EECs predicted by PRZM/EXAMS and PFAM in the screening-level assessment), but observed no persistent effects on the abundance and survival of fish or aquatic invertebrate communities. In particular, Kuhajda et al. (1996 [MRID 47587601]) measured peak malathion concentrations ranging from <LOD to 10.89 µg/L in 1993 and from 4.34 to 31.10 µg/L in 1994, but did not observe any significant effects on species abundance, number of taxa or diversity for fish or aquatic invertebrate communities over the three-year study. Although Kuhajda et al. (1996 [MRID 47587601]) observed a significant reduction in brain AChE in rough shiners (*Notropis baileyi*), the biochemical change did not have a significant effect on the survival of the shiners.

The reduction of lobsters in Long Island Sound as described in (Pearce and Balcom, 2005) was evaluated through two independent modeling exercises (Miller et al., 2005; Wilson et al., 2005). In both cases, estimated exposure concentrations of malathion in the Sound were lower than the lowest toxicity value available for adult lobsters. Moreover, all available lobster toxicity studies were rated as unacceptable by Cheminova because they did not follow standard testing guidelines and did not provide enough details to fully replicate the studies. Recent studies have failed to demonstrate a link between malathion exposure and the decline in lobster populations. Those populations have continued to decline in the absence of detectable concentrations of pesticides which bolsters the belief that warming temperatures is the main culprit (CDEEP, 2012; Collins, 2016; Middletown Press, 2016).

Mesocosm Summary

Ten mesocosm studies are available that studied the effects of malathion on aquatic communities (Ebke, 2002 [MRID 46525901]; Relyea, 2005, 2009 [MRID 48261129]; Sweilum, 2006; Groner and Relyea, 2011; Hua and Relyea, 2012, 2014; Halstead et al., 2014; Brogan and Relyea, 2015; Shrestha et al., 1987). Studies attempted to quantify effects to fish, aquatic-phase amphibians, periphyton, phytoplankton, macrophytes, zooplankton and macroarthropods. Test concentrations ranged from 0.6 to 2000 µg/L. Effects on cladocerans, the most sensitive taxon tested, were observed at concentrations ≥5.8 µg/L (Ebke, 2002 [MRID 46525901]; Relyea, 2005, 2009 [MRID 48261129]; Groner and Relyea, 2011; Hua and Relyea, 2012, 2014; Halstead et al., 2014; Shrestha et al., 1987). The effects observed on cladocerans are not unexpected, given that the acceptable 48-h LC50 for the water flea (*Daphnia magna*) is 0.70 µg a.i./L (Gries and Purghart, 2001a [MRID 47540303]). However, when measured, recovery of cladoceran populations occurred within eight days to seven weeks of when effects were originally observed (Ebke, 2002 [MRID 46525901]; Hua and Relyea, 2014; Brogan and Relyea, 2015; Shrestha et al., 1987). Other aquatic invertebrate taxa (e.g., copepods, rotifers, snails, amphipods, isopods, and insects) were considerably less sensitive to malathion. Reductions in copepod, amphipod and insect abundance were only observed in response to malathion exposures ≥40 µg/L (Relyea, 2005; Hua and Relyea, 2014; Shrestha et al., 1987). Copepod populations recovered within two weeks of the original effects being observed (Hua and Relyea, 2014; Shrestha et al., 1987). The study designs did not allow for measurement of recovery in amphipod and insect populations (Relyea, 2005; Hua and Relyea, 2014). Aquatic-phase amphibian survival was decreased for concentrations ≥ 3.1 µg/L (Groner and Relyea, 2011). Fish exposed to malathion at concentrations ≥500 µg/L had increased mortality and altered blood and muscle structure but these results are questionable due to poor experimental design and execution (Sweilum, 2006). Overall, snails, rotifers, isopods, periphyton, phytoplankton and macrophytes were resistant to malathion toxicity.

In addition, Brogan and Relyea (2013a,b; 2014; 2015) conducted microcosm and mesocosm studies demonstrating that malathion toxicity to cladocerans is substantially reduced in the presence of macrophytes. This reduction in toxicity was confirmed to be due to the photosynthetic activity of macrophytes, which increases the pH of the test system, leading to increased degradation of malathion. Furthermore, the initial findings of the microcosm studies (Brogan and Relyea, 2013a,b; 2014) were supported by a recent outdoor mesocosm study (Brogan and Relyea, 2015). Wildlife do not live in the inert environments used in standard toxicity tests, and factors such as pH, temperature, presence of organic matter and shading of water bodies, can influence the degree to which organisms are exposed to and affected by pesticides.

The pH of dilution water used in acceptable toxicity tests ranged from 7.46 to 8.5 for aquatic invertebrates, 6.84 to 8.6 for fish, and 8.0 to 8.2 for aquatic-phase amphibians (Breton et al., 2014a [MRID 49333901]). Although these pH values are representative of aquatic ecosystems in the United States, pH values exceeding 8 are common (Brogan and Relyea, 2014; 2015).

4.2.5 Birds, Reptiles and Terrestrial-phase Amphibians

Cheminova's Acute Threshold Values

Oral gavage exposure does not accurately reflect exposure in the field. In the field, natural dietary matrices, feeding patterns, and metabolism and elimination throughout the day mitigate the exposure. Comparison of an oral gavage metric to a total daily intake value assumes that the effects associated with an oral gavage dose could be achieved if the animal consumes the equivalent of the oral gavage dose over the course of a day. However, this approach will inevitably overstate risks. The fact that dietary LD50s are generally much higher than oral gavage LD50s is attributed to these factors. A possible exception is when the receptor of concern is a gorge-feeder (e.g., migrating Canada geese). In this case, a dietary endpoint from a surrogate non-gorge feeder may not be sufficiently conservative for screening purposes. If acute oral gavage studies are used as effects metrics at the screening level, the conservativeness of such endpoints should be noted and reflected in the risk characterization of the assessment.

Although acute dietary endpoints are more representative of pesticide exposure of birds in the field than oral gavage endpoints, the comparison between acute dietary LC50s and estimated residues on feed items in T-REX does not take into account food availability and food consumption in the wild. Major factors affecting food consumption, such as body weight, metabolic rate and the caloric density of the food are ignored in this approach. With a TDI already estimated in the T-REX model, it would make more sense to convert dietary LC50s to estimated dietary LD50s using the body weights and food intake rates of the test animals, and use these LD50s as effects metrics in the model.

As a conservative screening-level effects metric, Cheminova recommends that the oral gavage LD50 of 136 mg a.i./kg bw for the ring-necked pheasant (*Phasianus colchicus*) (Hubbard and Beavers, 2012a [MRID 48963305]) be used as the effect concentration for assessing acute risk to birds. Using the author-reported slope of 6.55, the chance of one in a million (0.0001%) of causing direct mortality to birds was estimated to be 25.6 mg a.i./kg bw. The concentration used to evaluate indirect effects based on 10% mortality to birds was estimated to be 25.6 mg a.i./kg bw (Table 4-25).

The use of avian toxicity data as a surrogate for herptiles is standard practice in the absence of appropriate data for this receptor group (EPA, 2004a). This extrapolation has little, if any, empirical support. That is, the extrapolation is made out of necessity due to a paucity of herptile toxicity data, not because it is scientifically justified. Birds and herptiles belong to different taxonomic classes, and therefore have different metabolic rates, diets, respiratory and reproductive systems and ecology in general. A comparison of the toxicity of organophosphate pesticides to herptiles and birds shows that birds were always more sensitive to organophosphates than terrestrial-phase amphibians (Appendix H). This suggests that their use as surrogate species for predicting effects to terrestrial-phase amphibians would likely overestimate risk. For reptiles, the most sensitive bird for a given organophosphate (which

would typically be adopted as the surrogate species in a risk assessment) was more sensitive than the most sensitive reptile for the corresponding pesticide in all cases except for phosphamidon. This finding also suggests that the use of surrogate avian receptor toxicity data are likely to result in an overestimate of risk to reptiles. Taxon-specific data should be used when available (EPA, 2011b). Therefore, the acute bullfrog study conducted by Fort (2015 [MRID 49693705]) should be used in the draft BE for malathion.

Cheminova recently commissioned a GLP acute oral toxicity test with adult bullfrog (*Lithobates catesbeianus*; Fort, 2015 [MRID 49693705]). This study was rated acceptable according to Cheminova's study evaluation criteria (Breton et al., 2014a [MRIDs 49333901; 2015 [49692301]). Fort (2015 [MRID 49693705]) estimated a female LD50 of 1,672 mg a.i./kg bw, respectively. Using the author-reported slope of 2.7, the chance of one in a million (0.0001%) of causing direct mortality to herptiles was estimated to be 29 mg a.i./kg bw. The concentration used to evaluate indirect effects based on 10% mortality to herptiles was estimated to be 561 mg a.i./kg bw (Table 4-25).

Cheminova's Chronic (Sublethal) Threshold Values

To assess chronic risk to avian species, EPA selected the chronic 21-week dietary NOEL and LOEL (reproduction) of 110 mg/kg and 350 mg/kg, respectively, diet for the northern bobwhite (*Colinus virginianus*) (Beavers et al., 1995 [MRID 43501501]) in their BE (EPA, 2016a) (Table 4-25). Cheminova is in agreement with EPA on the selection of this endpoint as this is the only chronic avian study rated acceptable based on Cheminova's study evaluation criteria. The study was conducted using GLP, standardized protocols, as well as using Cheminova-produced malathion with known purity and impurity composition. Cheminova calculated estimated daily doses using the measured dietary concentrations, feed consumption data and body weights of the birds (Breton et al., 2014a [MRID 49333901]). For direct effects, the NOEL was 12.6 mg a.i./kg bw/d while for indirect effects the LOEL was 42.8 mg a.i./kg bw/d (Appendix I). Body weight and feed consumption values were averaged over the 21-week exposure period.

Although a supplemental chronic toxicity study was available for terrestrial herptiles exposed to malathion (Holem et al., 2008), this study used an unconventional exposure regime whereby Western fence lizards were administered three oral gavage doses of technical malathion with a 27-day observation period following each dose. Thus, this study was inappropriate for estimating a chronic effects metric (Breton et al., 2015 [MRID 49692301]).

An acute-to-chronic ratio (ACR) was derived using the best available data from other terrestrial vertebrates. The studies used in the ACR calculation were rated either acceptable or supplemental based on Cheminova's study evaluation criteria (Breton et al., 2014a [MRID 49333901]). A literature search was conducted to acquire data to support an ACR for herptiles, with consideration of other chemicals. However, no studies reporting acute and chronic effects for terrestrial-phase herptiles were found.

Using acceptable and supplemental toxicity studies, ACRs for malathion were developed for three vertebrate species: northern bobwhite (*Colinus virginianus*), mallard (*Anas platyrhynchos*), and rat (*Rattus norvegicus*). The ACRs were developed using acute LD50s and chronic NOELs and LOELs (in mg a.i./kg bw/d; Rodgers, 2002 [MRID 48153114]; Beavers et al., 1995 [MRID 43501501]; Hubbard and Beavers, 2012b [MRID 48963307]; Pedersen and Fletcher, 1993 [MRID 42782101]; Moore, 2003 [MRID 48153112]; Schroeder, 1990 [MRID 41583401]). For bobwhite, mallard and rat, the calculated ACRs are 27.4, >20.6 and 5.10, respectively. These ACRs fall within the range of ACRs estimated by Layton et al. (1987) for mammals, which suggests only that these estimates are not wildly different from ACRs developed for other chemicals. ACRs were calculated using acute LD50s and chronic LOELs from the same studies listed above. For bobwhite, mallard and rat, the LOEL-based ACRs are 8.06, >10.2, and 3.28 (Appendix J).

The most conservative NOEL and LOEL-based ACRs of 27.4 and >10.2 were selected for herptiles. When the ACRs are applied to the author-reported LD50 of 1672 mg a.i./kg bw for female bullfrogs (*Lithobates catesbeianus*; Fort, 2015), the estimated chronic NOEL is 61.0 mg a.i./kg bw/d and the chronic LOEL is 164 mg a.i./kg bw/d. These values should be used to assess chronic risk to herptiles in the BE. The ACR-derived NOEL is more conservative than the survival and body weight NOEL of ≥96.5 mg a.i./kg bw obtained from Holem et al. (2008).

Table 4-25 Mortality and sublethal threshold values recommended by Cheminova for birds, terrestrial-phase amphibians and reptiles contrasted with those selected by EPA (2016a)

Taxon	*Recommended by Cheminova*				*EPA (2016a)*			
	Species; Reference(s); Cheminova Study Rating	***Effect Concentration from Original Study or SSD***	***Direct Effect Threshold[a]***	***Indirect Effect Threshold[b]***	***Species; Reference(s); Cheminova Study Rating***	***Effect Concentration from Original Study or SSD***	***Direct Effect Threshold***	***Indirect Effect Threshold***
Acute (Mortality) Effects								
Birds	Ring-necked pheasant (*Phasianus colchicus*); Hubbard and Beavers, 2012a [MRID 48963305]; **Acceptable**	Mortality (Dose-based) LD50 = 136 mg a.i./kg bw Slope = 6.55	25.6 mg a.i./kg bw	86.7 mg a.i./kg bw	Bird SSD; Multiple studies; **See footnote[c]**	Mortality (Dose-based) HC5 = 108 mg a.i./kg bw; Slope 6.6	20.6 mg a.i./kg bw	69 mg a.i./kg bw
					Northern bobwhite (*Colinus virginianus*); Gallagher et al. 2003 [MRID 48153106] **Acceptable**	Mortality (Dietary Concentration) LC50 = 2022 mg/kg diet; Slope 5.74	300 mg/kg diet	1210 mg/kg diet
Herptiles	Bullfrog (*Lithobates catesbeianus*) Fort, 2015 [MRID 49693705] **Acceptable**	Mortality (Dose-based) 1672 mg a.i./kg bw Slope = 2.7	29 mg a.i./kg bw	561 mg a.i./kg bw	Relied on bird threshold values			
Chronic (Sublethal) Effects								
Birds	Northern bobwhite (*Colinus virginianus*); Beavers et al., 1995 [MRID 43501501] **Acceptable**	Reproduction (Dose-based)[d] NOEL = 12.6 mg a.i./kg bw/d LOEL = 42.8 mg a.i./kg bw/d	12.6 mg a.i./kg bw/d	42.8 mg a.i./kg bw/d	Northern bobwhite (*Colinus virginianus*); Beavers et al., 1995 [MRID 43501501] **Acceptable**	Reproduction (Dietary Concentration) NOEL = 110 mg/kg diet LOEL = 350 mg/kg diet	110 mg/kg diet	350 mg/kg diet
					Ring-necked pheasant (*Phasianus colchicus*); Day et al., 1995 [E63276] **Unacceptable[f]**	AChE Inhibition (Dose-based) LOEL = 87.4 mg a.i./kg bw	87.4 mg a.i./kg bw	87.4 mg a.i./kg bw
Herptiles	Bullfrog (*Lithobates catesbeianus*); Multiple studies (ACR approach); **See footnote[e]**	Reproduction (Dose-based) ACR-derived NOEL = 61 mg a.i./kg bw/d ACR-derived LOEL = 164 mg a.i./kg bw/d	61 mg a.i./kg bw/d	164 mg a.i./kg bw/d	Relied on bird threshold values			

[a] For animals, direct effect thresholds for acute (mortality) effects represent one-in-one million mortality for either the most sensitive species or 5th centile species (HC5) from a species sensitivity distribution (SSD) and direct effect thresholds for chronic (sublethal) effects represent the most sensitive chronic NOEL.

[b] For animals, indirect effect thresholds for acute (mortality) effects represent 10% probability of effect for either the most sensitive species or 5th centile species (HC5) from a species sensitivity distribution (SSD) and indirect effect thresholds for chronic (sublethal) effects represent the most sensitive chronic LOEL or defensible low effect dose/concentration affecting x% of the test population (ED/ECx).

[c] SSD generated using LD50 values from 5 studies. Cheminova has rated 3 of these studies as unacceptable and 2 as acceptable (Breton et al., 2015 [MRID 49692301]). See details in Table 4-7 of this response document.

[d] Study reported NOEL and LOEL of 110 mg a.i./kg diet and 350 mg a.i./kg diet, respectively. Effect doses in mg a.i./kg bw/d were calculated by Intrinsik based on the measured dietary concentrations, feed consumption data and body weights of the birds (see Appendix I).

[e] Acute-to-chronic ratios (ACRs) describing the relationship between an acute LD50 and a chronic NOEL and between an acute LD50 and a chronic LOEL were derived using the best available data from other terrestrial vertebrates (Included data of both acceptable and supplemental quality). These ACRs were then applied to the female bullfrog LD50 of 1672 mg a.i./kg bw (Fort, 2015) to estimate a chronic NOEL and a chronic LOEL for terrestrial-phase herptiles. For further details, see Appendix J.

[f] Rated unacceptable in new study reviews completed for this response document. See Appendix D for full study review.

EPA's Acute Threshold Values

In Section 6 of Chapter 2, EPA (2016a) summarizes the toxicity data for birds that were considered in the BE. Additional toxicity data for reptiles and terrestrial-phase amphibians (collectively, herptiles) are presented in Sections 7 and 8 of Chapter 2. EPA (2016a) considered the toxicity data for herptiles to be insufficient for risk assessment and, therefore, concluded that threshold values for birds would be applied as surrogate thresholds to assess risk to herptiles.

Cheminova agrees that the herptile toxicity data presented by EPA (2016a) are of poor quality and are not suitable for risk assessment. For example, the studies reported by EPA (2016a) for reptiles generally observed no effects, except for AChE inhibition and mortality endpoints reported in Hall et al. (1982 [E36970]). Cheminova previously reviewed Hall et al. (1982 [E36970]) and found it to be of unacceptable quality due to the lack of study detail provided (Breton et al., 2015 [MRID 49692301]). Similarly, the data presented by EPA (2016a) for terrestrial-phase amphibians were generally representative of exposure pathways with limited ecological relevance and/or failed to observe significant effects. For example, the studies by Willens (2005 [E89001]) and Taylor et al. (1999 [E89577]) involved dermal exposures followed by a bacterial injection and the study by Kaplan and Glaczenski (1965 [E50823]) exposed organisms directly to liquid malathion in a glass jar.

To address the lack of suitable malathion toxicity data for herptiles, Cheminova has conducted a GLP acute oral toxicity test on the bullfrog (*Lithobates catesbeianus*) (Fort, 2015 [MRID 49693705]). This study has been rated acceptable according to Cheminova's study evaluation criteria (Breton et al., 2015 [MRID 49692301]). Since taxon-specific data are now available, Cheminova does not support using bird toxicity data to assess risk to herptiles. This is also supported by existing EPA guidance, which states that data for under-represented taxa are preferred over surrogate species data (EPA, 2011b). Therefore, Cheminova suggests that data from Fort (2015 [MRID 49693705]) should be applied to assess risk to herptiles rather than grouping herptiles with birds (see Table 4-25of this response document).

The mortality and sublethal thresholds recommended by EPA (2016a) for all birds and herptiles are summarized in Table 4-25 of this response document. For the mortality thresholds, EPA (2016a) proposed separate dose-based (mg a.i./kg bw) and concentration-based (mg a.i./kg diet) endpoints. The dose based threshold was based on an SSD derived using seven endpoints from five studies. None of these studies were evaluated for data quality in Appendix 2-3, "Open Literature Review Summaries for Malathion". However, all 5 studies were evaluated by Cheminova (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]). As noted in Table 4-25 of this response document, three out of these five studies were rated as unacceptable by Cheminova and only two were rated as acceptable. On the basis of the Cheminova study evaluation criteria, there are clearly insufficient data available to generate a dose-based acute SSD for birds. Therefore, Cheminova has recommended a dose-based acute threshold based on the best available scientific data identified in Breton et al., 2014 [MRID 49333901]; 2015 [MRID 49692301]) (see Tbale 4-26).

Table 4-26 Study classifications assigned by Cheminova for studies used to derive dose-based mortality SSD for birds by EPA (2016a)	
Rated Acceptable by Cheminova	***Rated Unacceptable by Cheminova***
MRID 48153114	E36916
MRID 48963305	Crabtree, D.G., 1965, Denver Wildlife Res. Center, USFWS as cited in RED
	MRID 00160000; [E50386]

EPA's Chronic (Sublethal) Threshold Values

The sublethal dose-based threshold recommended by EPA (2016a) was derived from Day et al. (1995 [E63276]), a study that was found to be unacceptable based on Cheminova's study quality criteria due to lack of details regarding study methods and lack of control results (Appendix D). Additionally, the endpoint reported in Day et al. (1995 [E63276]) is based on sublethal AChE inhibition. The suitability of this effect to derive a threshold value is questionable. First of all, inhibition of AChE is not biologically relevant unless it is directly associated with an observed, standard, adverse measure of effect (i.e., survival, growth, or reproduction). Furthermore, studies have shown that AChE inhibition in birds has been found to return to control levels within 24 hours of sublethal doses of malathion (Pym et al., 1984; Mehrotra et al., 1967). In birds, malathion is rapidly absorbed, filtered, and metabolized to non-toxic metabolites then excreted via urine (Cannon et al., 1993 [MRID 42715401]; Gupta and Paul, 1977). In addition, birds have demonstrated avoidance behavior to dietary items treated with malathion. In the laboratory, birds often reduce their feeding rate when exposed to acutely toxic pesticides in their food, particularly organophosphates and carbamates (Kononen et al., 1987; Bennett, 1989; Grue et al., 1997; EFSA, 2005; Fischer et al., 2005; Stafford, 2007; Springborn Smithers Laboratories, 2008). The reduction in feeding rate may be due to: (i) repellent taste or odor (e.g., methiocarb; Kononen et al., 1987), or (ii) post-ingestional toxicity, which is a common mechanism for acetylcholinesterase-inhibiting pesticides (Grue et al., 1997; Fischer et al., 2005). For malathion, avoidance has been noted in the field during field studies. Hill et al. (1971) and McLean et al. (1975) reported that bird populations may emigrate away from or avoid foraging in malathion-treated areas. Further, birds exhibiting sublethal signs of toxicity (e.g., lethargy, ruffled appearance, wing droop, loss of coordination, etc.) following exposure to malathion returned to normal condition within two hours to eight days and did not exhibit gross pathological changes. Clinical findings and necropsies of birds suggest that individuals recover quickly from sublethal toxicity (Breton et al., 2016 (in prep.)). Therefore, the link between this endpoint and apical endpoints (i.e., survival, growth and/or reproduction) is unclear particularly for wild bird species. As noted by NRC (2013), to properly incorporate sublethal effects into an ecological risk assessment, it is necessary to provide an explicit relationship between the sublethal effect in question and apical endpoints (i.e., survival, growth, and/or reproduction).

The sublethal concentration-based threshold recommended by EPA (2016a) was derived from a study identified as MRID 43510501 in Table 0-1 of Section 6.2 of Chapter 2 of the BE. However, in the reference list provided in Appendix 2-4, this MRID refers to a study of malathion/ malaoxon residue in/on winter wheat processed commodities. The correct MRID is provided in Section 6.4.2.2 of Chapter 2 of the BE, which identifies this study as Beavers et al. (1995 [MRID

43501501]). This study was also previously evaluated by Cheminova and was rated as acceptable (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]).

Most sensitive toxicity values for potential use as a refinement are not provided in Chapter 2 for birds.

Section 6.4.3 of Chapter 2 in EPA (2016a) also presents data from field studies for birds. Reference citations were not provided for the following studies:

- Kucera (1987)
- Engbring (1989)
- Parsons and Davis (1971)
- Giles (1970)

At a minimum, an MRID or ECOTOX ID should be made available. Cheminova was able to identify three of these studies (Breton et al., 2015 [MRID 49692301]) including Kucera (1987), Parsons and Davis (1971) and Giles (1970). Other bird field studies that were reviewed by Cheminova (Breton et al., 2015 [MRID 49692301]) but not by EPA (2016a) include Black and Zorb (1966); Custer and Mitchell (1987); George et al. (1995); Bejer-Petersen et al. (1972); Webber (1981); and McEwen and Ells (1975).

EPA (2016a) did not provide an overall summary of the findings of the field studies they reviewed and how they might be integrated into an overall weight of evidence approach. A summary and conclusion of the field studies follows (Breton et al. 2015 [MRID 49692301]).

A number of field studies have evaluated the effects of malathion applied at rates ranging from 0.15 to 1.035 lb/A on resident avian populations (Black and Zorb, 1966; Custer and Mitchell, 1987; George et al., 1995; Giles, 1970 [MRID 00058820]; Bejer-Petersen et al., 1972; Hill et al., 1971; McEwen et al., 1972 [MRID 00058747]; McLean et al., 1975; Webber, 1981; Howe et al., 1996; Kucera, 1987; Norelius and Lockwood, 1999; Parsons and Davis, 1971; Pascual, 1994; McEwen and Ells, 1975). All birds studied were wild and native to the study areas. For the vast majority of studies, no significant differences in bird abundance, survival, reproduction, or AChE activity were observed between treated and control plots. Some authors noted a reduction in insectivorous birds (e.g., horned lark, western meadowlark) following application and attributed the decrease to a reduction in arthropod prey base on the treated field because the reduction was not coupled with a decrease in AChE activity (Norelius and Lockwood, 1999; George et al., 1995). In one case, nestling deaths (coal tits and pied flycatchers) were considered by the authors as treatment-related because a decrease in brain ChE activity was also observed (Bejer-Petersen et al., 1972). However, this study was summarized in a review paper (Habig, 1995 [MRID 43860801]) which provided very few details, and the original report was not available for verification. The weight-of-evidence shows that birds are not being affected by malathion once applied in the field.

Clearly, EPA (2016a) should expand their review of field studies to include those mentioned by Cheminova and should also provide an overall summary that indicates how these field data may be used in a weight of evidence approach.

4.2.6 Mammals

Cheminova's Acute Threshold Values

Cheminova recommends using the oral gavage LD50 of 2,010 mg a.i./kg bw (reported by authors as 2,098 mg test substance/kg bw) for the rat as a conservative screening-level effects concentration for assessing acute risk to mammals (Moore, 2003 [MRID 48153112]) (Table 4-27). This is a GLP study conducted with technical grade malathion and was rated acceptable based on Cheminova's study evaluation criteria. The endpoint is supported by the results of other acceptable and supplemental studies (Fischer, 1991 [MRID 49127004]; Kuhn, 1996 [MRID 49127002]; Kynoch, 1986 [MRID 00159876]; and Terrell et al., 1978 [MRID 00113245]), all of which report higher oral gavage LD50s for mammals. However, as is the case for avian species, dietary exposure estimates are most appropriate for comparing with dietary toxicity data. Oral gavage is an unrealistic route of exposure for mammals and is generally over conservative for reasons previously noted above. Unfortunately, no acceptable subacute dietary studies with mammals were available. Accordingly, we opted to use an oral gavage study in its place, recognizing the added conservatism that comes with this approach. Using the raw data from Moore (2003 [MRID 48153112]), a probit slope of 11.5 was derived using SAS/STAT version 12.1. Using the author-reported slope of 11.5, the chance of one in a million (0.0001%) of causing direct mortality to mammals was estimated to be 776 mg a.i./kg bw. The concentration resulting in indirect effects causing 10% mortality to mammals was estimated to be 1560 mg a.i./kg bw (Table 4-27).

Cheminova's Chronic (Sublethal) Threshold Values

Cheminova recommends that the two-generation reproduction NOEL and LOEL for male parental body weight of 394 mg a.i./kg bw/d and 612 mg a.i./kg bw/d in rats (Schroeder, 1990 [MRID 41583401]), respectively, be used as the screening-level threshold value for assessing chronic risk to mammals (Table 4-28). This is a GLP and guideline-compliant study conducted with technical grade malathion and was rated supplemental based on Cheminova's study evaluation criteria only because it used technical malathion produced by the American Cyanamid Company. However, Cheminova is generally aware of the purity and impurity profile of the malathion used in American Cyanamid studies (see Hillwalker and Reiss, 2014 [MRID 49316501]). Although other acceptable chronic mammalian studies are available, Schroeder (1990 [MRID 41583401]) was the only two-generation reproduction toxicity test conducted for malathion. The NOEL and LOEL for parental body weight was reported to be 394 and 612 mg a.i./kg bw/d. These endpoints should be used for direct and indirect effects, respectively.

Table 4-27 Mortality and sublethal threshold values recommended by Cheminova for mammals contrasted with those selected by EPA (2016a)

Taxon	*Recommended by Cheminova*				*EPA (2016a)*			
	Species; Reference(s); Cheminova Study Rating	***Effect Concentration from Original Study or SSD***	***Direct Effect Threshold[a]***	***Indirect Effect Threshold[b]***	***Species; Reference(s); Cheminova Study Rating***	***Effect Concentration from Original Study or SSD***	***Direct Effect Threshold***	***Indirect Effect Threshold***
Acute (Mortality) Effects								
Mammals	Rat (female); Moore, 2003 [MRID 48153112] **Acceptable**	Mortality LD50= 2010 mg a.i./kg bw Slope = 11.5	776 mg a.i./kg bw	1560 mg a.i./kg bw	Rat; Mendoza, 1976; [MRID 45046301, E35348] **Unacceptable**	Mortality LD50 = 209 mg/kg bw; Slope 4.5	18.4 mg a.i./kg bw	108 mg a.i./kg bw
Chronic (Sublethal) Effects								
Mammals	Rat (male); Schroeder, 1990 [MRID 41583401] **Supplemental**	Parental body weight (males) NOEL=394 mg a.i./kg bw/d LOEL=612 mg a.i./kg bw/d	394 mg a.i./kg bw/d	612 mg a.i./kg bw/d	Rat; Daly, 1996 [MRID 43975201]. **Unacceptable for Malathion (see footnote)[c]**	AChE Inhibition LOEL = 1 mg/kg bw	1 mg/kg bw	1 m/kg bw

[a] For animals, direct effect thresholds for acute (mortality) effects represent one-in-one million mortality for either the most sensitive species or 5th centile species (HC5) from a species sensitivity distribution (SSD) and direct effect thresholds for chronic (sublethal) effects represent the most sensitive chronic NOEL.

[b] For animals, indirect effect thresholds for acute (mortality) effects represent 10% probability of effect for either the most sensitive species or 5th centile species (HC5) from a species sensitivity distribution (SSD) and indirect effect thresholds for chronic (sublethal) effects represent the most sensitive chronic LOEL or defensible low effect dose/concentration affecting x% of the test population (ED/ECx).

[c] The MRID provided refers to a chronic oral toxicity/ oncogenicity study for Malaoxon, not Malathion. The study referred to does report a decrease in red blood cell AChE at 6 months when exposed to 20 mg/kg-diet of malaoxon in feed, which is the effect EPA (2016a) states in Table 9-1 of Chapter 2 as the source of the 1 mg a.i./kg-bw LOEL.

EPA's Acute Threshold Values

In Section 9.2 of Chapter 2, EPA (2016a) summarizes the threshold values for mammals. For the mortality based thresholds, there were insufficient toxicity data to calculate SSDs. Therefore, EPA (2016a) derived direct and indirect effects thresholds for mortality from a single study (Mendoza, 1976 [MRID 45046301, E35348]) (Table 4-27 of this response document). EPA (2016a) provided a review of this study in Appendix 2-3, "Open Literature Review Summaries for Malathion", and classified this study as qualitative and suitable to derive the acute mortality threshold. EPA clearly notes in their problem formulation that only quantitative data can be used as a threshold value. The reliance on this study to generate a threshold value is questionable given that the study review notes that control performance was not reported in the LD50 studies. Therefore, it is unclear if the values in the treated pups were statistically different from control. This study was also rated as unacceptable by Cheminova due to lack of control data (Appendix D). Moreover, a 1-day old rat feeds only on the mother's milk. It would not be exposed to outside dietary exposure at that young age except through the milk thus the results are not representative of exposure possibilities in the wild. In fact, results of Fulcher (2001 [MRID 45566201) showed that in 4-day old pups, with potential exposure to malathion only through dam's milk, maternal exposures of 5 to 150 mg/kg bw/d (for approximately three week prior to post-natal day 4) resulted in no AChE inhibition in red blood cells, plasma and brain.

EPA's Chronic (Sublethal) Threshold Values

The sublethal threshold value relied on by EPA (2016a) is based on a LOEL of 1 mg a.i./kg bw for red blood cell AChE inhibition from Daly (1996 [MRID 43975201]) (Table 4-27 of this response document). The full reference for this study is listed as:

> Daly, I. (1996) A 24-Month Oral Toxicity/Oncogenicity Study of Malaoxon in the Rat via Dietary Administration: Final Report: Lab Project Number: 93-2234. Unpublished study prepared by Huntingdon Life Sciences. 4343 p. Relates to L0000076 and 43469901.

As can be seen in the study title, this study considers the effects of malaoxon (a metabolite of malathion) rather than malathion. Cheminova has previously reviewed this malaoxon study (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]) and rated it as acceptable. This study does report a decrease in red blood cell AChE at six months when exposed to 20 mg/kg-diet in feed, which is the sublethal endpoint value that EPA (2016a) indicates was used to derive their AChE inhibition LOEL of 1 mg a.i./kg-bw (see Table 9-1 and 9-2 of Chapter 2 of the BE and Table 4-27 of this response document). Therefore, it appears that EPA (2016a) incorrectly relied on a malaoxon study rather than a malathion study to determine this threshold for malathion.

Additionally, the suitability of AChE inhibition alone to derive a sublethal threshold value is questionable given that an explicit, quantitative and incremental relationship between AChE inhibition and effects to survival, growth, or reproduction, and ultimately fitness has not been

demonstrated. In the BE, EPA (2016a) has suggested relying on a LOEL of 1 mg a.i./kg bw as an effect threshold based on a 12-19% decrease in RBC AChE (although this LOAEL is from a malaoxon study rather than a malathion study; and notably, the effects range is erroneous, with actual inhibition being 19 to 21%). For comparison to known effects on mortality and/or reproduction, consider the two-generation rat reproduction study conducted by Schroeder (1990 [MRID 41583401]) as described above. Overall, the results of the Schroeder (1990 [MRID 41583401]) study suggest that the effect threshold selected by EPA (2016a) of 1 mg a.i./kg bw is considerably lower than the chronic exposure dose that would be required to cause significant impairment to either mortality or reproduction in rats. The link between blood AChE inhibition and survival, growth or reproduction need to be clearly established by EPA if a threshold based on AChE inhibition is used in the effects determinations. Simply choosing a NOEL based on treatment-related AChE inhibition only without other corroborative adverse effects at the same dose level is not acceptable.

In light of the poor quality data relied on by EPA (2016a) to derive effects thresholds for mammals, Cheminova has developed alternative effects thresholds based on the highest quality data identified in Breton et al. (2014a [MRID 49333901]; 2015 [MRID 49692301]). Endpoints were derived following the Interagency Interim Approaches provided by the Agencies (2013). Cheminova's recommended endpoints are contrasted with the endpoints recommended by EPA (2016a) in Table 4-27 of this response document.

Behavior, Growth, and Reproduction Sublethal Threshold Values

In addition to the threshold values summarized in Table 4-27 of this response document, EPA (2016a) also provided a table of most sensitive toxicity values for mammals for potential use as a refinement in Table 9-3 of Chapter 2 of the BE. The studies relied on as 'most sensitive endpoints' are listed in Table 4-28 of this response document and included three open literature studies and two registrant submitted studies. None of these studies were evaluated for data quality by EPA. Cheminova classified Schroeder (1990 [MRID 41583401]) as an acceptable study based on Cheminova study quality criteria.

Geraldi et al. (2008 [E153607]), in addition to being unacceptable based on Cheminova's scoring criteria, had other notable deficiencies. Malathion was sprayed homogenously on a monolayer of food pellets. This is not a standard "dietary" study whereby test substance is homogenously mixed into the meal diet. Further, only one concentration was tested. The mammalian toxicity studies used by EPA in the BE were all rated unacceptable by Cheminova (Table 4-28; Breton et al., 2014a [MRID 49333901]; Appendix D).

In Acker et al. (2011 [E162509]), it was not documented whether the behavioral tests were validated to detect meaningful and age-appropriate behavioral changes for pups 12 to 14 days old.

In Table 9-3 of Chapter 2, the Agency erroneously refer to the test species from Siglin (1985 [MRID 40812001]) as "rat." Siglin (1985 [MRID 40812001]) exposed *rabbits* to an irrelevant

environmental exposure pathway (dosed daily by oral gavage for 12 consecutive days). Further, Cheminova disagrees with EPA's interpretation of the developmental NOAEL/LOAEL. We believe that malathion had no effect on the number of resorptions in this study (see Robinson, 2002).

The treatment relationship of the increased resorptions at the mid- and hig-dose levels was re-evaluted by Robinson (2002) (an outside expert). The NOEL for developmental toxicity is >100 mg/kg bw/day (HDT).

The conclusions were as follows:

- The amount of lutenizing hormone used in the study was high compared to what is normally used, which could lead to super-ovulation and, therefore, a high incidence of preimplantation loss.

- There was no evidence of increased resoprtions in the range-finding study even at higher dose levels of malathion.

- The percentage of total resorptions was not statistically increased and was within the historical control range of data compiled by MARTA.

- When the resorptions are split out according to pre- and post-implantation loss from the combined results of the range-finding and definitive studies, there is no increase in the percentage of pre-implanation loss.

- The live litter size was higher at the high dose, which is unexpected with an increase in resoprtions. A smaller live litter size would be expected.

Table 4-28 Study classifications assigned by Cheminova for studies relied on as 'most sensitive endpoints' for mammals for potential use as a refinement in Table 9-3 of Chapter 2 of EPA (2016a)

Rated Acceptable by Intrinsik	***Rated Unacceptable by Intrinsik***
Schroeder, 1990 (MRID 41583401)	Geraldi et al., 2008 (E153607)
	Acker et al., 2011 (E162509)
	Samman et al., 1989 (E74457)
	Siglin, 1985 {MRID 40812001]

4.2.7 Terrestrial Invertebrates

Cheminova's Threshold Values

Cheminova recommends that the 48-hour contact LD50 of 0.189 µg a.i./bee and 48-hour oral LD50 of 1.66 µg a.i./bee for the honey bee (*Apis mellifera*) be used as the screening-level effects concentration for assessing risk to non-target terrestrial arthropods (Sindermann and Porch, 2013a [MRID 49270301]; Sindermann and Porch, 2013b [MRID 49270302]) (Table 4-29). This was the only GLP study for non-target terrestrial arthropods that used technical malathion and was rated acceptable based on Cheminova's study evaluation criteria. These endpoints were converted to an LD50 of 1.48 mg a.i./kg bw for contact and 13 mg a.i./kg bw for oral by dividing by the assumed weight of a foraging honey bee of 0.128 g (Mayer and Johansen, 1990). Probit slopes of 3.19 and 1.13 for contact and oral exposures, respectively, were calculated using raw data from the respective studies using SAS/STAT version 12.1. Using the probit slope of 3.19, the chance of one in a million (0.0001%) of causing direct mortality to invertebrates due to contact was estimated to be 0.0479 mg a.i./kg ww. The concentration resulting in indirect effects causing 10% mortality to invertebrates was estimated to be 0.587 mg a.i./kg ww (Table 4-29). For oral exposure, the chance of one in a million (0.0001%) of causing direct mortality to invertebrates was estimated to be 0.000808 mg a.i./kg ww. The concentration resulting in indirect effects causing 10% mortality to invertebrates was estimated to be 0.955 mg a.i./kg ww (Table 4-29).

Wüthrich (1991 [MRID 49086403]) investigated the acute toxicity of Fyfanon technical grade malathion (96.2% purity) to earthworms (*Eisenia fetida Savigny*) according to OECD guidelines (1984). The reported 7-day and 14-day LC50s for this study were 666 and 613 mg/kg dw soil, equating to approximately 641 and 590 mg a.i./kg dw soil, respectively. As this study was performed under GLP conditions using technical grade malathion of known composition and supplied by Cheminova, it was considered the best available data source for the development of an earthworm exposure benchmark. This study was rated acceptable based on Cheminova's data quality criteria (Appendix B).

EPA's Threshold Values

In Section 10.2 of Chapter 2, EPA (2016a) summarizes the threshold values for terrestrial invertebrates. These threshold values are also summarized in Table 4-29 of this response document. EPA (2016a) provided study reviews for two of the three studies relied on to generate these threshold values in Appendix 2-3, "Open Literature Review Summaries for Malathion" of the BE. Specifically, reviews were provided for Panda and Sahu (1999 [E052962]) and Robertson et al. (1975 [E89288]) but not for Lingappa et al. (1985 [E94337]). Both of the evaluated studies were considered 'quantitative' by EPA. However, EPA's review of Panda and Sahu (1999 [E052962]) raises many limitations of the acute and chronic study including that control mortality was not reported for earthworms. This alone invalidates the study for risk assessment. Similarly, for Robertson et al. (1975 [E89288]), the EPA review states that control mortality rates were unknown and that the company that supplied malathion for the study was

not reported. Additionally, the test material used by Panda and Sahu (1999 [E052962]) was a formulation (purity of 50%) where uncharacterized inerts likely impacted the study results.

Cheminova also completed study reviews of the three studies relied on by EPA (2016a) and found all three of them to be of unacceptable quality (see Appendix B). Lingappa et al. (1985 [E94337]), a two page journal article, provides very little information about the study methods and provides no details regarding the test item source or purity (the two malathion test substances are identified only as 'malathion technical' and 'malathion pure').

In light of the poor quality data relied on by EPA (2016a) to derive effects thresholds for terrestrial invertebrates, Cheminova has developed alternative effects thresholds based on the highest quality data identified in Breton et al. (2014 a,b [MRID 49333901, 49400601]; 2015 [MRID 49692301]). Endpoints were derived following the Interagency Interim Approaches (Agencies, 2013). Cheminova's recommended endpoints are contrasted with the endpoints recommended by EPA (2016a) in Table 4-29 of this response document.

Due to time imitations, Cheminova did not review all of the toxicity information provided in Chapter 10 of the BE. However, we did notice the following two assertions from EPA in their Lines-of-evidence section (Section 10.4 of the BE):

- "For the exposure unit 'ug/e.u.', the most sensitive endpoint available for terrestrial invertebrates is an LD_{50} value of 0.000375 µg a.i./organism for contact exposure to 2 to 3 day old female adult Anopheline mosquitoes (*A. albimanus*) (E111057). This endpoint is more sensitive than any of the available NOAEC or LOAEC values."
- "For the exposure unit 'ppm', the most sensitive endpoint available for terrestrial invertebrates is an LC_{50} value of 0.047 mg a.i./L (ppm) for mosquito (*Culex quinquefascatus)* (E82047). This endpoint is more sensitive than any of the available NOAEC or LOAEC values for terrestrial invertebrates."

It is totally inappropriate to be using intended target species as part of EPA's evaluation of the toxicity of malathion. These data should be omitted from the BE.

Table 4-29 Mortality threshold values recommended by Cheminova for terrestrial invertebrates contrasted with those selected by EPA (2016a)

Taxon	*Recommended by Cheminova*				*EPA (2016a) Values*			
	Species; Reference(s); Cheminova Study Rating	***Effect Concentration from Original Study or SSD***	***Direct Effect Threshold[a]***	***Indirect Effect Threshold[b]***	***Species; Reference(s); Cheminova Study Rating***	***Effect Concentration from Original Study or SSD***	***Direct Effect Threshold***	***Indirect Effect Threshold***
Acute (Mortality) Effects								
Pollinators	Honey bee (*Apis mellifera*); Sindermann and Porch, 2013a [MRID 49270301] **Acceptable**	Mortality (Contact Exposure) LD50=1.48 mg a.i./kg bw[c] Slope = 3.19	0.0479 mg a.i./kg ww	0.587 mg a.i./kg ww	Indian hive bee (*Apis cerana indica*); Lingappa et al., 1985 [E94337] **Unacceptable**	Mortality (Contact Exposure) LD50 (contact) = 1.64 mg/kg bw[e]; Slope 5	0.184 mg/kg bw	0.909 mg/kg bw
	Honey bee (*Apis mellifera*); Sindermann and Porch, 2013b [MRID 49270302] **Acceptable**	Mortality (Oral Exposure) LD50=13.0 mg a.i./kg bw[d] Slope = 1.13	0.000808 mg a.i./kg ww	0.955 mg a.i./kg ww				
Soil Invertebrates	Earthworm (*Eisenia foetida*) Wüthrich, 1991 [MRID 49086403] **Acceptable**	Mortality LC50 = 590 mg a.i./kg dw soil Slope = 8.29	158 mg a.i./kg dw	413 mg a.i./kg dw	Earthworm (*Drawida willsi);* Panda and Sahu, 1999 [E052962]; **Unacceptable**	Mortality LC50 =7.54 mg a.i./kg dw soil; Slope 4.5	0.66 mg a.i./kg dw soil	3.89 mg a.i./kg dw soil
Other Non-target Terrestrial Arthropods	-	-	-	-	Hemlock sawfly (*Neodriprion tsugae);* Robertson and Lyon, 1975 [E89288] **Unacceptable**	Mortality LR50 = 0.00875 lb a.i./A; Slope 5.6	0.0012 lb a.i./A	0.0052 lb a.i./A

[a] For animals, direct effect thresholds for acute (mortality) effects represent one-in-one million mortality for either the most sensitive species or 5th centile species (HC5) from a species sensitivity distribution (SSD) and direct effect thresholds for chronic (sublethal) effects represent the most sensitive chronic NOEL.

[b] For animals, indirect effect thresholds for acute (mortality) effects represent 10% probability of effect for either the most sensitive species or 5th centile species (HC5) from a species sensitivity distribution (SSD) and indirect effect thresholds for chronic (sublethal) effects represent the most sensitive chronic LOEL or defensible low effect dose/concentration affecting x% of the test population (ED/ECx).

[c] Original study reported an LD50 of 0.189 µg a.i./bee. This was converted to an LD50 of 1.48 mg a.i./kg bw by dividing by the assumed weight of a foraging honey bee of 0.128 g (Mayer and Johansen, 1990).

[d] Original study reported an LD50 of 1.66 µg a.i./bee. This was converted to an LD50 of 13.0 mg a.i./kg bw by dividing by the assumed weight of a foraging honey bee of 0.128 g (Mayer and Johansen, 1990).

[e] Original study reported an LD50 value of 0.072 ug a.i./bee. This was converted to an LD50 of 1.64 mg a.i./kg bw by dividing by an assumed weight of an *A. cerana indica* worker bee of 0.044 g (Dyer and Seeley,1987, as cited in EPA (2016a). Note: Reference cited but not provided by EPA).

4.2.8 Terrestrial Plants

Cheminova's Threshold Values

Two GLP terrestrial plant toxicity studies conducted on monocots and dicots have been submitted by Cheminova to EPA and should be considered best available scientific data (Sindermann et al., 2013a,b [MRID 49076001,49076002]). Both these studies were rated as acceptable based on Cheminova's study evaluation criteria. For monocots, the NOER and LOER of 4.7 and > 4.7 lb a.i./A, respectively, should be used for direct and indirect effects (Sindermann et al., 2013a,b [MRID 49076002,49076001]). For dicots, the NOER and LOER are 1.2 and 2.4 lb a.i./A for direct and indirect effects (Sindermann et al., 2013b [MRID 49076002]) (Table 4-30).

EPA's Threshold Values

In Section 11.2 of Chapter 2, EPA (2016a) summarizes the threshold values for terrestrial plants. These threshold values, summarized in Table 4-30 of this response document, were derived based on two registrant submitted studies (Sindermann et al., 2013a, 2013b [MRIDs 49076001; 49076002]) and one open literature study (Ahrens, 1990 [E068422]). EPA provided no quality review or evaluation of the registrant submitted studies in the BE. As noted above, these studies were reviewed by Cheminova and found to be of acceptable data quality (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]). As such, Cheminova also recommends deriving threshold values for terrestrial plants based on these studies (see Table 4-30).

For the open literature study (Ahrens, 1990 [E068422]), EPA (2016a) did provide a review in Appendix 2-3, "Open Literature Review Summaries for Malathion" of the BE. In their review of Ahrens (1990 [E068422]), EPA rates this study as quantitative. However, they also state in the limitations of the study that the purity of the test substance was not reported. In a review completed by Cheminova as part of this response document, this study was rated as unacceptable due to this lack of information on test substance purity, source, and contents of the formulation, as well as the lack of solvent control used (see Appendix D). Additionally, although EPA indicates in their study review of Ahrens (1990 [E068422]) that statistically significant reductions in plant fresh weight were observed for plants treated at a rate of 0.5 lb/acre, no indication of statistical significance is provided in the original study. Since raw data were not provided in the study, it is unclear how EPA determined statistical significance (and therefore identified a treatment LOER). Therefore, Cheminova suggests that terrestrial plant threshold values should only rely on the two registrant submitted studies (Sindermann et al., 2013a, 2013b [MRIDs 49076001; 49076002]).

Table 4-30 Threshold values recommended by Cheminova for terrestrial plants contrasted with those selected by EPA (2016a)

Taxon	Recommended by Cheminova				EPA (2016a)				
	Species; Reference(s); Cheminova Study Rating	Effect Concentration from Original Study or SSD	Direct Effect Threshold[a]	Indirect Effect Threshold[b]	Species; Reference(s); Cheminova Study Rating		Effect Concentration from Original Study or SSD	Direct Effect Threshold	Indirect Effect Threshold
All Terrestrial Plants	-	-	-	-	Pre-emergence	All spp. tested[c] Sindermann et al., 2013a, [MRID 49076001]; **Acceptable**	All Seedling Emergence Effects[d] NOER = 4.64 lb a.i./A LOER = >4.64 lb a.i./A	4.64 lb a.i./A	>4.64 lb a.i./A
					Post-emergence	Soybean (*Glycine max*); Ahrens, 1990 [E068422]; **Unacceptable**	Reduced weight NOER = 0.25 lb a.i./A LOER = 0.5 lb a.i./A	0.25 lb a.i./A	0.5 lb a.i./A
Monocots	All spp. tested[c]; Sindermann et al., 2013a, 2013b [MRIDs 49076001; 49076002]; **Acceptable**	All Seedling Emergence and Vegetative Vigor Effects[d] NOER = 4.7 lb a.i./A LOER = >4.7 lb a.i./A	4.7 lb a.i./A	>4.7 lb a.i./A	Pre-emergence	All spp. tested[c] Sindermann et al., 2013a, [MRID 49076001]; **Acceptable**	All Seedling Emergence Effects[d] NOER = 4.64 lb a.i./A LOER = >4.64 lb a.i./A	4.64 lb a.i./A	>4.64 lb a.i./A
					Post-emergence	All spp. tested[c] Sindermann et al., 2013b, [MRID 49076002]; **Acceptable**	All Vegetative Vigor Effects[d] NOER = 4.7 lb a.i./A LOER = >4.7 lb a.i./	4.7 lb a.i./A	>4.7 lb a.i./A
Dicots	*Brassica oleracea* (Cabbage); Sindermann et al., 2013b [MRID 49076002]; **Acceptable**	Vegetative Vigor, Dry Weight NOER = 1.2 lb a.i./A LOER = 2.4 lb a.i./A	1.2 lb a.i./A	2.4 lb a.i./A	Pre-emergence	All spp. tested[c] Sindermann et al., 2013a, [MRID 49076001]; **Acceptable**	All Seedling Emergence Effects[d] NOER = 4.64 lb a.i./A LOER = >4.64 lb a.i./A	4.64 lb a.i./A	>4.64 lb a.i./A
					Post-emergence	*Brassica oleracea* (Cabbage); Sindermann et al., 2013b [MRID 49076002]; **Acceptable**	Vegetative Vigor, Dry Weight NOER = 1.2 lb a.i./A LOER = 2.4 lb a.i./A	1.17 lb a.i./A	> 2.39 lb a.i./A

[a] For aquatic or terrestrial plants, direct effect thresholds represent the most sensitive NOEL or EC05 (whichever is lower).

[b] For aquatic or terrestrial plants, indirect effect thresholds represent the most sensitive LOEL or EC25 (whichever is lower).

[c] Monocots: *Allium cepa* (Onion), *Lolium perenne* (Ryegrass), *Triticum aestivum* (Wheat), and *Zea mays* (Corn). Dicots: *Brassica napus* (Oilseed Rape), *Brassica oleracea* (Cabbage), *Daucus carota* (Carrot), *Glycine max* (Soybean), *Lactuca sativa* (Lettuce), *Lycopersicon esculentum* (Tomato).

[d] Seedling emergence (pre-emergence) effects tested were emergence, survival, height and growth. Vegetative vigor (post-emergence) effects tested were height, dry weight, and survival.

4.3 Errors and Discrepancies in Aquatic and Terrestrial Threshold Values

EPA's threshold values are presented in several locations throughout the BE:

- Chapter 2;
- Appendix 3-6;
- AquaWoE_v1.0.xls ('Species Summary' and 'Spray Drift all' worksheets); and,
- TEDtool_v1.0.xls and TEDtool_v1.0_alt.xls ('inputs' worksheets).

Several discrepancies have been identified between the threshold values presented in Chapter 2 of EPA's BE, and the metrics used as inputs for the risk characterization presented in Appendix 3-6 and the AquaWoE_v1.0.xls / TEDtool_v1.0.xls files. In some cases, values presented in Chapter 2 are absent from the TED tool model inputs spreadsheets, and in other instances there are endpoints in Appendix 3-6 and the TED tool inputs that are not presented as threshold or endpoint values in Chapter 2 of the BE. This includes the presentation of some endpoints with no references available to identify the studies they originate from. There are also several instances of erroneous details in the study endpoints and/or references between these files. Details of these discrepancies and errors are further described in Table 4-31 and Table 4-32. Note that this discussion does not include reference to the quality of the studies presented. Some of these studies are discussed in Section 4 of this response document.

It is not clear how EPA selected some of the thresholds used in the modeling exercises. In Chapter 2, for each taxon, there is a table (or tables) of threshold values to be used in the Step 1 analysis. For some taxa (but not consistently for all), there are tables of endpoints to be used as 'potential refinements', which are presumably for the Step 2 analyses. However it is not explicitly explained which endpoints were selected for Step 2, how they were selected, or how they were used.

Table 4-31 Discrepancies between EPA thresholds and endpoints for aquatic organisms reported in Chapter 2 and Chapter 4 of EPA's BE

Taxon	*Endpoint*	*Chapter 2*		*Appendix 3-6 and AquaWoE_v1.0.xls*		*Comments*
		Value (µg/L)	*Reference*	*Value (µg/L)*	*Reference*	
Aquatic amphibians, freshwater fish and marine fish	Reproduction endpoint - NOAEC	690	Palmer et al. 2011c MRID 48617506	220	Palmer et al. 2011c MRID 48617506	The NOEC and LOEC were reversed in Table 2-2 of Chapter 2. Furthermore, these values are different from those reported in Section 2.4.2.2 of Chapter 2 (NOEC = 250 µg a.i./L and LOEC = 820 µg a.i./L), which were the author-reported values in Palmer et al. (2011 [MRID 48617506]). EPA does not describe how they derived the NOEC and LOEC values of 220 and 690 µg a.i./L in Chapter 2. Measured malathion concentrations are presented in Table 3 of Palmer et al. (2011 [MRID 48617506]). Day 21 concentrations were not included in the calculation of mean measured concentrations due to a sample handling or analysis error. However, EPA appears to have included the day 21 samples in their calculation of mean measured concentrations. Furthermore, EPA appears to have averaged all sample measurements together rather than averaging the day 0, 7, 14 and 21 averages. This practice biases the mean towards the day 0 and day 21 concentrations since four samples were analyzed on these days, whereas only two samples were analyzed for days 7 and 14.
	Reproduction endpoint - LOAEC	220		690		
Freshwater fish	HC5	38.6	SSD	45.2	SSD	The HC5 of 38.6 µg/L from the "All Fish" SSD was reported in the comments field of Table 2-2 of Chapter 2. The correct HC5 of 45.2 µg/L for freshwater fish was reported in Table 2-4 of Chapter 2.
Marine fish	Growth endpoint - NOAEC	>37	E5074	8.6	MRID	The NOEC of >37 ug a.i./L for effects growth and

Table 4-31 Discrepancies between EPA thresholds and endpoints for aquatic organisms reported in Chapter 2 and Chapter 4 of EPA's BE

Taxon	*Endpoint*	*Chapter 2*		*Appendix 3-6 and AquaWoE_v1.0.xls*		*Comments*
		Value (µg/L)	*Reference*	*Value (µg/L)*	*Reference*	
	Growth endpoint - LOAEC	>37		10.9	4878002; E995	reproduction in sheepshead minnow was presented for marine fish in Table 2-2 of Chapter 2. However, the growth endpoints for freshwater fish were presented in Appendix 3-6 and AquaWoE_v1.0.xls.
Marine invertebrates	Mortality - Direct	0.15	SSD	0.046	SSD	The mortality thresholds based on the marine invertebrate SSD were presented in Table 3-2 of Chapter 2. However, EPA reported the freshwater invertebrate thresholds for marine invertebrates in Appendix 3-6 and AquaWoE_v1.0.xls.
	Mortality - Indirect	0.88		0.22		
	Lowest LC50	0.4	E6793	0.4	Ashuer et al. 2011 E153561	Error in reference in Appendix 3-6 and AquaWoE_v1.0.xls.
Aquatic plants - vascular	Mortality - Direct	NR	-	100,000	Worthley. and Schott. 1971 E9184	These endpoints were not included in Table 4-1 or 4-2, which listed the aquatic plant thresholds and sensitive toxicity values for aquatic vascular plants in Chapter 2, respectively. In addition, the reference provided for the indirect mortality threshold (E9185) is incorrect and should be E9184.
	Mortality - Indirect	NR	-	100,000	E9185	
Aquatic non-vascular plants	Mortality - Direct	NR	-	10,000	NR	These endpoints were not included in Table 4-1, which listed the aquatic plant thresholds in Chapter 2. In addition, no reference was provided for these endpoints in Appendix 3-6 and AquaWoE_v1.0.xls. Agrawal and Manisha (2007; [E104317]) was cited for these values in Section 4.4.1 of Chapter 2.
	Mortality - Indirect	NR	-	10,000		

NR: not reported

Table 4-32 Discrepancies between EPA thresholds and endpoints for terrestrial organisms reported in Chapter 2 and Chapter 4 of EPA's BE

Taxon	*Threshold Type*	*Threshold Description*	*Chapter 2*		*Appendix 3-6 and TEDtool_v1.0.xls*		*Comments*
			Value	*Reference*	*Value*	*Reference*	
Mammals	Direct	1/million mortality	18.4 mg/kg bw	Mendoza, 1976 [E35348]	18.4 mg/kg bw	Mendoza, 1976 [E35348]	A bodyweight of 100 g was reportedly used to convert the dose-based value to dietary concentration (cell F70 of Appendix 3-6 and the 'inputs' tab of TEDtool_v1.0.xls). Firstly, the test organisms were day-old rats which would have weighed much less than 100 g. Secondly, it is not generally acceptable to convert a gavage dose-based endpoint to a dietary concentration. The oral gavage exposure route excludes the elements of natural dietary matrices, feeding patterns, metabolism and elimination throughout the day, and thus a simple conversion to dietary concentration is not biologically accurate. Further, EPA states in cell H95 of Appendix 3-6 that this calculation is from a WHO 2009 conversion factor for young rat, however specific details on this guidance are not provided.
					184 mg/kg diet		
	Indirect	10% mortality	108 mg/kg bw		108 mg/kg bw		
					1080 mg/kg diet		
	Direct and indirect	Lowest LD50	209 mg/kg bw		209 mg/kg bw		
					2090 mg/kg diet		
	Direct and indirect	Rat oral LD50	NR	-	1460 mg/kg bw	MRID 49127003	There is no mention of this MRID (Fisher, 1991) or the study endpoint in Chapter 2 of the BE.
	Direct and indirect	Rat dermal LD50	>2000 mg/kg bw	Kynoch 1986 MRID 00159877	>2000 mg/kg bw	Kynoch 1986 MRID 00159877	While this endpoint is provided in Chapter 2, it is not included in the tables of thresholds or potential refinements, or in the data arrays. Rather this study is presented in Table 9-7 "Toxicity Data from Registrant-submitted Studies for Malathion Based on Dermal Application Methods".
	Direct and indirect	Rat inhalation LC50	>5.2 mg/L	Jackson et al. 1986 MRID 00159878	>5.2 mg/L	Jackson et al. 1986 MRID 00159878	While this endpoint is provided in Chapter 2, it is not included in the tables of thresholds or potential refinements, or in the data arrays. Rather this study is presented in Table 9-7 "Toxicity Data from Registrant-submitted Studies for Malathion Based on Dermal Application Methods". It is not clear how EPA converted this metric to a dose-based value.
					>141 mg/kg bw		
	Direct and indirect	Sublethal (AChE inhibition)	1 mg/kg bw	Daly 1999 MRID 43975201	1 mg/kg bw	Daly 1999 MRID 43975201	This is a dietary study for which EPA converted the 20 mg/kg diet LOAEC, *for malaoxon*, to a dose-based endpoint assuming a bodyweight of 350 g. It is not clear how this conversion was conducted.

Table 4-32 Discrepancies between EPA thresholds and endpoints for terrestrial organisms reported in Chapter 2 and Chapter 4 of EPA's BE

Taxon	*Threshold Type*	*Threshold Description*	*Chapter 2*		*Appendix 3-6 and TEDtool_v1.0.xls*		*Comments*
			Value	*Reference*	*Value*	*Reference*	
			20 mg/kg diet (1 mg/kg bw)	Daly 1999 MRID 43975201	20 mg/kg diet	MRID 43942901	EPA incorrectly cited the MRID of 43942901 in Appendix 3-6, when the correct MRID for this study is 43975201. However, this study used *malaoxon* rather than malathion as the test substance.
	Direct	Growth NOEC	1700 mg/kg diet	Schroeder, 1990 MRID 41583401	1700 mg/kg diet	Schroeder, 1990 MRID 41583401	These endpoints are presented in Table 9-3 of Chapter 2, "Most Sensitive Toxicity Value for Different Effect Types for Mammals for Potential Use As a Refinement for Malathion". However, it is not clear how they will be used in Step 2 of the BE. Further, it is unclear why the LOEC is labeled as both a direct and an indirect threshold, as there is a NOEC value.
	Direct and indirect	Growth LOEC	5000 mg/kg diet		5000 mg/kg diet		
	Direct	Reproduction NOEC	25 mg/kg bw	MRID 40812001	825 mg/kg diet	MRIDs 00152569, 40812001	In Chapter 2 of the BE, this study is presented in Table 9-3 "Most Sensitive Toxicity Value for Different Effect Types for Mammals for Potential Use As a Refinement for Malathion". The test species is listed as a rat. However, this is a developmental rabbit study. The endpoint reported in Appendix 3-6 and the 'inputs' tab of TEDtool_v1.0.xls is reportedly converted to mg/kg diet, however the test organism weight is not provided and it is not clear how EPA converted this endpoint. Further, it is unclear why the LOEC is labeled as both a direct and an indirect threshold, as there is a NOEC value.
	Direct and indirect	Reproduction LOEC	50 mg/kg bw		1650 mg/kg diet		

Table 4-32 Discrepancies between EPA thresholds and endpoints for terrestrial organisms reported in Chapter 2 and Chapter 4 of EPA's BE

Taxon	*Threshold Type*	*Threshold Description*	*Chapter 2*		*Appendix 3-6 and TEDtool_v1.0.xls*		*Comments*
			Value	*Reference*	*Value*	*Reference*	
	Direct and indirect	Behavior LOEC	100 mg/kg bw	E162509	NR	-	This endpoint is presented in Table 9-3 of Chapter 2, "Most Sensitive Toxicity Value for Different Effect Types for Mammals for Potential Use As a Refinement for Malathion". However it is not clear how it will be used in Step 2 of the BE. It does not appear to have been utilized in Appendix 3-6 (or the 'inputs' tab of TEDtool_v1.0.xls).
	Direct and indirect	Growth LOEC	10 mg/kg bw	E74457	NR	-	This endpoint is presented in Table 9-3 of Chapter 2, "Most Sensitive Toxicity Value for Different Effect Types for Mammals for Potential Use As a Refinement for Malathion". However, it is not clear how it will be used in Step 2 of the BE. It does not appear to have been utilized in Appendix 3-6 (or the 'inputs' tab of TEDtool_v1.0.xls).
	Direct and indirect	Reproduction NOEC	7500 mg/kg diet	MRID 41583401	NR	-	This endpoint is presented in Table 9-3 of Chapter 2, "Most Sensitive Toxicity Value for Different Effect Types for Mammals for Potential Use As a Refinement for Malathion". However, it is not clear how it will be used in Step 2 of the BE. It does not appear to have been utilized in Appendix 3-6 (or the 'inputs' tab of TEDtool_v1.0.xls).
Birds	Direct and indirect	Sublethal	87.4 mg/kg bw	E63276	87.4 mg/kg bw	E63275	Firstly, the ECOTOX ID is incorrect in Appendix 3-6 and the 'inputs' tab of TEDtool_v1.0.xls. The correct ID is E63276, as reported in Chapter 2. Secondly, the organism weight applied in Appendix 3-6 is 1135 g, which is a default weight assigned to ring-necked pheasants. However, the ring-necked pheasants in the toxicity study weighed between 400 and 500 g. This apparent error has significant impact on the effects metrics used in the listed species assessment.
	Direct	Behavior NOEC	NA or 400 mg/kg diet	E89120; E90699	NA	E89120	In Appendix 3-6 and the 'inputs' tab of TEDtool_v1.0.xls EPA states that there is no NOEC, and that the LOEC Is for "sleeping time, chickens". This is obviously not a relevant ecological endpoint. In Chapter 2, this study is not presented in the tables of thresholds or potential

Table 4-32 Discrepancies between EPA thresholds and endpoints for terrestrial organisms reported in Chapter 2 and Chapter 4 of EPA's BE

Taxon	*Threshold Type*	*Threshold Description*	*Chapter 2*		*Appendix 3-6 and TEDtool_v1.0.xls*		*Comments*
			Value	*Reference*	*Value*	*Reference*	
	Direct and indirect	Behavior LOEC	400 or 800 mg/kg diet		400 mg/kg diet		refinements. Rather it is presented in Table 6-6 "Growth Effects in Birds Exposed to Malathion". The two field studies are presented together, with a NOEL/LOEL of <400 (NA) and 400 mg/kg diet for decreased body weight, and a NOEL/LOEL of 400 and 800 mg/kg diet for liver weight. It is not specified which endpoints are from which study. However, the measures of effect do not align with what is presented in Appendix 3-6 and the 'inputs' tab of TEDtool_v1.0.xls.
Herptiles	Direct and indirect	Lowest LD50	136 mg/kg bw (ring-necked pheasant)	MRID 48963305	2324 mg/kg bw (green anole)	E36970	It appears that rather than relying solely on avian toxicity data as a surrogate for terrestrial-phase amphibians and reptiles (which is stated to be the approach in Section 6.2 of Chapter 2), this green anole toxicity study was selected as the lowest LD50 value for reptiles (presented in Table 7-1 "Toxicity Data for Reptiles"). It was also converted to an LC50 presented in Appendix 3-6 and the 'inputs' tab of TEDtool_v1.0.xls using a body weight of 2 g derived from the literature. No other details of this conversion are provided by EPA. Study E36970 is presented in Section 7 of the BE (Table 7.1), "Effects Characterization to Reptiles". However, it is not clear from this Section how those data will be used in the effects determination. EPA states in Section 6.2 that birds will be used as surrogates for herptiles due to limited data for herptiles. Also, study E36970 was determined to be unacceptable based on Cheminova study quality criteria (see Section 4.2.5 of this response document)
	Direct and indirect	Lowest LC50	2022 mg/kg diet (bobwhite quail)	MRID 48153106	2324 mg/kg diet (green anole)		
	Direct	Sublethal	87.4 mg/kg bw (ring-necked	E63276	20 mg/kg bw (western fence lizard)	E104558 (Holem et al. 2008)	It appears that rather than relying solely on avian toxicity data as a surrogate for terrestrial-phase amphibians and reptiles (which is stated to be the approach in Section 6.2 of Chapter 2), this western

Table 4-32 Discrepancies between EPA thresholds and endpoints for terrestrial organisms reported in Chapter 2 and Chapter 4 of EPA's BE

Taxon	*Threshold Type*	*Threshold Description*	*Chapter 2*		*Appendix 3-6 and TEDtool_v1.0.xls*		*Comments*
			Value	*Reference*	*Value*	*Reference*	
			pheasant)				fence lizard toxicity study was selected for the sublethal values for reptiles (presented in Table 7-1 "Toxicity Data for Reptiles"). Holem et al. (2008) is presented in Section 7 of the BE (Table 7.1), "Effects Characterization to Reptiles". However, it is not clear from this Section how those data will be used in the effects determination. Although a supplemental chronic toxicity study for terrestrial herptiles, Holem et al. (2008) used an unconventional exposure regime whereby Western fence lizards were administered three oral gavage doses of technical malathion with a 27-day observation period following each dose. Thus, this study was inappropriate for estimating a chronic effects metric (Breton et al., 2015 [MRID 49692301]).
	Indirect	Sublethal			100 mg/kg bw (western fence lizard)		
	Not specified	Various	Various	Various	NR	-	There are several endpoints reported in both Table 7-1 "Toxicity Data for Reptiles" and 8-1 "Toxicity Data for Terrestrial-phase Amphibians" of Chapter 2 that are not applied in Appendix 3-6 or the 'inputs' tab of TEDtool_v1.0.xls. It is not clear how EPA decided between avian surrogate toxicity data and herptile data when available.
Terrestrial Invertebrates	Direct and indirect	Lowest LC50; sublethal	0.38 µg a.i./bee	MRIDs 05001991, 05004151	1.3 mg/kg food	Bee Rex calculator (based on LC50 of 0.38 µg a.i./bee; MRIDs 05001991, 05004151)	The 1.3 mg/kg food endpoint is not presented in Chapter 2. There is no mention of the use of Bee Rex in Chapter 1 or Chapter 2 of the BE.
	Direct and Indirect	Growth LOEC	45 600 mg/kg soil	NR	45 600 mg/kg soil	E120758	While this endpoint is presented as the growth LOEC for terrestrial invertebrates in Appendix 3-6 and the 'inputs' tab of TEDtool_v1.0.xls, in Chapter 2 the endpoint value is mentioned only in the text of Section 10.4.2 of the BE "Sublethal Effects to Terrestrial Invertebrates" (and without reference details).

Table 4-32 Discrepancies between EPA thresholds and endpoints for terrestrial organisms reported in Chapter 2 and Chapter 4 of EPA's BE

Taxon	*Threshold Type*	*Threshold Description*	*Chapter 2*		*Appendix 3-6 and TEDtool_v1.0.xls*		*Comments*
			Value	*Reference*	*Value*	*Reference*	
	Direct and Indirect	Reproduction LOEC	1100 mg/kg soil	E52962	1100 mg/kg soil	E52962	While this endpoint is presented as the reproduction LOEC for terrestrial invertebrates in Appendix 3-6 and the 'inputs' tab of TEDtool_v1.0.xls, in Chapter 2 the endpoint value is mentioned only in the text of Section 10.4.2 of the BE "Sublethal Effects to Terrestrial Invertebrates".
	Direct and Indirect	Growth LOEC	0.456 lb a.i./A	NR	0.456 lb a.i./A	E158669	While this endpoint is presented as the growth and reproduction LOEC for terrestrial invertebrates in Appendix 3-6 and the 'inputs' tab of TEDtool_v1.0.xls, in Chapter 2 the endpoint value is mentioned only in the text of Section 10.4.2 of the BE "Sublethal Effects to Terrestrial Invertebrates" (and without reference details).
	Direct and indirect	Reproduction LOEC					
Terrestrial Plants	Direct	Mortality	2.94 lb a.i./A (Dicots)	E162475	2.94 lb a.i./A (Monocots)	E162475	In Chapter 2, this study is not presented in the table of threshold values (Table 11-1). Rather this study is presented in Table 11-2 "Effects of Malathion on Pink Sundew and Venus Flytrap Survival". In Appendix 3-6 and the 'inputs' tab of TEDtool_v1.0.xls, the plant type is labeled as a monocot. However, it is a dicot study.
	Direct and indirect	Reproduction NOEC	NR	-	5.1 lb a.i./A	NR	The 5.1 lb a.i./A endpoint is not presented in Chapter 2. There is no reference provided in Appendix 3-6 or the 'inputs' tab of TEDtool_v1.0.xls. It is unclear where this endpoint comes from.
	Direct	Growth NOEC	1.17 lb a.i./A	MRID 49076002	NR	-	While these endpoints were presented in the threshold table (Table 11-1) in Chapter 2 of the BE, they were not included in Appendix 3-6 or in the 'inputs' tab of TEDtool_v1.0.xls.
	Indirect	Growth LOEC	2.39 lb a.i./A				

NR: not reported

4.4 Summary of Concern Regarding the Effects Characterization

Cheminova has a number of concerns with the effects characterization presented in Chapter 2 of EPA's draft BE for malathion. Cheminova's major issues with EPA's data selection process and presentation of selected effects metrics are summarized as follows:

- EPA is not transparent in its data quality evaluations and selection of effects thresholds and endpoints for 'potential refinement'. EPA has published several guidance documents to aid in the internal evaluation of toxicity studies (EPA, 2002, 2003, 2004a,b, 2011b). However, it is questionable whether these criteria were consistently followed by reviewers, and evaluations were not provided for the majority of studies presented in EPA's effects characterization. Furthermore, it appears that EPA included data in their SSDs from studies that were not formally evaluated by EFED.
- EPA's criteria for the evaluation of registrant-submitted studies are more stringent than those for open literature studies. A single, thorough set of data quality guidelines should be established and followed by EPA for each data point that is considered for use in every risk assessment.
- Most studies used by EPA as threshold values are classified as unacceptable for risk assessment based on Cheminova's data quality criteria (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]). The fundamental question of data quality and use of "best available data" does not appear to have been addressed in the draft BE.
- Cheminova is the only producer of technical malathion sold in the US. In 1992, Cheminova submitted an updated confidential statement of formula (CSF) with higher malathion purity and reduced impurities. EPA (2016a) does not appear to be accounting for relevance of the test chemical in their data quality evaluations as many of the studies used to construct SSDs in the BE had a chemical characterization identified as "unknown". This practice is scientifically unsound and goes against the recommendations made in NRC (2013).
- NOELs are the effects thresholds driving most, if not all of the risk designations. The use of NOELs in ecological risk assessment has long been criticized (Hoekstra and Van Ewijk, 1993; Moore and Caux, 1997; Landis and Chapman, 2011; Jager, 2012; Murado and Prieto, 2013) due to the inherent deficiencies of the metrics as a relative measure of toxicity, which include an absolute dependence on the selected treatment levels and sample size, and related issues of low statistical power. EPA stated in its Interagency Interim Approaches (Agencies, 2013) that ECx values would be considered. However, it seems that in most cases the EPA opted to circumvent data analyses and simply use the author-reported NOELs from toxicity studies. Although the use of NOELS may be practical in some instances (e.g., when sample size is large and/or when the data are not conducive to generating a meaningful dose-response), in a succeeding analysis, such as Step 2, the Agency should give precedence to more refined metrics (e.g., dose-response curves, benchmark doses) when possible.
- EPA (2016a) selected thresholds based on sublethal endpoints (e.g., biochemical, cellular, and behavioral effects) without providing evidence of any qualitative or

quantitative link between these endpoints and survival, growth or reproduction. Endpoints without a direct link to specific apical adverse effects are not considered to be biologically significant. EPA (2016a) should not rely on these endpoints when selecting their thresholds values and endpoints for 'potential refinement'.

- There is a mismatch between the exposure and effects metrics used to assess risk of acute mortality to listed species. EPA (2016a) predicted acute risk to aquatic organisms by comparing instantaneous aquatic peak EECs to threshold values derived from toxicity tests in which organisms were exposed to constant concentrations of malathion for much longer exposure durations (48 to 96 hours). However, malathion degrades very quickly in aqueous systems (half-lives of 0.3 to 3.3 days), and as such it is highly unlikely that aquatic organisms would be exposed to a 'peak' concentration of malathion for a 48- or 96-hour period. Furthermore, LC50 values observed in aquatic toxicity tests are much higher at shorter exposure durations for malathion. Therefore, the EPA (2016a) approach of comparing peak exposure concentration to effects thresholds derived based on 48- and 96-hour toxicity tests for fish and aquatic invertebrates is likely to overestimate risk.
- Cheminova disagrees with EPA's procedure for evaluating chronic risk to aquatic and terrestrial species. Chronic guideline studies typically use continuous pesticide exposures ranging from 21 days for aquatic invertebrates to greater than 10 weeks for birds and mammals. However, such exposures are unrealistic because malathion would, in reality, degrade rapidly between applications, particularly in marine environments. Pulse exposures far more relevant than maintained chronic exposures.
- A number of discrepancies were identified between the thresholds presented in Chapter 2 of the draft BE and the effects metrics used as inputs for the risk characterization presented in Appendix 3-6 and the AquaWoE_v1.0.xls / TEDtool_v1.0.xls files. In some cases, values presented in Chapter 2 are absent from the TED tool model inputs spreadsheets, and in other instances there are endpoints in Appendix 3-6 and the TED tool inputs that are not presented as threshold or endpoint values in Chapter 2 of the draft BE. There are also several instances of erroneous details in the study endpoints and/or references between these files.
- Finally, it is unclear how EPA selected some of the thresholds used in the modeling exercises. In Chapter 2, there is a table (or tables) of threshold values to be used in the Step 1 analysis for each taxon. For some taxa (but not consistently for all), there are tables of endpoints to be used as 'potential refinements', which are presumably for the Step 2 analyses. However, it is not explained which endpoints were selected for Step 2, how they were selected, or how they were used.

5 EFFECTS DETERMINATIONS (RISK CHARACTERIZATION)

5.1 General Comments

EPA's effects determination (the risk characterization) lacks complete transparency on how risk decisions or "calls" are made. For example, effects threshold values for aquatic and terrestrial species, presumably used to conduct Step 1 and 2, are presented in Chapter 2 of the BE (effects characterization). However, as shown in Section 4-3, these effects thresholds were not necessarily those that were used to make risk decisions in Steps 1 and 2. A great deal of effort was needed to decipher how risk decisions were made by comparing Chapter 2 threshold values with values used as inputs in EPA's predictive modeling tools. We found significant errors and discrepancies. At this point, we can confirm that EPA's Step 2 process used very few refinements and the end result are effects determinations conclusions that are based on hyperconservative approaches.

While investigating how the modeling tools, (e.g., TEDTool) were parameterized, we also found disturbing modeling decision rules, such as hyperconservative decision trees leading to "calls" based on unrealistic exposure and toxicity hypothesis. For instance, EPA notes in the BE that a "weight-of-evidence" (WoE) is used to make these calls. For the most part, the BE relies on a single line of evidence based on the risk quotient approach derived using exposure modeling predictions relative to a variety of toxicity thresholds, which is directly incongruous with the committee's conclusion that "RQs are not scientifically defensible for assessing the risks to listed species posed by pesticides" (NRC, 2013). We will further comment on these weight-of-evidence matrices in Section 5-2. Notwithstanding the fact that a true WoE (that uses independent lines of evidence) is not considered in making risk decisions, Cheminova has serious concerns regarding the use of a screening-level risk quotient approach to make regulatory decisions that is being passed off as refined analysis in Step 2. We have previously expressed these concerns in several documents (Breton, 2013a,b [MRID 49211702, 49211701], Breton et al. 2014b,c [MRID 49400601, 49400501]; Breton et al. 2016d,e [MRIDs Pending]). In short, a risk quotient exceeding 1 does not necessarily imply high risk. In fact, it implies that additional refined analysis is needed to make informed and realistic decisions as to the probability and magnitude of risk. A risk quotient approach provides no indication as to how small or large that probability might be. The threshold values used in Step 2 should no longer be the hyper-conservative 1-in-a-million threshold. An argument needs to be presented as to which level of effect will be acceptable. The one-in-a-million value would be more appropriate as a tier 1 assessment (Step 1) using the lowest endpoint, if relying on one data point only for a specific taxa (fish, aquatic invertebrates, etc.). For Step 2, a probabilistic approach is needed.

In EPA's WoE, toxicity is expressed as mortality, reproduction, growth, behavioral, sensory, indirect-prey, indirect-habitat, indirect-obligate, chemical stressors, and abiotic stressors. Each of these endpoints or attributes are assigned an equal weight, which is a logic flaw in the decision tree. Species undergoing sublethal effects such as behavioral, sensory and AChE inhibition effects are known to recover once exposure has ceased. These considerations were

completely ignored in EPA's modeling tools. The relevancy of these endpoints should be considered in EPA's single line of evidence. For example, when considering non-apical endpoints (such as behavioral, sensory and AChE inhibition effects) in their effects determinations, EPA has to link the causality of these endpoints to typical responses in ecological risk assessment. Of course, we are talking about mortality, growth and reproduction. As will be further discussed below, the risk conclusions for the direct-obligate relationships, chemical stressors and abiotic lines of evidence are not accounted for in the species call.

Confidence for each attribute in the risk quotient line of evidence is expressed as low, medium or high. Two points here: 1) it appears that EPA did not consider these confidence assignments in their "calls" for LAA and NLAA. We will expand on this point in Section 5-2. It is, therefore, a mystery why these confidence assignments are assigned in the first place, and 2) if only one of these attributes are assigned a medium or high designation, a LAA determination is declared. This approach completely ignores the results of the other attributes. Each attribute is considered on its own. This defies the meaning of "Weight-of-Evidence" where all lines of evidence are considered together to make a determination.

Moreover, although data on monitoring data, incident reports, mesocosm studies and field studies are presented in the BE, these other independent lines of evidence were not considered in the calls for NLAA or LAA. Cheminova has periodically provided updates of these data to EPA (Breton et al., 2013a,b [MRID 49211702, 49211701]; Breton et al., 2014b [49333901]). Updates to these data will also soon be provided to EPA in a refined ecological risk assessment for registration review of malathion (Breton et al., 2016 (in prep.); 2016a,b).

The science behind risk decisions for marine species and cave-dwelling terrestrial species is completely lacking. It is clear that calls for these species are completely determined by judgment of the EPA risk assessor with no information supporting these exposure hypothesis. EPA does acknowledge that there is uncertainty in these risk decisions. However, their assertion falls short of mentioning that these exposure hypothesis are completely unrealistic. We will provide more details comments on these qualitative assessments in Section 5-3.

As noted above, the EPA takes a highly conservative approach in assessing risks to listed species in their draft BE for malathion (EPA, 2016a). The use of risk quotients, a screening-level risk assessment (SLERA) approach, is indeed an uninformed and very conservative approach to conclude on effects determinations. If a risk quotient exceeds 1, then a refined probabilistic risk assessment needs be conducted to assess more environmentally realistic exposures and determine the magnitude of risk (NRC, 2013; EPA, 2004). Refined assessments should estimate the probability of effects of different magnitudes using concentration- and dose-response curves and probabilistic exposure modeling (Suter et al. 1993; EPA, 2004; NRC, 2013).

When assessing effects to an individual species using the risk curve approach, a concentration- or dose-response curve provides a suitable refined effects metric. When assessing indirect effects at the community level (e.g., aquatic invertebrate community, terrestrial plant community),

a species sensitivity distribution (SSD) provides a better understanding of the proportion of species likely to be affected by various levels of exposure. Overall, use of refined effects metrics such as concentration- or dose- response curves for individual species or SSDs for community receptors, joined with probabilistic exposure distributions, in a probabilistic risk assessment would provide a much more accurate description of risk than a deterministic risk quotient approach. Cheminova recommends that EPA recognize the limitations of a SLERA, actively participate in the design of a higher-tier risk assessment that considers all independent lines of evidence, and implements a true WoE approach to make regulatory decisions.

In their BE for malathion, EPA concluded that the use of malathion is LAA for the Kirtland's warbler (Setophaga kirtlandii) (Appendix 4-1; EPA, 2016a). Further, EPA estimated that the Kirtland's warbler would experience 15-35% mortality and reductions of up to 90% in reproductive fecundity when malathion is applied to pasture (Appendix 4-7; EPA, 2016a). Such effects were predicted to occur each year and that is just for one use pattern. Such effects are highly unlikely in the real world, however, given that the size of the Kirtland's warbler population is at its historical maximum. The current population size is nearly 10 times larger than it was at the time of listing and close to twice as large as the threshold stated in the primary objective (FWS, 2012a). Furthermore, the population size has surpassed recovery goals every year since 2001. FWS (2012b) reported that 2012 was a banner year for the Kirtland's warbler population, stating that it had exhibited a remarkable recovery of 12 times its population size over 25 years. It has been reported that a recommendation from FWS could come as early as 2017 to remove the Kirtland's warbler from the endangered species list (The Detroit News, 2016a,b). The evidence with respect to the recovery and health of the Kirtland's warbler population in the US is clearly inconsistent with the finding of the highly conservative conclusions of EPA's BE for malathion.

In their effects determination for malathion, EPA (2007) concluded that the use of malathion was LAA for the California red-legged frog (CRLF; *Rana draytonii*, formerly known as the subspecies *Rana aurora draytonii*). EPA made a similar determination of LAA for the delta smelt (DS; *Hypomesus transpacificus*) and for all three distinct population segments (DPSs) of the California tiger salamander (CTS; *Ambystoma californiense*) (EPA, 2010b). The Agency also concluded that malathion may modify the designated critical habitat of the DS and CTS. These conclusions were based on screening-level risk estimates.

Cheminova had a number of concerns with these EPA effects determinations and subsequently conducted refined effects determinations for the CRLF, DS, CTS and the KW. The purpose of these refined effect determinations was to illustrate the proper application of refined probabilistic methods when evaluating direct and indirect effects to these species. To assess the effects of malathion to the CRLF, DS, CTS and KW and their respective prey and habitat, the effects determinations completed by Cheminova used a tiered approach that started with a screening-level assessment followed by more refined probabilistic analyses. The overall approach is conceptually similar to the tiered approach that EPA uses for ecological risk assessment (EPA, 1998; 2004; NRC, 2013), in that increasingly refined analyses are only conducted for use patterns for which concern was not eliminated in a previous tier. Thus, only those use patterns

for which a presumed risk remains proceed to the next tier. Cheminova's detailed refined effects determinations for these species are provided in Breton et al. (2013a [MRID 49211702]; 2016a,b) and in Moore et al. (2016).

In the SLERA of Cheminova's EDs, estimated environmental concentrations (EECs) of malathion in surface water were predicted using the Pesticide Root Zone Model/Exposure Analysis Modeling System (PRZM/EXAMS). Additionally, the Pesticides in Flooded Applications Model (PFAM) was used in the CTS and DS assessments and the Tier II Rice WQ model was used for rice scenarios in the CRLF assessment. For the refined analyses, the Soil and Water Assessment Tool (SWAT) was used for the CRLF and DS assessments. The CRLF analysis also used the Vegetative Filter Strip Modeling System (VFSMOD). A custom approach to modelling malathion was used for the CTS given its unique habitat, which included the PRZM model and the Variable Volume Water Model (VVWM) provided as part of the Surface Water Concentration Calculator (SWCC).

To ensure that the best available data were used in selecting measures of ecological effects, Cheminova developed study evaluation criteria that were applied uniformly, in a transparent manner, to GLP studies conducted using standard guidelines, open-literature studies and other reports. For an example of such study evaluation criteria see Breton et al. (2014a [MRID 49333901]).

The refined effects determination used a similar approach as the screening-level effects determination except that distributions were used instead of conservative deterministic point estimates as recommended by NAS. Exposure distributions were integrated with the appropriate dose-response curve or acute or chronic species sensitivity distributions (SSD) to derive risk curves. Each risk curve illustrates the cumulative probability of effects of differing magnitude. The area under the risk curves (% AUC) were used to categorize risk for each of the scenarios as either *de minimis*, low, intermediate or high. Other lines of evidence, including aquatic field studies, mesocosm studies, surface water monitoring data, and incident reports, were considered in the weight-of-evidence risk characterization for the CRLF, DS and CTS.

For the CRLF, the screening-level effects determination ruled out risk to the terrestrial-phase CRLF, which were therefore not considered in the refined assessment. The refined effects determination indicated *de minimis* risk to aquatic-phase CRLF and fish (prey). Although the quantitative assessment suggested low risks to invertebrates (prey) for some scenarios and intermediate risk for a single scenario, consideration of results from other lines of evidence suggests that invertebrate prey populations of the CRLF are not likely to be adversely affected. In the refined effects determination for the DS, acute direct risk was categorized as de *minimis* for all scenarios. Acute and chronic risk to aquatic invertebrate prey was categorized as *de minimis* to low with the exception of one scenario for which chronic risk was classified as intermediate. Chronic effects to aquatic species are highly unlikely because malathion degrades almost instantly in aquatic environments, particularly in marine environments. Thus, malathion is unlikely to be present in the environment at chronic exposure levels that would result in effects to aquatic taxa. The weight-of-evidence indicates that malathion uses would rarely be

associated with acute and chronic effects to the aquatic invertebrate prey of DS, and in instances where adverse effects are observed, recovery is expected to occur quickly once exposures have ceased.

The conservative screening-level effects determination for the CTS indicated that potential risk to aquatic- and terrestrial-phase CTS, aquatic-phase amphibian prey of the CTS and the mammalian species on which terrestrial-phase CTS depend for habitat are unlikely. The refined assessment found overall risk to be low for indirect effects to CTS via reduction of their aquatic invertebrate prey.

The weight-of-evidence, which included consideration of field studies, mesocosm studies, monitoring data and incident reports, in the refined effects determinations completed by Cheminova indicated that malathion use is not likely to adversely affect the CRLF, DS or CTS and their respective prey. Additional details on how these lines of evidence were considered are presented in Cheminova's respective refined effects determinations. The refined assessments incorporated more realistic and site-specific input parameters in comparison to the overly conservative assumptions used by the EPA in their effects determinations for these species. Therefore, the results of Cheminova's refined effects determinations reach scientifically defensible conclusions that are appropriate for use in regulatory decision-making for malathion.

For the Kirtland Warbler, a refined avian risk assessment was conducted using models developed to simulate breeding season as well as spring and fall migration (Moore et al., 2016). This assessment applied species-specific life history data (e.g., feeding, breeding and migration data) and chemical-specific toxicity and fate data to estimate acute and chronic risk to Kirtland's warbler potentially exposed to malathion. Results indicate very low acute and chronic risk of malathion to Kirtland's warbler, with no exceedances of the most sensitive avian LD50, and one exceedance of the chronic NOEL out of the 15 use patterns modeled. The probability of risk of acute exposure during migration was determined to be 0% for both spring and fall migrations. The maximum predicted effect on breeding was an average reduction in fecundity of 0.198% for chronic exposure in breeding areas after aerial application to alfalfa.

The above clearly shows that EPA are using highly conservative approaches to develop their effects determinations. These approaches are based on unrealistic exposure scenarios and use a single line of evidence that is itself flawed with logic, filled with mathematical errors, and is non-transparent. All of which misleads the public into thinking that listed species and the critical habitat on which they depend are imperiled by malathion. Many of these points will be tackled in the following sections. Section 5.2 presents our analysis of the model outputs, decision trees and weight-of-evidence matrices for aquatic and terrestrial effects determinations. Section 5.3 presents comments on EPA's qualitative assessment of listed species. Lastly Section 5-4 discusses the assumptions and evaluation of the mosquito adulticide use of malathion.

5.2 Weight-of-Evidence Tools and Species and Critical Habitat Calls

The vast majority of the species and critical habitats considered in the BE (1686/1782 species, and 763/795 critical habitats) screened through Step1 and were assessed using the Agency's

WoE tools (https://www.epa.gov/endangered-species/provisional-models-endangered-species-pesticide-assessments#woe). The exceptions were only:

1. Species presumed extinct, who received the call of “No Effect”.
2. Species that no longer occur in the US, who received the call of “No Effect”.
3. Species that only exist in captivity, who received the call of “No Effect”.
4. Species found outside the action area, who received the call of “No Effect”.
5. Sea turtles, whales, deep sea fish, marine mammals (excluding whales), cave-dwelling invertebrates, lichens, pinnipeds and otters were all assessed separately.

This section focuses on the species and critical habitat calls made in the WoE tools. The WoE tools are a series of Excel workbooks available on the Provisional Models webpage (https://www.epa.gov/endangered-species/provisional-models-endangered-species-pesticide-assessments#woe). The tools include “root files”:

- TEDtool_v1.0.xlsx
- TEDtool_v1.0_alt.xlsx
- Data input template.xlsx
- TerrWoE_v1.0.xlsx
- AquaWoE_v1.0.xlsx

As well as species template files:

- Species_Animal_Template.xlsx
- Species_Aquatic_Template.xlsx
- Species_Aquatic_Plant_Template.xlsx
- Species_Plant_Template.xlsx

According to the “WoE_read_me” text file that comes with the WoE tools (i.e., in the downloadable zip file), to use the tools properly the five root files must be opened simultaneously, and the correct species-specific template file must also be opened. On the ‘WoE Matrix’ worksheet of the template file the species can be changed by entering species number in cell C3. This action will update the species ‘WoE Matrix’ and the ‘Summary Sheet’ to reflect the details of the selected species.

Table 1-8 in Section 1.4 describes the thresholds to be used in the effects determinations. For terrestrial animals, the table lists four thresholds:

1. Mortality – direct effects: 1/million mortality (from Table 1.6 in the BE)
2. Mortality – indirect effects: “Concentration (or dose) that would result in a decrease of 10% of individuals (i.e., the EC_{10}). This is calculated by using HC_{05} of SSD of LC_{50}/LD_{50} or EC_{50} values and representative slope. If SSD cannot be derived, most sensitive LC_{50}/LD_{50} or EC_{50} will be used.”

3. Sublethal – direct effects: “Lowest available NOAEC/NOAEL or other scientifically defensible effect threshold (EC_x) that can be linked to survival or reproduction of a listed individual will be used.”
4. Sublethal – indirect effects: “LOAEC/LOAEL for growth or reproduction will be used (see text for details).”

However, many more thresholds were used in the WoE tools and the species and critical habitat calls. There is clearly inconsistencies in the approach presented in the analysis plan, and what was actually carried out in the effects determinations. The text of the analysis plan refers only to mortality and sublethal threshold categories, whereas the WoE tools consider measures of effects falling into ten distinct categories of effects, seven of which are employed in the species and critical habitat calls for terrestrial species (See Section 5.2.2 below). One of these is mortality, the rest can be considered sublethal. For instance, the Agency states that its sublethal threshold for direct effects will be the “Lowest available NOAEC/NOAEL or other scientifically defensible effect threshold (EC_x) that can be linked to survival or reproduction of a listed individual will be used.” However, in the WoE tools, the EPA considers exceedances of the following thresholds in their risk designations, and their species calls:

1. growth NOELs and LOELs, and application-rate based growth thresholds in lb a.i./A,
2. reproduction NOELs and LOELs, and application-rate based reproduction thresholds in lb a.i./A,
3. behavioral NOELs and LOELs, and application-rate based behavioral thresholds in lb a.i./A,
4. the assigned direct sublethal threshold in mg/kg diet (which is assessed in the behavioral risk designation calculations), and;
5. sensory NOELs and LOELs, and application-rate based sensory thresholds in lb a.i./A.

This inconsistency needs to be dealt with for the final BE.

5.2.1 Aquatic Species and Critical Habitat Calls

On the ‘Summary Sheet’ worksheet of the Species_Aquatic_Animal_Template.xls and Species_Aquatic_Plant_Template.xls files, risk conclusions for ten categories of measures of effects, as well as the “Species Call” and “Critical Habitat Call” are presented for whichever species is selected, based on species number, in cell C3 on the ‘WoE Matrix’ worksheet in the same file. Table 5-1 lists the risk conclusions and their possible designations.

Table 5-1 Summary of potential species risk conclusions from WoE matrix[a]

Measure of Effects/Calls	*Risk Designations*	*Confidence Designations*
Mortality	LOW, MED, HIGH, unknown or NA	LOW, MED, HIGH or NA
Growth		
Reproduction		
Behavioral[b]		
Sensory[b]		
Indirect-Prey[b]		
Indirect-Habitat		
Indirect-Obligate		
Chemical Stressors		

Abiotic Stressors		
Species Call[c]	LAA or ""[d]	NA
Habitat Call[c]	NLAA, LAA or NA	NA

NA = Not applicable

[a] From the 'Summary Sheet' worksheet in the Species_Aquatic_Animal_Template.xls and Species_Aquatic_Plant_Template.xls files

[b] Measure of effects in the Species_Aquatic_Animal_Template.xls file only

[c] The Species Call and Habitat Call refer to the effects determinations made for species and their critical habitat, respectively.

[d] If not "LAA", Species Call is left blank

The "Species Call" is found in cells X6 and R6 on the 'Summary Sheet' for aquatic animals and aquatic plants, respectively, and is a function of risk designations assigned to some, but not all, of the measures of effects presented in Table 5-1. A likely to adversely affect (LAA) "Species Call" is made if the risk designation for one or more of:

- mortality,
- growth,
- reproduction,
- behavioral (animals only),
- sensory (animals only),
- indirect - prey (animals only), or
- indirect – habitat.

is MED or HIGH. Otherwise, the entry for the "Species Call" is blank. It is perplexing that the EPA would even have separate risk designations of MED and HIGH, since either finding results in an LAA call.

The confidence in the risk designations for measures of effects are not accounted for in the "Species Call" formulae (cells X6 and Y6 on the 'Summary Sheet' worksheet). In fact, for aquatic animals, the effects determination is computed as LAA so long as at least one of seven risk designations listed above is medium or high (MED or HIGH), regardless of the confidence designations. This is not expressed in the analysis plan (Section 1.4 of Chapter 1 of the BE), and is contradictory to a legitimate weight of evidence approach that accounts for evidence both for and against a particular risk hypothesis.

Also, risks associated with indirect-obligate relationships, chemical stressors and abiotic stressors are not accounted for in the "Species Call". Given the absence of documentation surrounding these categories of effects and associated risk and confidence designations, it is unclear whether this apparent omission was intentional or not. Some clarification from the EPA is required.

The risk designations for all categories of effects considered in the "Species Call" originate on the 'WoE Matrix' worksheet in the same file. Unfortunately, columns J through IF of the 'WoE Matrix' worksheet are hidden and locked. Without unlocking these cells, the only way to determine their contents is to copy the worksheet into a new unlocked workbook. This was done to determine exactly how the EPA established their risk designations for their numerous

categories of effects. In the sections below, we comment on the calculations used to establish HIGH, MED and LOW risk designations for the various categories of effects in the Species_Aquatic_Animal_Template.xls and Species_Aquatic_Plant_Template.xls files that were subsequently used to establish the species and critical habitat calls for aquatic animals.

The following subsections cover the categories of effects that were considered in the species and critical habitat calls made on cells X6 and Y6 of the 'Summary Sheet.' Because of the insufficient time to review the BE and its attachments within the 60-day public comment period, these comments do not address the following categories of effects found in the 'WoE Matrix': indirect-obligate relationships, chemical stressors and abiotic stressors. Notably, the risk designations for these categories were ultimately not used in the species and critical habitat calls made in the WoE tools.

The critical habitat call is given in cell Y6 and S6 of the "Summay Sheet" worksheet of the Species_Aquatic_Animal_Template.xlsx and Species_Aquatic_Plant_Template.xlsx files, respectively. Any LAA determinations for critical habitat are entirely dependent on the species call also being LAA. Given the concerns and inconsistencies identified with the species calls discussed below, the critical habitat calls are also highly questionable.

5.2.1.1 Mortality

The risk designation for mortality is determined in cell ED9 of the 'WoE Matrix' worksheet. Based on the formula in the cell, the risk designation for aquatic animals and plants is LOW if neither the minimum nor the maximum EECs from all bins and HUC2 regions exceed the mortality endpoints outlined in Appendix K. If the maximum EECs but not the minimum EECs exceed the mortality endpoints, risk is designated as MED. Finally, if both minimum and maximum EECs exceed any mortality endpoint, the risk designation is HIGH.

These calculations are inconsistent with the methods for establishing risk conclusions described in Attachment 1-9 of the BE. On page 9 of Attachment 1-9, EPA states that, when assessing direct effects to animals and plants, MED risk conclusions may be made for a line of evidence if exposure exceeds an endpoint where no effects to species are observed (e.g., one-in-a-million chance of mortality or NOEC) but not an endpoint where effects were observed (e.g., EC25, LC50, LOAEC). However, as described above, EPA's calculations for concluding MED risk in the 'WoE Matrix' worksheet are not based on which endpoints are exceeded by EECs, but rather whether exceedances of any threshold or endpoint were associated with the minimum or maximum EECs.

Furthermore, as described in Sections 5.2.1.2 and 5.2.1.3 below, EPA compared EECs based on spray drift only to their spray drift effects thresholds (Cells AJ9:AZ10 of the 'WoE Matrix' worksheet) to calculate the "Distance to Threshold". This information is considered when making risk conclusions for indirect effects. Although EPA calculates the "Distance to Threshold" for direct effects endpoints in the 'Spray Drift all' worksheet of the AquaWoE_v1.01.xls file, this information is not considered when making risk conclusions for direct effects. EPA provides no

rationale as to why the spray drift only calculations would be made for indirect effects and not direct effects conclusions.

5.2.1.2 Sublethal Effects

The risk designations for sublethal endpoints are determined in the following cells of the 'WoE Matrix' worksheet:

- growth: cell ED19
- reproduction: cell ED26
- behavior (animals only): cell ED33
- sensory (animals only): cell ED41

As described for the mortality endpoints, risk for the growth, reproduction, behavior and sensory lines of evidence are designated as LOW if neither the minimum nor the maximum EECs exceed effects endpoints, MED if only the maximum EECs exceed effects endpoints, and HIGH if both the minimum and maximum EECs exceed one or more effects endpoints (See Appendix K for further details). Again, these calculations are inconsistent with the methods for establishing risk conclusions described in Attachment 1-9 of the BE. Also, as discussed in Section 5.2.1.1, EPA did not consider the spray drift only calculations made in the 'Spray Drift all' worksheet of the AquaWoE_v1.01.xls file when making their risk conclusions for direct effects for the growth, reproduction, behavior and sensory lines of evidence.

In their RQ calculations, EPA divides peak EECs by all sublethal thresholds, regardless of the exposure duration used to derive the endpoint. The majority of sublethal endpoints for aquatic taxa (e.g., survival, growth, reproduction and behavior) were obtained from chronic studies and were associated with exposure durations of up to 40 days. Thus, comparison of these endpoints to peak EECs is inappropriate and considerably overestimates risk to aquatic species. Furthermore, these calculations are inconsistent with the approach outlined in Table 1-7 of Chapter 1 of the BE, where EPA states that "estimated exposure values or field-scale monitoring studies relevant to the duration of exposure that elicited the effect(s)" will be compared to endpoints for growth, reproduction, behavior and sensory function.

5.2.1.3 Indirect Effects - Prey

The risk designations for indirect effects due to prey item exposures are determined in cells ED54 of the 'WoE Matrix' worksheet of the Species_Aquatic_Animal_Template.xls file. Based on the formulas in these cells, only LOW or HIGH risk can be concluded (See Appendix K for further details). This is inconsistent with EPA's methods for applying their weight of evidence approach in Step 2 (Attachment 1-9), which states that MED risk conclusions may be made if "exposure is below both the mortality and sublethal indirect threshold for some animal taxa but above for other animal taxa. This may be influenced by available information on the relative importance of the animal taxa to the assessed species (e.g., primary dietary item)".

Risk is designated as LOW if minimum and maximum EECs are below the indirect mortality and sublethal thresholds for prey items of the listed species AND if the "Distance to Threshold" calculated for prey items in the 'Spray Drift all' worksheet of the AquaWoE_v1.0.xls file is zero feet (See Appendix K for further details). As mentioned above in Section 5.2.1.1, the comparison of EECs for spray drift only to effects metrics is inconsistent with the approaches taken to assess direct effects to aquatic animals and plants.

In the RQ calculations performed for indirect effects to prey (cells AG40:AR42 of the 'WoE Matrix' worksheet), aquatic-phase amphibians are not included as potential prey items. Although this may not affect EPA's results since the same effects metrics were used for aquatic-phase amphibians, freshwater fish and marine/estuarine fish, it does not allow for separate effects metrics to be used for aquatic-phase amphibians in cases where amphibian data are available.

Furthermore, as mentioned above in Section 5.2.1.2 peak EECs are compared to sublethal effects thresholds, which represents a mismatch between exposure and effects data. EPA's sublethal thresholds were often obtained from chronic toxicity studies and, therefore, the calculated RQs for these endpoints are extreme overestimates of risk to aquatic species.

5.2.1.4 Indirect Effects - Habitat

The risk designations for indirect effects due to prey item exposures are determined in cells ED61 of the 'WoE Matrix' worksheet of the Species_Aquatic_Animal_Template.xls and Species_Aquatic_Plant_Template.xls files. Based on the formulas in these cells, only LOW or HIGH risk can be concluded. However, as was observed for prey item risk conclusions (Section 5.2.1.3), Attachment 1-9 of the BE indicates that, when evaluating indirect effects due to impacts on plants (i.e., habitat), MED risk conclusions can be made if the "exposure is below the lowest EC25 (terrestrial) or EC50 (aquatic) for some plant types but above for other plant types."

Risk is designated as LOW if minimum and maximum EECs are below the indirect mortality and sublethal thresholds for prey items of the listed species AND if the "Distance to Threshold" calculated for prey items in the 'Spray Drift all' worksheet of the AquaWoE_v1.0.xls file is zero feet (See Appendix K for further details). As mentioned above in Section 5.2.1.1, the comparison of EECs for spray drift only to effects metrics is inconsistent with the approaches taken to assess direct effects to aquatic animals and plants.

5.2.2 Terrestrial Animal Species and Critical Habitat Calls

On the 'Summary Sheet' worksheet of the Species_Animal_Template.xls file risk conclusions for ten categories of effects, as well as the species call and critical habitat calls are presented for whichever species is selected, based on species number, in cell C3 on the 'WoE Matrix' worksheet in the same file. Table 5-2 below lists the measures of effects and their possible risk designations.

Table 5-2 Summary of potential species risk conclusions from WoE matrix[a]

Categories of Effects/Calls	*Risk Designations*	*Confidence Designations*
Mortality	LOW, MED, HIGH, unknown or NA	LOW, MED, HIGH or NA

Growth		
Reproduction		
Behavioral		
Sensory		
Indirect-Prey		
Indirect-Habitat		
Indirect-Obligate		
Chemical Stressors	User input[b]	User input[b]
Abiotic Stressors	User input[b]	User input[b]
Species Call[c]	LAA or ""[d]	NA
Habitat Call[c]	NLAA, LAA or "NA"	NA

NA: not applicable

[a] From the 'Summary Sheet' worksheet in the Species_Animal_Template.xls file

[b] These risk designations and associated confidence designations are pulled from the "Chemical abiotic – general" worksheet in the Data input template.xlsx file. These designations are text inputs (i.e., not formula based).

[c] The Species Call and Habitat Call refer to the effects determinations made for species and their critical habitat, respectively.

[d] If not "LAA", Species Call is left blank

The species call is found in cell X6 on the 'Summary Sheet', and is a function of risk designations assigned to some, but not all, of the categories of effects presented in Table 5-42. A likely to adversely affect (LAA) species call is made if the risk designation for one or more of:

- mortality,
- growth,
- reproduction,
- behavioral,
- sensory,
- indirect-prey, or;
- indirect-habitat

is MED or HIGH. Otherwise, the entry for the species call is blank. It is perplexing that the EPA would even have separate risk designations of MED and HIGH, since either finding results in an LAA call.

There is no accounting for the confidence designation in the call formulae (cells X6 and Y6 on the 'Summary Sheet' worksheet). In fact, for terrestrial animals the effects determination is computed as LAA so long as at least one of seven risk designations is medium or high (MED or HIGH), no matter what the confidence designations are (Table 5-42). This is not expressed in the analysis plant of Section 1.4, and is contradictory to a legitimate weight of evidence approach that accounts for evidence both for and against a particular risk hypothesis.

Risks associated with indirect-obligate relationships, chemical stressors and abiotic stressors are not accounted for in the species call. Given the absence of documentation surrounding these categories of effects and associated risk and confidence designations, it is unclear whether this apparent omission was intentional or not. Some clarification from the EPA is required.

The risk designations for all categories of effects considered in the species call originate on the 'WoE Matrix' worksheet in the same file. Unfortunately, columns J through IF of the 'WoE Matrix'

worksheet are hidden and locked. Without unlocking these cells, the only way to determine their contents is to copy the worksheet into a new, unlocked workbook. This was done to determine exactly how the Agency established their risk designations for their numerous categories of effects. The categories are discussed below. We first start with an evaluation of the Species_Animal_Template.xlsx that was used to establish the species and critical habitat calls for terrestrial animals. Subsequently, we discuss the the Species_Plant_Template.xlsx used to evaluate the species and critical habitat calls for terrestrial plants.

For terrestrial animal species assessed with the WoE tools, the 'WoE Matrix' and the species and critical habitat calls are made in the Species_Animal_Template.xlsx. The following subsections cover the categories of effects that were considered in the species and critical habitat calls made on cells X6 and Y6 of the 'Summary Sheet.' Because of the insufficient time to review the BE and its attachments within the 60-day public comment period, these comments do not address the following categories of effects found in the 'WoE Matrix': indirect-obligate relationships, chemical stressors and abiotic stressors. Notably, the risk designations for these categories were ultimately not used in the species and critical habitat calls made in the WoE tools.

Since either a MED or HIGH risk designations for any of the categories of effects in the bullet list above results in an LAA species call in the WoE Matrix, it is questionable why the Agency would want to make the distinction. In terms of the method for distinguishing between MED and HIGH risk designation, the process used by the Agency is dubious and not at all intuitive (See Appendix L). In Attachment 1-9, the EPA states that a MED risk designation is applied "if exposure exceeds the threshold or lowest endpoint (if a threshold is not available) but not an endpoint where effects were observed." In some instances, only specific exposure units are considered in the designation of MED risk (e.g., mortality of terrestrial invertebrates). In other cases, thresholds at which effects were observed may be exceeded by some exposure estimate, and a risk designation of MED may still apply (e.g., growth). Also, in many instances approaches seem to vary depending on the application rate being considered. There appears to be a paucity of consistency across the categories of effects considered in the species call. That said, for the species call of terrestrial animals, no credence was given to the differences between MED and HIGH risk designations. As such, this section focuses on the risk designations that differentiate LAA and NLAA calls; that is, LOW versus MED/HIGH risk designations.

For sublethal thresholds discussed below, EPA has decided to use NOELs as threshold values. Repeatedly, if a NOEL is exceeded by a conservative estimate of on-field exposure, the species call is 'Likely to Adversely Affect' (LAA). There is no justification for such a conclusion, given that no effects are observed at the threshold value in the supporting toxicity test. Also, by definition the upper bound exposure estimates are in fact unlikely. In the context of the protection goals, there is no evidence to suggest that NOEL exceedance would result in adverse effects to individual fitness to a species.

These NOELs are compared to peak exposure estimates, with no accounting for the fact that the exposures in the chronic toxicity tests supporting the effects metrics likely exceeded one day and may have been weeks, months or even years before effects were observed in the LOEL treatment group. The conclusion that a NOEL exceedance for one day establishes that a species is likely to be adversely affected is inadequate on its own, let alone that the exposure estimates are on-field, upper bound, and worst-case (See Appendix L).

5.2.2.1 Mortality

The risk designation for mortality is determined in cell DI10 of the 'WoE Matrix' worksheet. Based on the formula in the cell, the criteria for LOW, MED and HIGH risk for terrestrial animals are presented in Tables L-1 and L-2 of Appendix L.

A crucial error in the WoE Matrix worksheet with respect to the determination of the risk designation for mortality of terrestrial vertebrates is that *dose-based thresholds* in units of mg/kg bw are compared with estimates of *concentration in diet* in units of mg/kg diet, as opposed to body burden estimates for the listed species. This occurs at six times in the determination of the risk designation for mortality for all terrestrial vertebrate species (see Table L-1 in Appendix L). Concentrations in food are generally higher than dose estimates. On average, for malathion upper bound dietary estimates in mg/kg diet for the multiple application scenario were on average 15 times higher than associated dose based estimates in mg/kg bw. Thus, this error results in a considerable and overestimation of exposure, and ultimately risk, for terrestrial vertebrate species in the EPA's biological evaluations.

Even with this error corrected, the approach to mortality risk designation is lacking, particularly in light of the NRC (2013) recommendations. *If the estimated 1/million mortality threshold is exceeded by any of the conservative estimates of on-field exposure, the species call is LAA*. Thus, the species call, as it relates to the mortality risk designation is based on a hyper-conservative risk quotient, and does not account for, among other things: the potential exposure distribution on treated fields (i.e., upper bound residues and T90 half-lives are assumed), or the fact that time on treated fields will vary among individuals, among species and over time. Further, the Agency provides no evidence to support the 1/million mortality threshold *on treated fields* as being directly relevant to a listed species individual fitness. If a species doesn't regularly use managed lands to which pesticides are applied, the 1/million mortality threshold on treated fields is tremendously inappropriate.

For terrestrial invertebrates, in addition to the 1/million threshold, the EPA also compares the maximum single application rate to a mortality NOEL. *If the NOEL is exceeded the species call is LAA*. As indicated above, such a comparison is totally inappropriate.

5.2.2.2 Growth and Reproduction

The risk designations for growth and reproduction are determined in cells DI19 and DI26 of the 'WoE Matrix' worksheet. Based on the formula in the cells, the criteria for LOW, MED and HIGH risk for terrestrial animals are presented in Tables L-3 through L-6 in Appendix L. Measures of

effects to growth were not mentioned as sources of sublethal thresholds in Table 1-8 of the analysis plan (Section 1.4). The driving RQs for the growth and reproduction risk designations are peak (one-day) upper bound exposure estimates following the maximum application rates (single and multiple) divided by the growth NOELs. *If a NOEL is exceeded the species call is LAA*.

The species call and critical habitat call is also dependent on whether or not application-rate based thresholds for growth and reproduction are exceeded. For malathion, no such thresholds were provided for mammals, birds or terrestrial herptiles. However, cells calculating the risk designations erroneously use the *reproduction* threshold for the growth risk designation for birds and the *behavioral* threshold for the reproduction risk designation for birds (See Tables L-3 and L-5 in Appendix L).

For invertebrates, growth and reproduction NOELs in mg/kg soil are also considered, and directly impact the species call. It appears that in error, the calculation considering this threshold does not check that the listed invertebrate is in fact a soil-dwelling invertebrate. For invertebrates that do not inhabit soil it is completely illogical to consider such a threshold. *If a NOEL is exceeded the species call is LAA.*

5.2.2.3 Behavioral and Sensory

The risk designation for behavioral and sensory effects is determined in cell DI33 and DI41 of the 'WoE Matrix' worksheet. Based on the formula in the cells, the criteria for LOW, MED and HIGH risk for terrestrial vertebrate animals are presented in Tables L-7 through L-10 in Appendix L.

As with the growth and reproduction risk designations, the behavioral and sensory risk designations are LOW only if there are no NOEL exceedances in one-day peak upper bound exposure estimates following the maximum applications (single maximum and multiple applications).

Further, as was the case for birds in the risk designations for growth and reproduction, the sensory application-rate based threshold was considered for the behavioral risk designation, and the mortality rate-based threshold was considered for the sensory risk designation. Fortunately, for malathion, no values were provided for these thresholds, and therefore, the error was not propagated through the assessment. However, these errors should be addressed in the final BE of the WoE tools.

Finally, in the calculation for the behavioral risk designation is a check for exceedances of the direct sublethal threshold (mg/kg diet). For birds and terrestrial herptiles, this approach is as described in the analysis plan, a reproduction NOEL. However, for mammals a threshold of 20 mg/kg diet based on AChE inhibition is employed (See Table L-7 in Appendix L). For dose-based direct sublethal thresholds, AChE inhibition thresholds were used bor birds and mammals, and a motility threshold was used for terrestrial herptiles. *If any direct sublethal threshold, or any behavioral NOEL is exceeded the species call is LAA*.

For invertebrates, a behavioral NOEL in mg/kg soil is also considered, and directly impact the species call (though for malathion, no such metric was applied). Again, it appears that in error, the calculation considering this threshold does not check that the listed invertebrate is in fact a soil-dwelling invertebrate.

5.2.2.4 Indirect – Prey

The risk designation for indirect effects due to exposure of prey base/dietary items is determined in cell DI54 of the 'WoE Matrix' worksheet. Based on the formula in the cell, the criteria for LOW and HIGH risk for terrestrial vertebrate animals are presented in Table L-11 in Appendix L. Notably, there is no MED risk designation for indirect effects to prey in the WoE tools.

In Attachment 1-9, the Agency said that for indirect effects due to impacts on animals: "MED: If exposure is below both the mortality and sublethal indirect threshold for some animal taxa but above for other animal taxa. This may be influenced by available information on the relative importance of the animal taxa to the assessed species (e.g., primary dietary item)." Here is another example of where the EPA described a method that was ultimately not implemented in the effects determinations.

Of the prey/feed items consumed by the listed species, if any of their indirect thresholds in Table L-11 are exceeded by the upper bound exposure estimates with the maximum application rates (single maximum or multiple applications), the risk designation for indirect effects associated with prey/feed items is set to HIGH in cell DI54. This leads to a LAA call for the species. Several crucial mistakes were identified in the calculation of this risk designation.

First, the risk designation calculation is counting exceedances of indirect dietary thresholds of prey for feed items (i.e., thresholds for the diets of the prey) that are unlikely to be consumed by the prey of the listed species. For instance, in the assessment of California red-legged frog (CRLF), the risk designation for effects to prey is set to HIGH if the concentration of the pesticide in large carrion, small birds and small mammals exceeds the indirect dietary threshold for small mammals (prey of CRLF) for one day. However, it is implausible that CRLF, which consume mostly invertebrates, would be dependent on small mammals that only consume other vertebrates in pesticide treated areas with upper bound residues. In all likelihood, mammals that consume vertebrates, are too large to be prey of CRLF. Thus, this assessment of CRLF is implausible.

Second, in the Interagency Interim Approaches document, the threshold for indirect sublethal effects in Step 2 was deemed either a LOEC for growth or reproduction (Agencies, 2013). However, in the risk designation for indirect effects due to effects to prey/feed items, the dose-based indirect sublethal thresholds for mammal and bird prey was based on AChE inhibition, as was the diet-based indirect sublethal thresholds for mammals. Estimated reductions in the AChE inhibition of prey is NOT evidence of adverse effects to the predator, and should not be

construed as such. Erroneously in the risk designation for indirect effects due to effects on prey/feed items, if these exceptionally low thresholds are exceeded, the species call is LAA.

5.2.2.5 Indirect – Habitat

The risk designation for indirect effects due to exposure of habitat is determined in cell DI61 of the 'WoE Matrix' worksheet. Based on the formula in the cell, the criteria for LOW, MED and HIGH risk for terrestrial vertebrate animals are presented in Table L-12.

First, there is a check to determine whether the species is associated with aquatic habitat. If so, terrestrial plant thresholds cannot be exceeded by either run-off to dry area, run-off to semi-aquatic area and drift EECs. As described in the Interagency Interim Approaches document, the most sensitive LOECs and EC25s were used to determine the risk designation for indirect effects to habitat. However, these thresholds were compared directly with the highest single application rate (which for malathion reportedly only applies to home owner garden use on woody ornamentals). It is questionable whether or not a LOEC or EC25 exceedance on a residential property could in fact lead to adverse effects to listed species fitness. Given that *any LOEL or EC25 threshold exceedances results in a LAA call for the species*, this warrant further investigation.

5.2.2.6 Critical Habitat Call for Terrestrial Animals

The critical habitat call is given in cell Y6 of the "Summay Sheet" worksheet of the Species_Animal_Template.xlsx. If the species has an assigned critical habitat and there is less than or equal to 0.005% overlap with any one assessed use pattern, then the critical habitat call is "Not Likely to Adversely Affect" (NLAA). Alternatively, if there is overlap >0.005%, and the species call is "Likely to Adversely Affect" (LAA), than the critical habitat call is also LAA. Otherwise, the critical habitat call is assigned "NA," or not applicable.

Given the concerns regarding, and the errors identified in the species calls discussed above, the critical habitat calls in the BEs also bear miscalculations. These mistakes may be rectified by addressing the issues in the species call calculations.

5.2.3 Terrestrial Plant Species and Critical Habitat Calls

On the 'Summary Sheet' worksheet of the Species_Plant_Template.xls file, risk conclusions for ten categories of measures of effects, as well as the species call and "Critical Habitat Call" are presented for whichever species is selected, based on species number, in cell C3 on the 'WoE Matrix' worksheet in the same file. Table 5-3 lists the measures of effects and their possible risk designations.

Table 5-3 Summary of potential species risk conclusions from WoE matrix[a]

Measure of Effects/Calls	*Risk Designations*	*Confidence Designations*
Mortality	LOW, MED, HIGH or unknown	unknown, LOW, MED or HIGH
Growth	LOW, MED, HIGH or unknown	unknown, LOW, MED or HIGH
Reproduction	LOW, MED, HIGH or unknown	unknown, LOW, MED or HIGH
Behavioral	NA	NA

Table 5-3 Summary of potential species risk conclusions from WoE matrix[a]		
Measure of Effects/Calls	***Risk Designations***	***Confidence Designations***
Sensory	NA	NA
Indirect/Pollinator/Diaspore Dispersal	LOW or HIGH	LOW, MED or HIGH
Indirect-Habitat	LOW, MED or unknown	LOW, MED or HIGH
Indirect-Obligate	NA, LOW or HIGH	NA, LOW, MED or HIGH
Chemical Stressors	User input[b]	User input[b]
Abiotic Stressors	User input[b]	User input[b]
Species Call	LAA or ""	NA
Habitat Call	NLAA, LAA or "NA"	NA

[a] From the 'Summary Sheet' worksheet in the Species_Animal_Template.xls file

[b] These risk designations and associated confidence designations are pulled from the "Chemical abiotic – general" worksheet in the Data input template.xlsx file. These designations are text inputs (i.e., not formula based).

The species call is found in cell X6 on the 'Summary Sheet', and is a function of risk designations assigned to some, but not all, of the measures of effects presented in Table 5-3, if the call is not already specified for the species in a list presented in columns AP through AR on the same sheet. If the call is not already specified in the list, a likely to adversely affect (LAA) species call is made if the risk designation for one or more of:

- mortality,
- growth,
- reproduction,
- behavioral,
- sensory,
- indirect/pollinator/diaspore dispersal, or;
- indirect-habitat

is HIGH. Otherwise, the entry for the species call is blank.

The list with predetermined Species and Critical Habitat Calls (i.e., effects determination) in the "Summary Sheet" of the Species_Plant_Template.xlsx file is of unknown origin. The list presents fixed calls for 127 plants species, with no formulae or reference to the origin of the calls. It is unclear how these determinations were made.

For listed terrestrial plants, the risk designations for mortality, growth and reproduction are made in cells DI10, DI15 and DI20 of the 'WoE Matrix' worksheet of the Species_Plant_Template.xlsx. By tracing the precedents of the formulae, the risk designation formulae was deciphered and is summarized in Tables L-13 through L-15 in Appendix L.

All of the aforementioned risk designation calculations involve comparing NOELs for the particular effects category to the maximum application rates. However, in contrast to the assessment of indirect effects to listed species due to potential effects to habitat, these risk designation formulae do not consider whether or not the species is semi-aquatic, and does not consider runoff exposures to either dry or semi-aquatic areas. It is questionable why the EPA

would take a more involved approach to indirect effect to plants as habitat elements than to direct effects to listed terrestrial plants.

That said, application rates to treated fields are suspect exposure estimates for listed plant species. Managed and disturbed agricultural fields are unlikely to provide suitable habitat for many listed species. Species requirement should be taken into account at this stage, and the potentially treated field should not necessarily be considered habitat.

For risk of indirect effects to listed terrestrial plants due to exposure of pollinators or seed dispersers, the risk designation is determined in cell DI32 on the “WoE Matrix” worksheet of the Species_Plant_Template.xlsx. It seems that in error, this designation is not a function of exposure but is based solely on whether or not the listed species relies on biological vectors for pollination services and relies on biological vectors for diaspore dispersal. If text in C32 express that the listed plant is not reliant on biological vectors (i.e., “Species does not rely on biological vectors for pollination services. Species does not rely on biological vectors for diaspore dispersal”), the risk designation is LOW, otherwise it is HIGH. This conclusion is entirely independent of actual exposure of biological vectors and predicted effects on the listed plant species, and is therefore invalid.

For risk of indirect effects to listed terrestrial plants due to exposure of habitat, the risk designation is determined in cell DI39 on the ‘WoE Matrix’ worksheet of the Species_Plant_Template.xlsx. Details of the risk designation calculation are presented in Table L-16 in AppendixL. There is no HIGH risk designation for indirect effects to terrestrial plants due to effects to exposed habitat (as measured by effects to plants). As in the risk designation for indirect effects to listed terrestrial animals due to effects to habitat, NOELs were compared to exposure estimates. However, in contrast to the assessment of indirect effects to listed species due to potential effects to habitat, these risk designation formulae do not consider whether or not the species is semi-aquatic, and does not consider runoff exposures to either dry or semi-aquatic areas. This same inconsistency was observed for direct effects to listed plants above. It is questionable why the Agency would take a different more rigorous approach to risk designations for animal habitat than plant habitat.

5.2.4 Summary

In summary, the species calls are in fact based on a binary assessment of whether or not the most sensitive effects thresholds are exceeded by the highest exposure point estimates. If even one effects threshold is exceeded, the species call is LAA. Confidence designations are not considered in the effects determinations. Several apparent errors in the risk designation calculations were identified including mismatched threshold and exposure units and mismatched thresholds and risk designations. Overall the species calls lack actual risk estimates. As noted by NRC (2013): “The RQ approach does not estimate risk—the probability of an adverse effect—itself but rather relies on there being a large margin between a point estimate that is derived to maximize a pesticide’s environmental concentration and a point estimate that is derived to minimize the concentration at which a specified adverse effect is not expected.” The BE would be more robust if complete effects and exposure distributions were

considered, and EPA were to evaluate the probability associated with exceeding various levels of effect. This would be consistent with the NRC (2013) recommendation to use probabilistic methods.

5.3 Effects Determinations of NLAA/LAA: Qualitative Analyses

EPA presents their qualitative analyses for sea turtles, whales, deep sea fish, marine mammals, and cave dwelling invertebrates in Section 7 of Chapter 4 of the BE. EPA made species calls and critical habit calls (if applicable) of “LAA” for all sea turtle and cave-dwelling invertebrate species, and “NLAA” for all whale and deep sea fish species except for the killer whale (Southern resident DPS). For marine mammals (excluding whales), EPA made species calls and critical habit calls (if applicable) of “LAA” for the Guadalupe fur seal, southern sea otter, Steller sea lion, Hawaiian monk seal, Pacific harbor seal and West Indian Manatee, and “NLAA” for the northern sea otter (Southwest Alaska DPS), bearded seal, Pacific walrus, ringed seal and polar bear.

Although this section is titled “Qualitative Analyses”, in most cases, EPA (2016a) derived quantitative estimates of exposure and compared these to effects thresholds to characterize risk. As described in other sections of this response document, Cheminova takes issue with many of the effects metrics selected for the qualitative assessments, with the use of surrogate bins to estimate EECs for marine and estuarine environments, and with the comparison of dietary exposure concentrations to dietary effects metrics. Furthermore, EPA (2016a) makes unrealistically conservative assumptions regarding the potential for dermal exposure to sea turtles and dietary exposure to cave-dwelling invertebrates. Many of these assumptions are based solely on professional judgement and not any reliable data. All the quantitative assessments were deterministic and did not consider the likelihood of species actually being exposed to malathion. Furthermore, even when EPA (2016a) stated that the likelihood of exposure was low (e.g., cave-dwelling invertebrates), species still received LAA effects determinations.

Throughout the qualitative analyses, EPA (2016a) categorizes the risk and confidence as low, medium and high for various lines of evidence, including those based on professional judgement. Although EPA’s criteria for establishing low, medium and high conclusions for risk and confidence are provided in Attachment 1-9 of the BE, these criteria are only based on EEC exceedances of effects thresholds and cannot be applied for qualitative information. Thus, there is no transparency in EPA’s risk and confidence conclusions for several aspects of their qualitative analyses.

5.3.1 Sea Turtle Analysis

Comment 1
Chapter 4
Section 7.1.1
Dietary toxicity data sub-section

EPA divided avian dietary endpoints (in mg a.i./kg diet) by BCFs for relevant food items (i.e. aquatic plants, aquatic invertebrates, and fish) to calculate aquatic thresholds for sea turtles (in µg a.i./L), as shown in Table 5-4. As discussed in Section 2, the BCF of 131 µg a.i./kg ww per µg a.i./L is reported to be from MRID 43106401, which corresponds to a group of documents (procedure and raw data) for a study conducted by Forbis and Leak, 1994a,b [MRID 43106401, 43106402] and Kammerer and Robinson, 1994 [MRID 43340301]. This is a registrant submitted study that actually reports a BCF for bluegill (*Lepomis macrochirus*) of 103. Because EPA fails to provide a discussion on the study and data used to determine the BCF, it is impossible to identify the discrepancy between the BCFs. As such, it seems that EPA reported a BCF of 131 from this study in error.

An aquatic invertebrate BCF of 72 was estimated using the Kow (based) Aquatic BioAccumulation Model (KABAM). As previously mentioned in Section 2 of this response document, aside from stating that KABAM will be used, there is no discussion in the TEDtool or chapter 3 on any of the assumptions or data used for this modeling.

Comment 2
Chapter 4
Section 7.1.1
Table 4-7.2

The aquatic thresholds presented in Table 4-7.2 of Chapter 4 of the BE are based on the assumption that if concentrations in prey items (plants, aquatic invertebrates and fish) reach a level equal to an avian dietary effects threshold, then sea turtles will be adversely affected. As previously discussed in Section 2, this approach does not take account for differences between the gross energies and assimilation efficiencies of the laboratory test diet and prey items and food intake rates of receptors in the wild. Pesticide concentrations in the diet are not exposure estimates and as such, the direct comparison of pesticide concentrations in dietary items to dietary LC50s is inappropriate.

Comment 3
Chapter 4
Section 7.1.1
Exposures in estuaries and near shore areas sub-section

On page 4-10, EPA (2016a) states that EECs for bin 2 (low flow), bin 3 (medium flow), and bin 5 (low volume static) were used as surrogates for intertidal nearshore areas (bin 8), subtidal nearshore waterbodies (bin 9), and tidal pools (bin 8), respectively. It is unclear why EPA (2016a) has only one designated bin for both intertidal nearshore areas and tidal pools when separate surrogate freshwater bins are assigned to the two types of environments. Furthermore, the use of freshwater bins as surrogates for estuarine and marine environments leads to extreme overestimation of EECs. See the comments included in Section 0 for further details.

Comment 4
Chapter 4
Sections 7.1.1 and 7.1.2

The following ranges of EECs are provided for the qualitative assessment of sea turtles:

- On page 4-10 of Chapter 4, EPA (2016a) indicates that 4-day average EECs for bins 2, 3, and 5 ranged from 94 to 6530 µg a.i./L for estuaries and nearshore areas (HUCs 1-3, 8, 12, 13, 17-21).
- On page 4-15 of Chapter 4, EPA (2016a) states that 4-day average EECs for bins 3 and 4 ranged from 47 to 244 µ a.i./L for freshwater environments (HUCs 1-3, 8, 12, 13, 17-21).
- On page 4-15 of Chapter 4, EPA (2016a) states that peak EECs for bins 2, 3, and 5 ranged from 12 to 26,200 µg a.i./L for estuaries and near shore areas (HUCs 1-3, 8, 12, 13, 17-21).
- On page 4-16 of Chapter 4, EPA (2016a) states that peak EECs for bins 3 and 4 ranged from of 6-5,240 µg a.i./L for freshwater environments (HUCs 1-3, 8, 12, 13, 17-21).

These EECs are one to four orders of magnitude higher than any measured concentration for malathion in estuarine/marine environments (≤5.5 µg a.i./L; Smalling and Orlando, 2011). EPA stated that “the utility of these data are limited in that they represent ambient monitoring data for which the applications of malathion in the watershed of the sampled estuaries are not defined and are not expected to capture peak exposures”. As stated in EPA’s guidance for the “Evaluation and Use of Water Monitoring Data in Pesticide Aquatic Exposure Assessments” (EPA, 2014), even if not used quantitatively in the assessment, monitoring data can still be useful for comparison to modeled EECs to assess the realism of estimated concentrations. Instead, EPA completely dismisses the surface water monitoring data presented in Appendix 1-10 of their BE and by Smalling and Orlando (2011) and does not discuss the realism of the EECs generated for surrogate bins in the context of observed measured concentrations of malathion.

Comment 5
Chapter 4
Section 7.1.1
Exposures in freshwater environments (for Green Sea Turtle only) sub-section

On page 4-15, EPA (2016a) states that bins 3 and 4 were selected as the surrogate bins for the freshwater habitat of green sea turtle. However, it appears as though EPA did not actually model EECs for bins 3 and 4, but instead estimated EECs for these bins assuming that they were 5 times and 10 times lower than the EECs calculated for bin 2. EPA (2016a) should have used actual bin 3 and bin 4 EECs calculated from their Pesticide Water Calculator (PWC) rather than dividing bin 2 EECs by factors of 5 and 10, respectively. Additional comments regarding the conservativeness in the calculation of freshwater EECs are detailed in Section 0.

Table 5-4 Derivation of aquatic effects thresholds for sea turtles

Food item	*BCF Description*	*BCF value*	*Mortality threshold*[a]		*Lowest LC50*[b]		*Sublethal threshold*[c]		*Lowest NOEC for reproduction*[d]		*Lowest LOEC for reproduction*[e]	
			mg a.i./kg diet	*µg a.i./L*	*mg a.i./kg diet*	*µg a.i./L*	*mg a.i./kg diet*	*µg a.i./L*	*mg a.i./kg diet*	*µg a.i./L*	*mg a.i./kg diet*	*µg a.i./L*
Aquatic plants	Empirical	23	300	13043	2022	87913	110	4783	110	4783	350	15217
Aquatic invertebrates	KABAM estimate	72	300	4167	2022	28083	110	1528	110	1528	350	4861
Fish	Empirical	131	300	2290	2022	15435	110	840	110	840	350	2672

[a] One-in-one million effects on mortality based on northern bobwhite (*Colinus virginianus*) LC50 of 300 mg a.i./kg diet and slope of 5.74 (Gallagher et al., 2003 [MRID 48153106]). This study was rated acceptable by Cheminova (Appendix [Study Evaluations]).

[b] Lowest dietary LC50 of 2022 mg a.i./kg diet for the northern bobwhite (*Colinus virginianus*; Gallagher et al., 2003 [MRID 48153106]). This study was rated acceptable by Cheminova (Appendix [Study Evaluations]).

[c] Reproduction NOEC of 110 mg a.i./kg diet for the northern bobwhite (*Colinus virginianus*; Beavers et al., 1995 [MRID 43501501]). This study was rated acceptable by Cheminova (Appendix [Study Evaluations]).

[d] Reproduction NOEC of 110 mg a.i./kg diet for the northern bobwhite (*Colinus virginianus*; Beavers et al., 1995 [MRID 43501501]). This study was rated acceptable by Cheminova (Appendix [Study Evaluations]).

[e] Reproduction LOEC of 350 mg a.i./kg diet for the northern bobwhite (*Colinus virginianus*; Beavers et al., 1995 [MRID 43501501]). This study was rated acceptable by Cheminova (Appendix [Study Evaluations]).

5.3.2 Whale and Deep Sea Fish Analysis

Comment 1
Chapter 4
Section 7.2.1

EPA (2016a) made "NLAA" determinations for all whales and deep sea fish except for the killer whale (Southern resident DPS). On page 4-2 of Chapter 4, EPA (2016a) states that the killer whale is an obligate with the Pacific salmon. In Chapter 1, EPA states that obligate relationships occur "when one species is interdependent with or highly reliant on another species in a way that one cannot survive without the other". However, Table 4-7.8 of EPA's BE indicates that killer whales consume other fish species (e.g. herring), squid and marine mammals in addition to salmon. Although NMFS (2008) states in their recovery plan that southern resident killer whales have a strong preference for Chinook salmon, other fish, squid and marine mammals are also consumed. These other prey items could replace salmon in the killer whale diet if reductions in salmon were to occur. As such, there is no obligate relationship between the killer whale and Pacific salmon, and a "LAA" determination should not be made for the killer whale (Southern resident DPS) based on effects to salmon.

5.3.3 Marine Mammals (excluding whales) Analysis

Comment 1
Chapter 4
Section 7.3.1
Dietary toxicity data sub-section

The same approach used in the sea turtle analyses was used to convert mammalian dietary toxicity values to aquatic thresholds for marine mammals. As previously noted in Comment 1 of Section 5.3.1, the BCF of 131 µg a.i./kg ww per µg a.i./L is reported to be from MRID 43106401, which corresponds to a group of documents (procedure and raw data) for a study conducted by Forbis and Leak, 1994a,b [MRID 43106401; 43106402] and Kammerer and Robinson, 1994 [MRID 43340301]. This is a registrant submitted study that actually reports a BCF for bluegill (*Lepomis macrochirus*) of 103. Because EPA fails to provide a discussion on the study and data used to determine the BCF, it is impossible to identify the discrepancy between the BCFs. As such, it seems that EPA reported a BCF of 131 from this study in error.

An aquatic invertebrate BCF of 72 was estimated using the Kow (based) Aquatic BioAccumulation Model (KABAM). As previously mentioned in Comment 1 of Section 5.3.1 of this response document, aside from stating that KABAM will be used, there is no discussion in the TEDtool or Chapter 3 on any of the assumptions or data used for this modeling.

The aquatic thresholds derived by EPA (2016a) for marine mammals are presented in Table 1-2. As noted in Cheminova's comments on the mammalian effects metrics presented in Chapter 2 of EPA's BE (Section 4.2.6 - Mammals), several of EPA's effects thresholds were deemed

unacceptable. EPA (2016a) used the reproduction endpoints from Siglin (1985 [MRID 40812001]) to derive aquatic effects thresholds. This study was rated unacceptable by Cheminova since it used an irrelevant environmental exposure pathway. Moreover, Robinson (2002 [MRID 45626801]) reevaluated the study by Siglin (1985 [MRID 40812001]) and raises important issues on histroical control lab data as well as the use of leutinizing hormone on the evaluation of development effects (resorptions and abortions). The use of the leutinizing hormone in the study renders it artificial and unsuitable for use in evaluating potential ecological effects of a chemical. Robinson (2002 [MRID 45626801]) concludes that there was no effects on development with a NOEL of 100 mg a.i./kg per day, thus, supporting EPA's inappropriate use of data from Siglin (1985 [MRID 40812001]).

In addition, EPA (2016a) lists the source of the sublethal threshold of 1 mg a.i./kg bw was listed as MRID 43942901 in Table 4-7.14 of Chapter 4, but as MRID 43975201 in Table 9-1 of Chapter 2. Daly (1996 [MRID 43975201]) is the correct reference as the Daly (1996 [MRID 43942901]) study did not include a 20 mg/kg diet (1 mg/kg bw/d) treatment level. However, the Daly (1996 [MRID 43975201]) study considers the effects of malaoxon (a metabolite of malathion) rather than malathion. Cheminova has previously reviewed this malaoxon study (Breton et al., 2014a [MRID 49333901]; 2015 [MRID 49692301]) and rated it as acceptable. Notably, this study does report a decrease in red blood cell AChE at six months when exposed to 20 mg/kg-diet in feed, which is the sublethal endpoint value that EPA (2016a) indicates was used to derive their AChE inhibition LOEL of 1 mg a.i./kg-bw (see Tables 9-1 and 9-2 of Chapter 2 of the BE). Therefore, it appears that EPA (2016a) incorrectly relied on a malaoxon study rather than a malathion study to determine this threshold.

Additionally, the suitability of AChE inhibition to derive a sublethal threshold value is questionable given that an explicit relationship between AChE inhibition and effects to survival, growth, or reproduction has not been demonstrated by EPA (2016a). In the BE, EPA (2016a) has suggested relying on a LOEL of 1 mg a.i./kg bw as an effect threshold based on a 12-19% decrease in RBC AChE (although this appears to be from a malaoxon study rather than a malathion study). For comparison to known effects on mortality and/or reproduction, consider the two generation rat study carried out by Schroeder (1990 [MRID 41583401]) as described above. Overall, the results of the Schroeder (1990 [MRID 41583401]) study suggest that the effect threshold selected by EPA (2016a) of 1 mg a.i./kg bw is considerably lower than the chronic exposure dose that would be required to cause significant impairment to either mortality or reproduction in rats. The link between blood AChE inhibition and survival, growth or reproduction needs to be clearly established.

Finally, the use of endpoints from studies conducted with rodents is inappropriate for the assessment of pinnipeds, mustelids, manatees and bears. EPA (2016a) mentions this as an uncertainty but does comment on the relative sensitivity of marine mammals and rodents for malathion or other chemicals.

Comment 2
Chapter 4
Section 7.3.1
Table 4-7.15

As mentioned for the sea turtle analysis (Comment 2 of Section 5.3.1), the aquatic thresholds presented in Table 4-7.15 of Chapter 4 are based on the assumption that if concentrations in prey items (plants, aquatic invertebrates and fish) reach a level equal to an avian dietary effects threshold, then sea turtles will be adversely affected. This approach does not take account for differences between the gross energies and assimilation efficiencies of the laboratory test diet and prey items and food intake rates of receptors in the wild. Pesticide concentrations in the diet are not exposure estimates and as such, the direct comparison of pesticide concentrations in dietary items to dietary LC50s is inappropriate.

Comment 3
Chapter 4
Section 7.3.1
Exposures in estuaries and near shore areas sub-section

As was done in the sea turtle analysis (Comment 3 of Section 5.3.1),
EECs for bin 2 (low flow), bin 3 (medium flow), and bin 5 (low volume static) were used as surrogates for intertidal nearshore areas (bin 8), subtidal nearshore waterbodies (bin 9), and tidal pools (bin 8), respectively. It is unclear why EPA (2016a) has only one designated bin for both intertidal nearshore areas and tidal pools when separate surrogate freshwater bins are assigned to the two types of environments. Furthermore, the use of freshwater bins as surrogates for estuarine and marine environments leads to extreme overestimation of EECs. See the comments included in Section 3 for further details.

Comment 4
Chapter 4
Sections 7.3.1 and 7.3.2

The same EECs derived for the sea turtles analysis were also used in the qualitative assessment of marine mammals (See Comment 4 of Section 5.3.1). As stated above, these EECs are one to three orders of magnitude higher than any measured concentration for malathion in estuarine/marine environments (≤5.5 µg a.i./L; Smalling and Orlando, 2011). EPA stated that “the utility of these data are limited in that they represent ambient monitoring data for which the applications of malathion in the watershed of the sampled estuaries are not defined and are not expected to capture peak exposures”. As stated in EPA’s guidance for the “Evaluation and Use of Water Monitoring Data in Pesticide Aquatic Exposure Assessments” (EPA, 2014), even if not used quantitatively in the assessment, monitoring data can still be useful for comparison to modeled EECs to assess the realism of estimated concentrations. Instead, EPA completely dismisses the surface water monitoring data presented in Appendix 1-10 of their BE and by Smalling and Orlando (2011) and does not discuss the realism of the

EECs generated for surrogate bins in the context of observed measured concentrations of malathion.

Comment 5
Chapter 4
Section 7.3.1
Exposures in freshwater environments (for Manatee and Steller sea lion only) sub-section

As was noted for the sea turtle analysis (Comment 5 of Section 5.3.1), EPA (2016a) states on page 4-9 that bins 3 and 4 were selected as the surrogate bins for the freshwater habitat of manatees and Steller sea lions. However, it appears as though EPA did not actually model EECs for bins 3 and 4, but instead estimated EECs for these bins assuming that they were five times and 10 times lower than the EECs calculated for bin 2. EPA (2016a) should have used actual bin 3 and bin 4 EECs calculated from PWC rather than dividing bin 2 EECs by factors of five and 10, respectively.

Table 5-5 Derivation of aquatic effects thresholds for marine mammals (excluding whales)

Food item	*BCF Description*	*BCF value*	*Mortality threshold*[a]		*Lowest LC50*[b]		*Sublethal threshold*[c]		*Lowest NOEC for reproduction*[d]		*Lowest LOEC for reproduction*[e]	
			mg a.i./kg diet	*µg a.i./L*	*mg a.i./kg diet*	*µg a.i./L*	*mg a.i./kg diet*	*µg a.i./L*	*mg a.i./kg diet*	*µg a.i./L*	*mg a.i./kg diet*	*µg a.i./L*
Aquatic plants	KABAM estimate	23	184	8000	2090	90,870	20	870	825	35,870	1650	71,739
Aquatic invertebrates	lowest of empirical	72	184	2556	2090	29,028	20	278	825	11,458	1650	22,917
Fish	lowest of empirical	131	184	1405	2090	15,954	20	153	825	6298	1650	12,595

[a] One-in-one million effects on mortality based on the rat LD50 of 209 mg/kg bw and slope of 4.5 (Mendoza, 1976 [MRID 45046301, E35348]). This study was rated unacceptable by Cheminova (Appendix D).

[b] Lowest dietary LC50 of 2090 mg/kg diet calculated based on the rat LD50 of 209 mg/kg bw (Mendoza, 1976 [MRID 45046301, E35348]). This study was rated unacceptable by Cheminova (Appendix D).

[c] AChE inhibition NOEC of 20 mg a.i./kg diet calculated based on the dose-based NOEL of 1 mg/kg bw in rats (*Rattus norvegicus*; Daly, 1996 [MRID 43975201]). The MRID provided refers to a chronic oral toxicity/ oncogenicity study for Malaoxon, not Malathion. In Table 4-7.14 of Chapter 4, EPA erroneously lists the MRID as 43942901. This MRID refers to a 24-month oncogenicity study for malathion that did not include the 20 mg/kg diet treatment level.

[d] Reproduction NOEC of 825 mg a.i./kg diet for the rabbit, which EPA erroneously presents as rat toxicity data (Siglin, 1985 [MRID 40812001]). This study was rated unacceptable by Cheminova (Appendix D).

[e] Reproduction LOEC of 1650 mg a.i./kg diet for the rabbit, which EPA erroneously presents as rat toxicity data (Siglin, 1985 [MRID 40812001]). This study was rated unacceptable by Cheminova (Appendix D).

5.3.4 Cave Dwelling Invertebrate Species Analysis

Comment 1
Chapter 4
Section 7.4

As shown in Table 4.7-19 of Chapter 4, the species call and critical habitat call was “LAA” for all species assessed in EPA’s Cave Dwelling Invertebrate Species Analysis. EPA (2016a) does not attempt to estimate exposures of contaminated food items that are “washed into a cave and then ingested”. Furthermore, they do not use spatial data to characterize the likelihood of pesticide drift off-field reaching the caves inhabited by these species. Although EPA acknowledges that the likelihood of exposure is low, EPA made “LAA” determinations for all arthropods that rely on food items within the cave because the potential for effects to a single organism cannot be precluded. This is an extremely conservative assumption and goes against the professional judgement provided by EPA.

EPA (2016a) provides hardly any details on how exposures were calculated for arthropods that rely primarily on food items from outside the cave and no information on the effects thresholds against which exposures were compared. They only indicate that application rates used were 0.5 and 2 lb a.i./A, that a foliar half-life of 6.1 days was used for leaf litter and that the daily fractions retained in mammalian and avian carcasses were assumed to be 0.27 and 0.81, respectively. No references are provided for the foliar half-life or retained fraction data, and no references to the equations or tools used to estimate exposures are available. It is also unclear whether EPA is assuming that leaf litter and carcasses are exposed to the full rate applied to the field or off-field drift. As such, it is impossible to verify EPA’s calculations. For consumption on feces, EPA cites four literature studies that measured pesticides in guano and carcasses found in cave systems (Eidels et al., 2007; Land, 2001; MacFarland, 1998; and Sandel, 1999). However, no information of the pesticides detected or the level of contaminants measured was provided and full references for the studies were not provided in Chapter 4. Furthermore, metabolism studies show that malathion is eliminated mainly as non-toxic metabolites in feces and is not detected in tissues within days of exposure. O’Brien et al. (1961) found that malathion and malaoxon represented only 5.93 and 0.819% of residues measured in cow feces (0.474 and 0.066% of ingested residues), and neither malathion nor malaoxon were detected in feces in studies conducted with rats and chickens (Reddy et al., 1989 [MRID 41367701]; Cannon et al., 1993 [MRID 42715401]). Residues in tissues represented less than 1% of the ingested dose (Bourke, 1968; Reddy et al., 1989 [MRID 41367701]) and again, neither malathion nor malaoxon were detected (Cannon et al., 1992 [MRID 42581401]; 1993 [MRID 42715401]). Thus, cave-dwelling arthropod exposures to malathion from consumption of feces and carcasses is negligible and would not adversely affect these species.

For indirect effects, EPA concluded that “the chance that the impacts on the availability of animal feces, carcasses, and leaf litter would reach a level that would adversely affect the listed terrestrial invertebrates due to the loss of food is very low”. However, EPA (2016a) still made LAA determinations for habitat for all arthropod species because reductions in food items

cannot be precluded. Again, this is an extremely conservative assumption and even goes against EPA's professional judgement.

5.4 Comments on Mosquitocide Use

Appendix 4-5 of the BE (Terrestrial species with species range and/or critical habitat overlap only with mosquito adulticide uses) and Appendix 3-3 (Spray drift considerations for malathion) describes the EPA approach to addressing potential risk of mosquitocide use. There are numerous problems with EPA's assessment. These are outlined below and in more detail in Appendix M.

Comment 1
B5(ED)-1 – Paragraph 1

The Agency states: "Additionally the application rates for the mosquito adulticide uses are generally lower than those for other uses (*e.g.* agricultural and non-agricultural uses). Therefore, if a listed species range or critical habitat overlaps with other potential use sites, those uses are expected to be protective of the mosquito adulticide uses (*i.e.*, potential exposures are expected to be higher with most of the non-mosquito adulticide uses)."

Although this statement is generally true, it entirely mis-represents the exposure potential and potential risk associated with use of malathion as an adulticide. The maximum application rate for aerial application of malathion as an adulticide is 0.234 lb a.i./A. This is less than half of the lowest single application rate for agricultural use (i.e., Flax – 0.5 lb a.i./A, max. 3 applications, 7 day interval). For non-thermal aerosol applications by ground equipment, the maximum application rate is 0.06 lb a.i./A. For the thermal fog ground applications, the maximum rate is 0.11 lb a.i./A. All of these should be mentioned and assessed. Therefore, the adulticide application rate is very relevant to the assessment of risk assuming exposure *may* occur. Exposure and, therefore, risk is likely to be considerably lother non-agricultural use patterns and should be assessed separately from those use patterns. In fact, FIFRA, as amended by FQPA, requires that public health mosquito control be assessed separately from all other uses (FIFRA, Subtitle C – Public Health Pesicides SEC. 230 (a) Section 2(bb) (7 USC 136(bb)).

Comment 2
B5(ED)-1 – Paragraph 1

The Agency states: "A limited number of terrestrial species (listed in Table A 4-5.1) are identified where the only use that overlapped with their species range is the mosquito adulticide use for malathion and mosquito adulticide and wide area use (*e.g.,* general outdoor treatments around perimeters and ant mounds for pests) for chlorpyrifos."

This statement is unlikely to be true for malathion and is also likely untrue for chlorpyrifos wide area use which will not be addressed here. What is entirely missing from the discussion is **why** the Agency believes all species and critical habitat are expected to be exposed. There is no discussion in the appendix regarding why the Agency believes malathion adulticide use could

occur across the entire US. Presumably, this is due to the lack of restriction on the label regarding where the adulticide could be applied. However, there are no restrictions on agricultural use labels (with few exceptions) that spatially prevent a crop from being grown. To account for this 5 to 10 years of the Cropland Data Layer (CDL) is used to capture change over time. The flexibility in the adulticide labels is critical to the use of the product as an adulticide and provides the American Mosquito Control Association, Center for Disease Control (CDC), and Department of Defence (DoD) the ability to apply the product to manage public health threats as they occur, where they occur.

The assumption that malathion adulticide could potentially be applied anywhere is flawed within the context of the BE. There are readily available data and information (e.g., American Mosquito Control Associations (AMCA) surveys, CA and FL databases, and Cheminova sales information) that quantitatively identify the locations where adulticide active ingredients have been and are currently being applied. These data and information to support it are found in Appendix M and illustrated in Figure 5-1. The spatial area identified is considerably smaller than the area considered in the BE which corresponds to the vast majority of the United States and its territories. Therefore, the number of species (and critical habitat) that may potentially be exposed to malathion is considerably fewer than all listed species except for those identified as lacking exposure (i.e., Storm petrel) as the Agency states in the BE.

The spatial data characterizing adulticide use represent the best available scientific and commercial data characterizing the application of mosquito adulticide throughout the U.S. Therefore, it is no different from utilizing the best scientific and commercially available data to characterize crop footprints (e.g., Crop Data Layer, AgCensus data etc.) for individual crops over the last 5-10 years, as data permit. The re-registration period for pesticides is every 15 years under the *Food Quality Protection Act* (FQPA). Re-registration is the federal action and as such must be evaluated in the context of the ESA. Thus, spatial changes in use patterns such as adulticide application are re-evaluated every 15 years and significant changes will be captured at that point. This may include heavier use due to virus outbreaks for which the mosquito acts as a vector (e.g., malaria, Zika virus). Over the 15-year registration period, there could also be reduced use if no public health threat presents itself. **No other use pattern being evaluated by the Agencies is treated in the same way.** This is why we use spatial and other data to capture changes to crop use patterns over time. Because they are additive (if additional land is put into production it is added to the crop footprint, but any land previously identified as having a specific crop that no longer has that crop is not removed) it is a conservative ‘picture’ of where one might reasonably anticipate the pesticide could be used at the time of the re-registration review.

Another factor not considered in the BE is the timing of the adulticide applications across the country. Mosquitocide use in general will ebb and flow based on the life cycle of the many different varieties of mosquitoes found in the United States, and the need to initiate control during specific months, or maintain control throughout a year. Latitude plays a role as well given that many of the southern states may apply adulticides (and/or larvicides) more frequently than northern states. Warmer temperatures in the southern states encourage growth of mosquitoes

and may also influence the frequency of application for public health reasons (e.g. the threat of disease is more frequent).

Given the available data available from AMCA surveys, CA and FL databases, and Cheminova sales information identifying the mosquitocide 'crop footprint' the 'best available scientific and commercial data' as stipulated in the ESA, to characterize the potential for exposure is being used. Therefore, a blanket assumption that an adulticide could be used 'everywhere' is entirely untenable and unsupported within the context of the ESA.

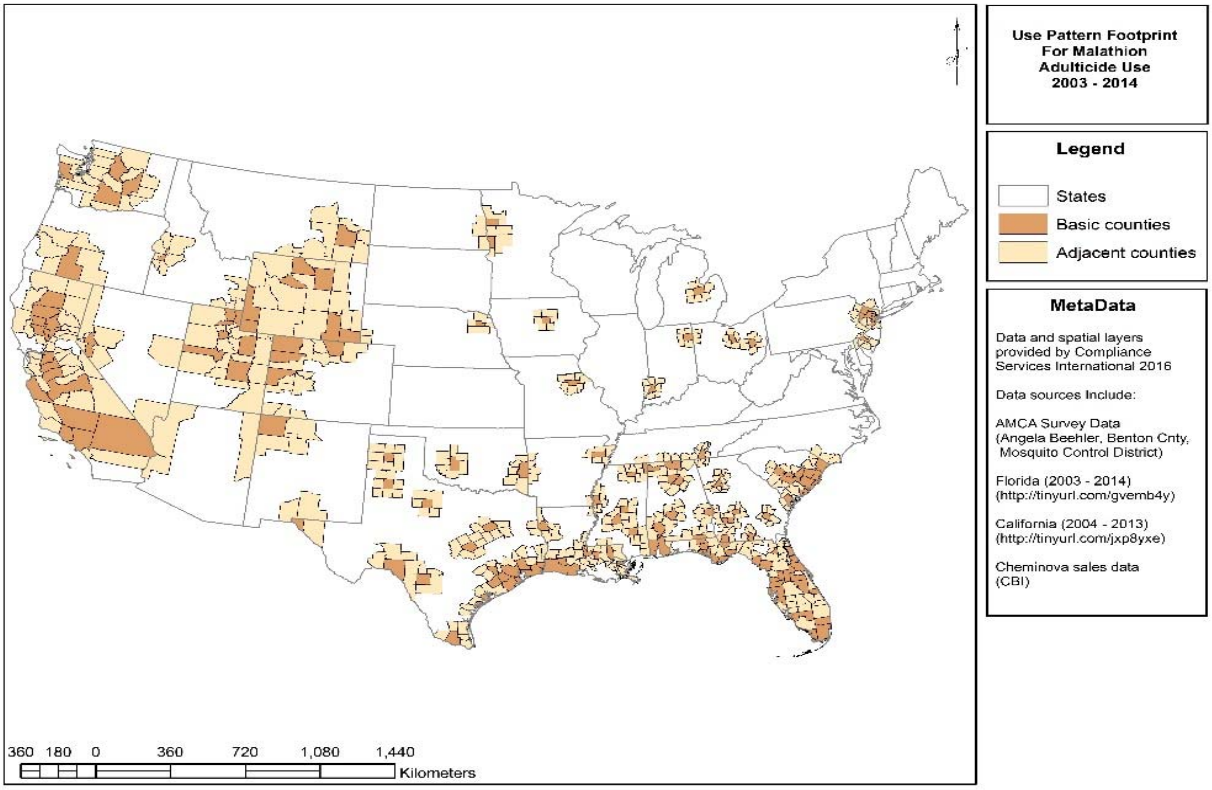

Figure 5-1 Use pattern footprint for malathion adulticide use 2003 -2014

Comment 3
Appendix 4-5 B5 (ED)-2 and Appendix 3-3 B3(FC) - 4

The Agency states: "Therefore, modeling of ground applied adulticides could not be conducted. However, in 2013, EPA (DP Barcode 407817, 3/28/2013) conducted a comparison of ground and aerial applications of adulticides using open literature information and other modeling and concluded that the maximum deposition was similar between the two methods of application . Based on this analysis, ground deposition fractions are considered to be the same as those expected for aerial applications."

It is unclear in the BE how the decision that ground applied adulticides were determined to have the same deposition fractions as aerial applications. The DP Barcode 407817, 3/18/2013) is provided as an additional resource for the assumption. After contacting EPA, it was determined that this DP Barcode is for the following reference:

EPA, 2013. Memorandum: Etofenprox: Public Health – Mosquito Control. Label Amendment to Remove Application Restriction to Cropland and Pastureland. Summary of Analytical Chemistry and Residue Data. DP Barcode: D396053; MRID No. 48612201 and 48612202.

Although the DP Barcodes appear incorrect in the BE, the memorandum describes the basis for this assumption as from an 'IR4 study and dissertation by Schleiser, 2012 and a new physics-based model'. No other data or information are provided requiring the reader to then follow up by finding the dissertation (if possible). If the EPA based their spray drift modeling on the work of Schleier et al. (2012) then there are significant issues with respect to the validity of the modeling in the MULTI-Disp model which is based on Schleier et al. 2012 (see Appendix M).

5.5 Summary of Concern Regarding the Risk Characterization

We have noted a number of problems with the effects determinations made in the draft BE. Our major concerns include the following:

- There is an overall lack of transparency in how species and critical habitat calls were made.
- EPA used risk quotients alone in the effects determinations and did not provide valid (probability-based) risk estimates.
- There are major discrepancies between the Interagency Interagency Approaches (Agencies, 2013), the analysis plan (Section 1.4), Chapter 4 text, and what was actually carried out in the WoE tools.
- Species calls and critical habitat calls were made for all uses of malathion, assuming that all label uses can be made anywhere in the United States, without drawing any distinctions between use patterns, locations and co-occurrence.
- With the exception of the Agency's overly conservative RQs, other lines of evidence were not directly considered in species and critical habitat calls in the weight-of-evidence tools (e.g., incident reports, monitoring data, other toxicity data etc.).
- EPA inappropriately gave equivalent "weights" to exceedances of thresholds associated with direct effects to survival, growth or reproduction, as they did to exceedances of sublethal thresholds not necessarily linked to adverse effects on individual fitness (e.g., endpoints for avoidance behavior, AChE inhibition, etc.).
- There are critical errors in the comparison of effects thresholds and exposure estimates in the WoE tools.
- The spatial extent of the potential malathion adulticide application is grossly exaggerated and simply not defensible when the best available scientific and commercial data are considered. Thus, the spatial extent of the action area in the BE is grossly overestimated.

Cheminova strongly urges EPA to follow the EPA agency-wide guidelines for ecological risk assessment (EPA, 1998) as well as the more recent NRC (2013) recommendations, by incorporating genuine (probabilistic) risk estimates in their biological evaluations.

6 CONCLUSION

EPA's effects determinations for listed aquatic and terrestrial species potentially exposed to malathion are not scientifically defensible. In addition to conceptual errors, we also have documented numerous serious transcription and calculation errors that affected risk designations.

In contrast to the NRC (2013) recommendations, risk quotients (RQs) were used to determine risk designations in Step 2. Although RQs are practical for a cursory screen, to avoid wasting resources in instances were risks are expected to be negligible, RQs should not be used to make risk conclusions and regulatory decisions. RQs do not communicate risk. When applied correctly, they may indicate there is a possibility of adverse effects. The NRC specifically concluded that RQs are not scientifically defensible for assessing the risks to listed species posed by pesticides.

In direct contrast, the EPA has maintained its use of RQs. This ignores the fact that the NRC (2013) recommended the use of probabilistic approaches "that require integration of the uncertainties (from sampling, natural variability, lack of knowledge, and measurement and model error) into the exposure and effects analyses by using probability distributions rather than single point estimates for uncertain quantities." Prime examples of such an approach for malathion include Cheminova's refined effects determinations conducted on the Kirtland's warbler (KW), the California tiger salamander (CTS) and the delta smelt (DS) (Moore et al., 2016, Breton et al. 2016a,b). Cheminova had previously submitted a refined effects determination for the California Red-legged frog (CRLF) (Breton et al., 2013a [MRID 49211702]). The refined CRLF, DS, CTS and KW effects determinations, conducted by Cheminova, demonstrate that when higher tier assessments are carried out using realistic exposure and effects assumptions and consideration of all lines of evidence, the conclusion of these effects determinations are quite different than those predicted by EPA. These types of assessments are 'refined' assessments that should be applied once risks are identified using highly conservative risk estimates (i.e., risk quotients) in screening-level ecological risk assessment approaches (e.g., NRC, 2013; EPA, 2004; EPA, 1998).

In addition, Cheminova is particularly concerned with the major lack of transparency necessary for evaluation, and reproduction of the Agency's results. We were able to discern that EPA used threshold values from studies deemed invalid by the Agency themselves, or deemed acceptable for quantitative use when criteria for quantitative use were not met. The Agency also used toxicological measures of effects or attributes that were not empirically linked to apical ecological risk assessment endpoints (mortality, growth and reproduction) or fitness. As a result, the weight-of-evidence approach was flawed. Moreover, numerous lines of evidence were unaccounted for in the species and critical habitat calls, including toxicity data other than those used in the derivation of threshold values, field studies, monitoring data and incident reports.

With respect to exposure estimates, compounding conservatism occurred with the use of “upper bound” exposure estimate inputs, resulting in unrealistically high deterministic values. The Agency should first estimate the likelihood of exposure. Co-occurrence of potential habitat with potential use sites does not equate to exposure, and moreover, does not equate to peak on-field exposure, as assumed by EPA.

Combined, the draft BE offers nothing less than catastrophic effects determinations for the majority of listed species. This conclusion cannot stand in light of the over sixty years of use experience with malathion, often under less restrictive use directions than currently allowed. Moreover, as shown with the Kirtland’s Warbler example, the size of the Kirtland’s warbler population is currently at its historical maximum, which is nearly 10 times larger than it was at the time of listing and close to twice as large as the threshold stated in the primary objective (FWS, 2012a,b). The evidence with respect to the recovery and health of the Kirtland’s warbler population in the US is clearly inconsistent with the finding of the highly conservative conclusions of EPA’s draft BE for malathion.

This document will also be supplemented by a problem formulation, and a Step 1 and Step 2 ESA that will be submitted to EPA after the public comment period. These documents will provide an alternate, more scientifically defensible and resource-efficient approach than is reflected in EPA’s draft BE for malathion. Cheminova acknowledges that the Agency will, in accordance with its letter denying the requests for extension of the public comment period, provide additional opportunities for further feedback on the approach to the biological evaluations of listed species.

Cheminova requests that EPA give careful consideration to the detailed comments provided in this document, and rectify the errors and oversights. Critically, the Agency must incorporate real risk estimates (i.e., the probabilities of exceeding various magnitudes of effects) in their biological evaluations, as was concluded by NRC (2013).

7 REFERENCES

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Appendix A
Comments on the Problem Formulation of the BE

Page 1-6.

EPA Statement: Malathion was first registered as an insecticide in 1965.

Comment: EPA's statement is factually incorrect. Malathion was first registered by the United State Department of Agriculture in 1956.

Page 1-7.

EPA Statement: Malathion is an organophosphate used as an insecticide on a wide variety of terrestrial food and feed crops, terrestrial non-food crops, aquatic food, non-agricultural indoor, outdoor use sites, and for wide area public health uses.

Comment: Malathion is not approved for use in non-agricultural indoor use sites as indicated in EPA's statement. It is used in gain storate facilities and in mushroom houses; which are agricultural indoor use sites.

EPA Statement: Based on an Office of Pesticide Programs Information Network (OPPIN) query (conducted Feb. 2015), there is currently 1 active technical registrant that sponsors guideline studies on malathion, and there are 96 active registrations (43 Section 3's, 53 Section 24c Special Local Needs, and 0 Section 18 Emergency Exemptions) from 21 registrants, which include formulated end-use products and technical grade malathion (See Appendix 1-2).

Comment: A query of NPIRS on May 16, 2016, identified 98 active registrations [3 active technical product registrations, 39 end-use product registrations (3 of which are registered by CTX-Cenol and are known to be suspended), and 56 Section 24c Special Local Need registrations}.

EPA Statement: Malathion can be applied in a dust, liquid or encapsulated form.

Comment: There are no encapsulated formulations of malathion registered in the United States. Other than a single dust product registration labeled for stored grains, all are liquid products identified as emulsifiable concentrates, ready-to-use solutions, soluble concentrates, and flowable concentrates.

EPA Statement: Registered labels for agricultural products require 25-foot (ground and non-ULV applications), or 50-foot (ULV aerial applications) no-spray buffer zones adjacent to "any water body."

Comment: While we support the buffer zones mentioned by EPA in its statement, we note that this is not consistent with the requirements in the RED. In EPA's June 30, 2009, letter to registrants requiring changes to labels to reflect the requirements in the RED, the following requirements are identified on in Table 30 page 10:

Table A-1 Summary of Labeling Changes for Malathion		
Description	***Amended Labeling Language***	***Placement on Label***
Buffer Zones	**Buffer Zones for Aerial Application** When making a Non-ULV application with aerial application equipment, a minimum buffer zone of 25 feet must be maintained along any water body. When making a ULV application with aerial application equipment, a minimum buffer zone of 50 feet must be maintained along any water body.	In the Directions for Use section in a section titles: "Buffer Zones for Aerial Application":

Thus, buffer zones were only required for aerial applications, with 25 foot buffers for non-ULV applications and 50-foot buffers for ULV applications. Buffer zones for ground applications were not a requirement of the RED and we suspect that these do not appear on all agricultural labels. We recommend that EPA confirm whether the requirements of the RED have been met for all agricultural labels.

Pages 1-7 and 1-8.

EPA Statement:

All registered labels for agricultural use also include the following spray drift requirements when spraying in the vicinity of aquatic areas:

- Droplet Size
  - Use the largest droplet size consistent with acceptable efficacy. Formation of very small droplets may be minimized by appropriate nozzle selection, by orienting nozzles away from the air stream as much as possible, and by avoiding excessive spray boom pressure.
  - For ground boom and aerial applications, use only medium or coarser spray nozzles according to ASAE (S572) definition for standard nozzles, or a volume mean diameter (VMD) of 300 microns or greater for spinning atomizer nozzles. In conditions of low humidity and high temperatures, applicators should use a coarser droplet size.
- Wind Direction and Speed
  - Make aerial or ground applications when the wind velocity favors on-target product deposition (approximately 3 to 10 mph). Do not apply when wind velocity exceeds 15 mph. Avoid applications when wind gusts approach 15 mph. For all non-aerial applications, wind speed must be measured adjacent to the application site on the upwind side, immediately prior to application
- Temperature Inversion
  - Do not make aerial or ground applications into areas of temperature inversions. Inversions are characterized by stable air and increasing temperatures with increasing distance above the ground. Mist or fog may indicate the presence of an inversion in humid areas. Where permissible by local regulations, the applicator may detect the

presence of an inversion by producing smoke and observing a smoke layer near the ground surface. In conditions of low humidity and high temperatures, applicators should use a coarser droplet size.

- Additional Requirements for Ground Applications
  - For ground boom applications, apply with nozzle height no more than 4 feet above the ground or crop canopy.

Comment:

The RED also required the following language:

- Spray should be released at the lowest height consistent with pest control and flight safety. Applications more than 10 feet above the crop canopy should be avoided.

  This requirement has apparently been missed by EPA because of a placement error in Table 30 on page 12 of EPA's June 30, 2009 memorandum. EPA mistakenly placed this statement under the header "Additional Requirements for Ground Applications". It should have been under a separate header called "Additional Requirements for Aerial Applications." We are aware of at least one label where EPA's Registration Division mistakenly required this statement as part of the additional requirements for ground applications. EPA needs to review the malathion agricultural labels to make sure this error is corrected. And, this requirement of the RED needs to be reflected in the Agency's risk assessments.

Page 1-26.

EPA Statement: All of the mitigations proposed in the Malathion Registration Eligibility Decision (RED, May 2009) have been addressed; therefore, there are no outstanding mitigations from the RED process for malathion.

Comment: We disagree with this statement. There are a number of uses on other registrants' labels (i.e., not Cheminova/FMC) that do not reflect the conditions of re-registration as specified in the RED. For example, there are labels that include mosquito larvicide uses (CTX-CENOL Inc., EPA Company No.: 45385) that have not been supported since the 1988 Registration Standard. These should have been removed long before the RED was issue, but certainly should have been removed during the label review process after the RED.

Appendix B
Comments on Appendix 1-1
(Regulatory History and Past Assessments for Malathion)

Malathion Registration Eligibility Decision, 2006

EPA Statement: In 2006, the Agency completed a screening-level ecological risk assessment in support of the Re-registration Eligibility Decision (RED) for malathion (EPA 2006a).

Comment: The EPA statement is misleading as written because it implies that an ecological risk assessment for malathion was prepared in 2006. It is not true that EPA completed an ecological risk assessment in 2006. The ecological risk assessment that was conducted by the Environmental Fate and Effects Division (EFED) for malathion was completed in 1999 and issued for public comment in December of 2000. This is acknowledged in EPA's memorandum dated February 3, 2005 (DP barcode: D312678), providing the "Status of the ecological risk assessment for malathion". As stated in that memorandum "This memo accompanies the ecological risk assessment (December 6, 2000; DP barcode D238903 and D238906) conducted on uses of malathion that are being supported by the registrant Cheminova." Furthermore, in Section 1.1.4 of this appendix, EFED says that the ecological risk assessment was conducted in 1999 and not in 2006:

> "Although the RED was published in 2006, following completion of the organophosphate cumulative assessment, the ecological risk assessment was compiled in 1999…"

Obviously, EFED's ecological risk assessment was conducted well before EPA finalized the RED for malathion.

EFED Statement: The ecological risk assessment in the RED concluded that use of malathion poses a high risk of mortality to fish and aquatic invertebrates from acute toxicity. Almost all uses are expected to pose a high risk of adversely affecting aquatic invertebrate populations, especially in urban streams and wetlands. High acute risk is also expected to fish and amphibians for uses with higher application rates or repeated applications. Numerous incidents of fish kills confirm the acute risk to fish.

Comment: This statement is misleading. Because EFED's risk assessment was completed in 1999, and the RED with its required label changes was not finalized until 2006, EFED has not actually evaluated the use patterns that the RED deemed eligible for re-registration. Thus, it is not appropriate to state that the use patterns permitted by the RED result in the same conclusion as the pre-RED use patterns evaluated by EFED. This is highlighted in EPA's statement on page 95 of the RED:

> "When assessed with maximum application values, most scenarios resulted in RQs above the Agency's LOC. ***However, the Agency expects that acute and chronic RQs to aquatic organisms will be greatly reduced, with RQs for some scenarios being below the Agency's LOC, when reduced application rates are assessed." [emphasis added]***

In addition, EFED did not actually evaluate risk. In its assessments, it derived Risk Quotients (RQs) and compared them to Level of Concern based on EFED policy. As noted in the report by NAS (NRC, 2013):

> "The RQ approach does not estimate risk—the probability of an adverse effect—itself but rather relies on there being a large margin between a point estimate that is derived to maximize a pesticide's environmental concentration and a point estimate that is derived to minimize the concentration at which a specified adverse effect is not expected."

> "Furthermore, the committee concludes that RQs are not scientifically defensible for assessing the risks to listed species posed by pesticides or indeed for any application in which the desire is to base a decision on the probabilities of various possible outcomes."

So, it is entirely inappropriate for EFED to state that there is high risk to fish and invertebrates when it has not evaluated the RED-required use patterns and it is not truly evaluated risk.

EFED Statement: Use of malathion is generally not expected to pose a high risk of mortality to terrestrial wildlife (birds, mammals, and reptiles, terrestrial stages of amphibians) although the acute level of concern (LOC) is exceeded for some uses with high application rates and repeated applications. Use of malathion poses a risk of impairing reproduction in birds, and may cause other sublethal effects in wildlife.

Comment: As with the previous comment on aquatic species, it is not appropriate for EFED to claim there is risk based on use of RQ approach. As noted by NAS (NRC, 2013), risk needs to be determined based on the probability of an effect occurring. EFED's 1999 risk assessment for the RED did not consider probability of an effect occurring, so it has not determined risk. Furthermore, EFED did not assess risk to these organisms using the use patterns deemed eligible for reregistration by the 2006 RED.

EFED Statement: The ecological risk assessment in the RED concluded that use of malathion could potentially harm all taxa of threatened and endangered animals. Risk quotients exceeded the level of concern for threatened and endangered species of fish, aquatic invertebrates, birds, and mammals.

Comment: This statement is factually incorrect and is misleading. First, the EFED risk assessment conducted in 1999 to support the RED did not actually assess the use patterns deemed eligible for reregistration in the 2006 RED. Second, as noted previously, NAS (NRC, 2013) concluded that EFED's use of the RQ approach does not constitute an evaluation of risk because it did not evaluate the probability of an effect occurring. And finally, on page 66 of the RED, the Agency stated the following:

> "The ecological assessment that EPA conducted for this RED does not, in itself, constitute a determination as to whether specific species or critical habitat may be harmed by the pesticide. Rather, this assessment serves as a screen to determine the need for any species specific

assessment that will evaluate whether exposure may be at levels that could cause harm to specific listed species and their critical habitat."

EFED needs to modify its statement to correctly reflect the limits of the analysis it conducted in 1999 and the ultimate conclusions of the RED.

Organophosphate Cumulative Assessment, and Malathion Re-registration Eligibility Decision, 2006

EPA Statement: Because the Agency had determined that malathion shares a common mechanism of toxicity with structurally-related organophosphate insecticides, a cumulative human health risk assessment for the organophosphate pesticides was necessary before the Agency could make a final determination of reregistration eligibility of malathion. This cumulative assessment was finalized in 2006 (EPA 2006b).

Comment: The 2006 update to the OP Cumulative Risk Assessment can be accessed by copying and pasting the following link into your browser: https://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2006-0618-0002.

It is not clear why EFED references EPA's 2006 Cumulative Risk Assessment in this BE. However, there are perhaps some important conclusions from that assessment that could be relevant for ecological risk assessment. For example, EPA concluded that the cumulative exposure to the OPs considered in the assessment were below the Agency's level of concern. Another relevant point for the ecological risk assessment for malathion is that the Agency concluded that malathion is by far the least potent of the organophosphates as measured by brain cholinesterase inhibition in rats (see the figures below which were extracted from the Agency's 2006 update to the OP cumulative risk assessment). These points should be acknowledged and highlighted by EFED in its problem formulation document.

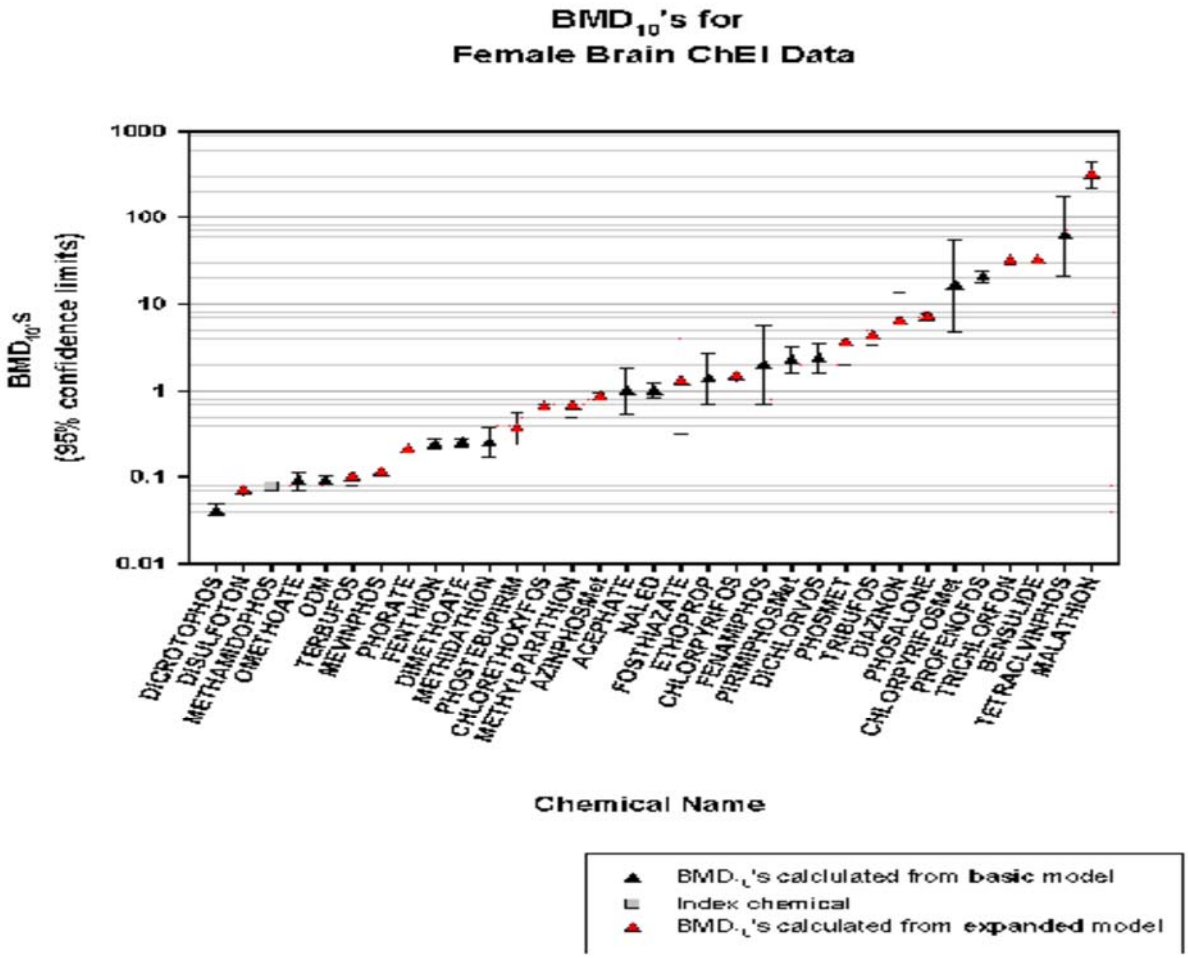

Figure B-1 Plot of BMD10s and the 95% confidence limits for female rat brain ChE inhibition for the OPs

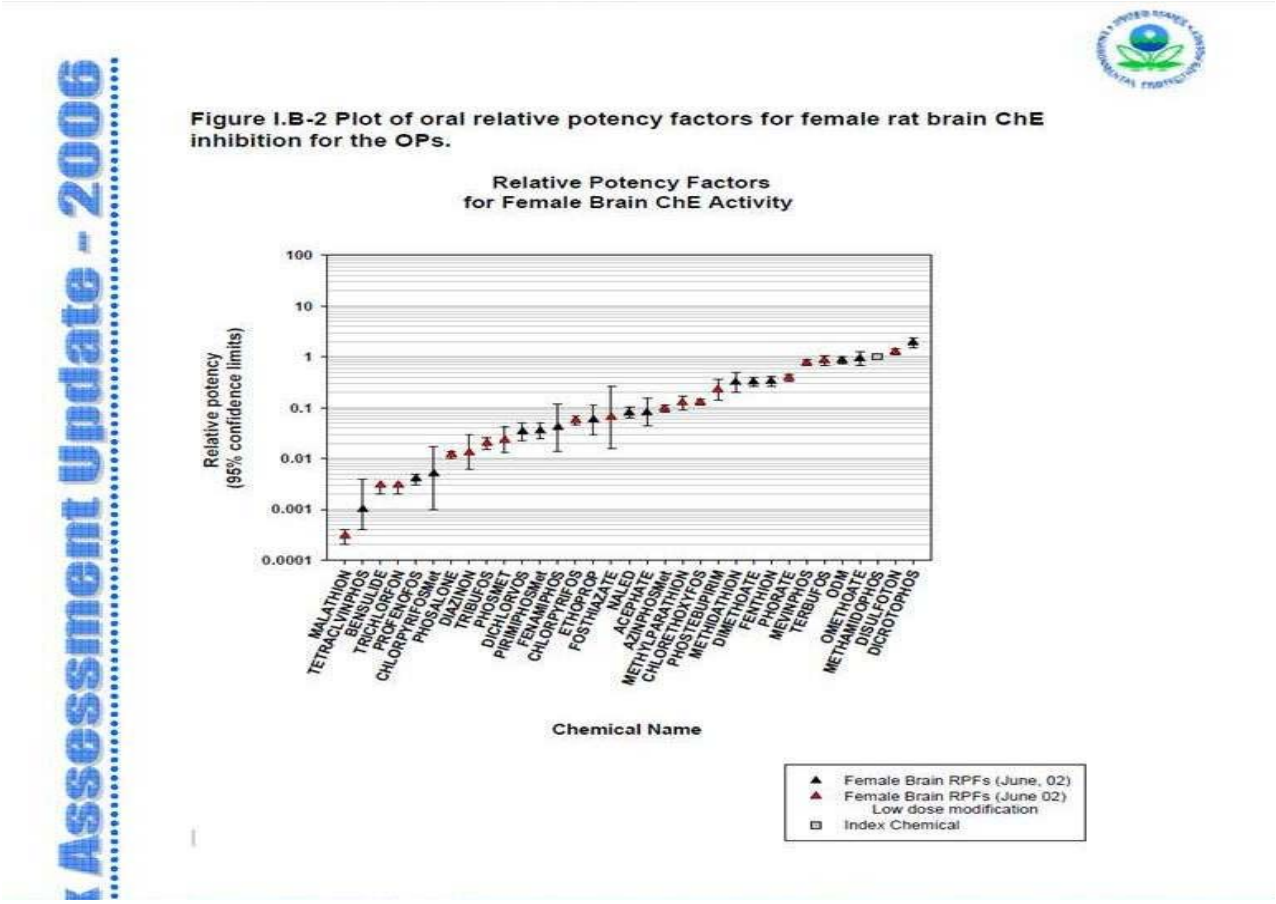

Figure B-2 Plot of oral relative potency factors for female rat brain ChE inhibition for the OPs.

California Red-legged Frog Endangered Species Assessment

EPA Statement: The Agency recently completed an endangered species risk assessment of the potential effects of malathion and maloxon on the threatened California red-legged frog (*Rana aurora draytonii*; CRLF) arising from uses of malathion (EPA 2007). Uses included in this 2007 assessment reflected some post-RED mitigations. This endangered species risk assessment was part of the *Center for Biological Diversity (CBD) vs. EPA et al*. (Case No. 02-1580-JSW(JL)) settlement entered in the Federal District Court for the Northern District of California on October 20, 2006. The assessment resulted in a determination that the use of pesticide products containing malathion is likely to adversely affect the CRLF. This determination is based on the potential for malathion use to both directly and indirectly affect the species and result in modification to designated critical habitat.

Comment: Under the Endangered Species Act, it is not appropriate for EPA to provide the Services with a citation to only EFED's evaluation of the California Red-legged frog (CRLF) without also providing the comments that the applicant prepared in response to that assessment and also the highly refined risk assessment that the applicant conducted and provided to EPA.

These documents are referenced in the table below (Table B-1). Our comments noted a number of deficiencies with the EPA screening-level assessment which actually did not assess risk since it relied on the RQ approach that NAS has rejected. Our highly-refined assessment utilized probabilistic approaches to determine the level of potential risk and concluded that risks to the California red-legged frog were *de minimus*.

Table B-1 Documents provided to EPA by the registrant in relation to the CRLF

Submitted Document - Reference	***Date Submitted to EPA***	***EPA MRID***
Breton, R.; Kara, Y.; Moore, D.; et al. (2013). Cheminova's Comments on EPA's Malathion Effects Determination for the California Red-Legged Frog. Unpublished study prepared by Intrinsik Environmental Sciences, Inc. 146p.	09/09/13	49211701
Breton, R.; Rodney, S.; Kara, Y.; et al. (2013). Refined Effects Determination for California Red-Legged Frog Potentially Exposed to Malathion. Unpublished study prepared by Intrinsik Environmental Sciences, Inc. and Stone Environmental, Inc. 1009p.	09/09/13	49211702
Hanzas, J.; Estes, T.; Winchell, M.; et al. (2013). Refined Exposure Modeling of Malathion for the California Red-Legged Frog. Project Number: 112482. Unpublished study prepared by Stone Environmental, Inc. 128p	09/09/13	49211703

EPA Statement: Toxicity values used in this document are in some cases different than those used in the malathion RED and those used in the current assessment of risk to the Delta smelt (DS) and California tiger salamander (CTS). Although the RED was published in 2006, following completion of the organophosphate cumulative assessment, the ecological risk assessment was compiled in 1999, prior to the regular incorporation of open literature ecotoxicological (ECOTOX) data into EFED risk assessments. Review of the open literature data resulted in a number of lower toxicity endpoints used in the CRLF assessment.

Comment: As noted in Cheminova's comments on EPA's effects determination for the CRLF (Breton et al., 2013 [MRID 49211701]) and Section 4 of this response document, the Agency's evaluation of the quality of the data from the open literature was inadequate. Cheminova has developed a robust set of quality acceptance criteria and applied that to all ecological effects data (open literature and guideline GLP data; Breton et al., 2014 [MRID 49333901]; 2015 [MRID 49692301]). Only data that were deemed acceptable or supplemental were used in our refined risk assessment.

EPA Statement: In this current assessment for the DS and CTS, open literature data have been further evaluated and toxicity endpoints have been further revised. Some of the toxicity endpoints were revised higher relative to those used in the CRLF document, and thus some of the RQs have decreased in this current assessment relative what was reported in the CRLF assessment.

Comment: The applicant (Cheminova and FMC) has also submitted responses to EPA's evaluations of the Delta Smelt (DS) and California Tiger Salamander (CTS). With our comments on this draft BE, we are also submitting highly-refined risk assessments for these two species.

As with the Agency's evaluation of the CRLF, we noted a number of deficiencies with EFEDs evaluation of the DS and CTS. And, our highly refined risk assessments, which utilized probabilistic approaches to estimating risk, generally concluded *de minimus risk* to these two species.

Table B-2 Documents provided to EPA by the registrant in relation to the DS and CTS

Submitted Document - Reference	*Date Submitted*	*EPA MRID*
Teed, R.; Rodney, S.; Clemow, Y.; et al. (2015). Cheminova's Comments on EPA's Malathion Effects Determination for Delta Smelt and the California Tiger Salamander: Final Report. Project Number: 60440, AF/5228/CN, 378FYF. Unpublished study prepared by Intrinsik Environmental Sciences, Inc. 200p	08/06/2015	49692201
Breton, R.; Manning, G.; Greer, C.; et al. (2015). Addendum to Breton et al. (2014 [MRID 49333901]): Additional Ecotoxicological Data, Updates to Proposed Screening-Level Effects Metrics, and Presentation of Field and Mesocosm Studies for the Registration Review of Malathion. Project Number: 232/112A, 103FYF, 108. Unpublished study prepared by Intrinsik Environmental Sciences, Inc. 180p	08/06/2015	49692301
Breton, et al, 2016. Refined Effects Determination For California Tiger Salamanders Potentially Exposed To Malathion.	June 10, 2016	Pending
Breton, et al, 2006. Refined Effects Determination For Delta Smelt Potentially Exposed To Malathion.	June 10, 2016	Pending

Pacific Anadromous Salmonids Endangered Species Assessment

The Agency completed an endangered species risk assessment of the potential effects of malathion on 26 listed Evolutionarily Significant Units (ESUs) of Pacific salmon and steelhead arising from FIFRA regulatory actions regarding use of malathion (EPA 2004). This risk assessment was part of the *Washington Toxics Coalition vs. EPA* (Case No. C01-132C) order entered in the Federal District Court for the Western District of Washington on July 2, 2002. The assessment concluded that malathion is toxic to fish as well as to organisms that serve as food for threatened and endangered Pacific salmon and steelhead. The final conclusion was that the uses (at that time) of malathion (and its degradate malaoxon) may affect 24 of these ESUs.

Comment: Because this assessment was conducted in 2004, long before the Agency completed its RED (with required use restrictions) for malathion, this assessment is not relevant to the current evaluation process under registration review.

EPA Statement: On November 18, 2008, the National Oceanic Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS) issued a final biological opinion on the effect of pesticide products containing malathion, chlorpyrifos, or diazinon to 28 listed Pacific salmonids (National Marine Fisheries Service, 2008). This opinion concluded that the effects of registration of pesticide products that contain malathion or the two other active ingredients is likely to jeopardize the continued existence of 27 of the 28 species of Pacific salmonids. They concluded that these pesticides are not likely to jeopardize the continued existence of Ozette Lake Sockeye salmon, but may adversely affect that species. Furthermore, they concluded that registration of these products is likely to destroy or adversely modify 25 of the 26 critical habitats that have been designated for these Pacific salmonids. The only critical habitat that they

concluded would not be adversely modified is that of the Ozette Lake Sockeye salmon. This Biological Opinion is available on the internet (http://www.nmfs.noaa.gov/pr/pdfs/pesticide_biop.pdf).

Comment: In the discussion of the BiOp, it is wholly inappropriate for EFED to exclude the fact that the Agency itself found serious flaws with the draft BiOp (September 15, 2008 letter to James H. Leahy (Director, Office of Protected Resources, National Oceanic and Atmospheric Administration, National Marine Fisheries Service) from Debra Edwards (Director, EPA's office of Pesticide Programs). EFED also must acknowledge that the U.S. Court of Appeals for the Fourth Circuit vacated the 2008 biological opinion (Dow AgroSciences LLC v. National Marine Fisheries Service, 4th Cir., No. 11-2337, 2/21/13). The court ruled Feb. 21, 2013, that the biological opinion was "arbitrary and capricious" under the Administrative Procedure Act. Because the courts vacated this BiOp, it is inappropriate for EPA to refer to it in this and other regulatory documents.

References

Breton, R.L., Y. Kara, D.R.J. Moore, S. Rodney and S. Teed. 2013. Comments on EPA's Malathion Effects Determination for the California Red-Legged Frog. Unpublished study performed by Intrinsik Environmental Sciences, Inc., Ottawa, ON, Project No. 60275, for Cheminova, Inc., Arlington, VA. Final report dated September 6, 2013. [MRID 49211701].

Breton, R., G. Manning, Y. Kara, S. Rodney and K. Wooding. 2014. Cheminova's Ecotoxicological Study Evaluation Criteria, Study Evaluations and Proposed Screening-level Effects Metrics for the Registration Review of Malathion. Unpublished report prepared by Intrinsik Environmental Sciences, Inc., Ottawa, ON, Project No. 60320, for Cheminova, Inc., Arlington, VA. Final report dated March 4, 2014. [MRID 49333901].

Breton, R., G. Manning, Y. Clemow, S. Rodney and K. Wooding. 2015. Addendum to Breton et al. (2014 [MRID 49333901]): Additional Ecotoxicological Data, Updates to Proposed Screening-Level Effects Metrics, and Presentation of Field and Mesocosm Studies for the Registration Review of Malathion. Unpublished study performed by Intrinsik Environmental Sciences Inc., Ottawa, ON, Project No. 60320 for Cheminova, Inc., Arlington, VA. [MRID 49692301].

EPA (U.S. Environmental Protection Agency). 2004. Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, U.S. Environmental Protection Agency: Endangered and Threatened Species Effects Determinations. Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC. January 23, 2004 [online]. Available: https://www.epa.gov/sites/production/files/2014-11/documents/ecorisk-overview.pdf.

EPA (US Environmental Protection Agency). 2006a. Reregistration Eligibility Decision for Malathion: Case No. 0248. Office of Prevention, Pesticides and Toxic Substances. Office of Pesticide Programs. Washington, D.C. EPA 738-R-06-030. pp 188. EPA (US Environmental Protection Agency). 2007. Risks of Malathion Use to Federally Listed California Red-legged frog (Rana aurora draytonii). Pesticide Effects Determination. Environmental Fate and Effects Assessment Division, Office of Pesticide Programs, Washington, DC. October 19, 2007.

EPA (US Environmental Protection Agency). 2006b. Organophosphate Cumulative Assessment and Malathion Reregistration Eligibility Decision. Environmental Fate and Effects Assessment Division, Office of Pesticide Programs, Washington, DC.

EPA (US Environmental Protection Agency). 2007. Risks of Malathion Use to Federally Listed California Red-legged frog (*Rana aurora draytonii*). Pesticide Effects Determination. Environmental Fate and Effects Assessment Division, Office of Pesticide Programs, Washington, DC. October 19, 2007.

NMFS (National Marine Fisheries Service). 2008. Endangered Species Act Section 7 Consultation Biological Opinion Environmental Protection Agency Registration of Pesticides Containing Chlorpyrifos, Diazinon, and Malathion.

NRC (National Research Council) 2013. Assessing Risks to Endangered and Threatened Species from Pesticides. Committee on Ecological Risk Assessment under FIFRA and ESA, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies, National Research Council of the National Academies. The National Academies Press, Washington, DC. http://www.nap.edu/catalog.php?record_id=18344.

Appendix C
Data Evaluation Records (DERs) received by Cheminova as of June 3, 2016 for Registrant-submitted Studies Referenced in the Malathion Biological Evaluation

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion Technical	Fletcher, D. (1988) 42-day Neurotoxicity Study with AC 6,601 Technical in Mature White Leghorn Hens: Report No. BLAL 87 DN109. Unpublished study prepared by Bio-Life Associates, Ltd. 69 p.	16 FYF	Malathion Registration Task Force	12/20/1988	40939301	Yes	Acceptable
Cythion 57 EC	Burgess, D. (1989) Acute Flow-through Toxicity of Cythion 57% EC to Daphnia magna: Report No. 37394. Unpublished study prepared by Analytical Bio-Chemistry Labs Inc. 197 p.	11 FYF	MALATHION REREGISTRATION TASK FORCE	3/14/1989	41029701	Yes	Acceptable
Malathion (Cythion Tech)	Bowman, J. (1989) Acute Flow-Through Toxicity of Cythion Technical to Sheepshead Minnow (Cyprinodon variegatus): Report No. 37397. Unpublished study prepared by Analytical Bio-chemistry Laboratories, Inc. 205 p. (18 FYF)	18 FYF	MALATHION REREGISTRATION TASK FORCE	7/20/1989	41174301	Yes	Acceptable
Malathion Technical (Cythion)	Burgess, D. (1989). Acute Flow-through Toxicity of Cythion Technical to Mysid Shrimp (Mysidopsis bahia): Project ID 37398. Unpublished study prepared by Analytical Bio-Chemistry Laboratories, Inc. 211 p. (019 FYF)	19 FYF	MALATHION REREGISTRATION TASK FORCE	8/3/1989	41189201	Yes	Acceptable
Malathion 57% EC	Hoxter, K.; Jaber, M. (1989). Cythion Insecticide (6601 57% EC): Honey Bee Toxicity of Residues of Foliage: Report No. 130-145. Unpublished study prepared by Wildlife International Ltd. 55 p. CHA Doc. No. 21 FYF.	21 FYF	MALATHION REREGISTRATION TASK FORCE	8/21/1989	41208001	Yes	Acceptable
Malathion (57 EC)	Bowman, J. (1989) Acute Flow-Through Toxicity of Cythion 57% to Sheepshead Minow (Cyprinodon variegatus): Report No. 37396. Unpublished study prepared by Analytical Bio-Chemistry Laboratories, Inc. 245 p. (24 FYF)	24 FYF	MALATHION REREGISTRATION TASK FORCE	10/2/1989	41252101	Yes	Acceptable

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion 57% EC	Sweeney, R. (1989) Addendum 1: Cythion Insecticide (AAC6601 57% EC) Honey Bee Toxicity of Residues on Foliage – Malathion and Malaoxon in Alfalfa Green Forage and Analysis of Spray Solutions: Lab Project Number: 38212. Unpublished study prepared by Analytical Bio-Chemistry Laboratories, Inc. 230 p. CHA Doc. No. 27 FYF	27 FYF	MALATHION REREGISTRATION TASK FORCE	10/30/1989	41284701	Yes	Acceptable
Malathion (57 EC)	Rabe, B. (1989) AC 6,601 57% EC Acute Effect on New Shell Growth of the Eastern Oyster (Crassostrea virginica) Under Flow-Through Conditions: Lab Project Number: 93000/0200/3140. Unpublished study prepared by Hunter/ESE 40 p.	30 FYF	MALATHION REREGISTRATION TASK FORCE	12/12/1989	41320201	Yes	Unacceptable
Malathion Technical	Pedersen, C. (1989). AC 6,601 Technical: Toxicity and Reproduction Study in Bobwhite Quail: BLAL Study # 88 QR 32. Unpublished study prepared by Bio-Life Associates, Ltd. in association with American Cyanamid Co. 69 p.	17 FYF	MALATHION REREGISTRATION TASK FORCE	1/25/1990	41367801	Yes	Supplemental
Malathion Technical	Fletcher, D. (1989) 42-Day Neurotoxicity Study with AC 6,601 Technical in Mature White Leghorn Hens: Lab Report No. 87 DN 109. Unpublished study prepared by Bio-Life Associates, Ltd. 69 p.		MALATHION REREGISTRATION TASK FORCE	01/26/90	41371401	Yes	Acceptable
Malathion Technical	Cohle, P. (1989) Early Life Stage Toxicity of Cythion to Rainbow Trout (Oncorhynchus mykiss) in a Flow-through System: Lab Report Number: 37400. Unpublished study prepared by Analytical Bio-Chemistry Laboratories, Inc.	34 FYF	MALATHION REREGISTRATION TASK FORCE	3/24/1990	41422401	Yes	Acceptable
Malathion Technical (Cythion)	Forbis, A. (1990). Acute Flow-through Toxicity of Cythion Technical to Mysid Shrimp (Mysidopsis bahia): Lab Project Number: 38414. Unpublished study prepared by Analytical Bio-chemistry Laboratories, Inc. 184 p. (036 FYF)	36 FYF	AmCy on behalf of MALATHION REREGISTRATION TASK FORCE	5/4/1990	41474501	Yes	Acceptable

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion Technical (Cythion)	Blakemore, G.; Burgess, D. (1990). Chronic Toxicity of Cythion to Daphnia magna Under Flow-through Test Conditions: Lab Project Number: 37399. Unpublished study prepared by Analytical Bio-Chemistry Laboratories, Inc. 391 p.	44 FYF	MALATHION REREGISTRATION TASK FORCE	12/7/1990	41718401	Yes	Acceptable
Malathion (57 EC)	Wade, B.; Wisk, J. (1992). Effect of Cythion Insecticide 57 Percent EC on New Shell Growth in the Eastern Oyster under Flow-through Conditions: Lab Project Number: 3913032-0200-3140. Unpublished study prepared by ESE, Inc. 52 p.	60 FYF	Cheminova	3/23/1992	42249901	Yes	Acceptable after considering 43213201
Malathion Technical	Pederson, C.; Fletcher, D. (1993). AC 6,601 Technical: Toxicity and Reproduction Study in Mallard Ducks: Lab Project Number: 90 DR 39. Unpublished study prepared by Bio-Life Assocs. 223 p.	69 FYF	Cheminova	5/21/1993	42782101	Yes	Acceptable
[14C]Malathion	Forbis, A.; Leak, T. (1994) Uptake Depuration, and Bioconcentration of (carbon 14)-Malathion by Bluegill Sunfish (Lepomis macrochirus) under Flow-Through Test Conditions: Lab. Project Number: 40542: XBL-92151. Unpublished study prepared by ABC Labs, Inc. and XenoBiotic Labs, Inc. 96 p. 76 FYF	76 FYF	Cheminova	2/1/1994	43106401	Yes	Acceptable
[14C]Malathion	Forbis, A.; Leak, T. (1994) Raw Data Report for: Uptake, Depuration, and Bioconcentration of (carbon 14)-Malathion by Bluegill Sunfish (Lepomis macrochirus) under Flow-Through Test Conditions: Lab Project Number: 40542R: XBL-92151. Unpublished study prepared by ABC Labs, Inc. and XenoBiotic Labs, Inc. 1096 p. 76 FYF RD.	76 FYF RD	Cheminova	2/1/1994	43106402	Yes	Acceptable
Malathion (57 EC)	Habig, C. (1993). Cheminova's Response to EPA's Review of the Malathion Oyster Shell Deposition Study: Lab Project Number: 3913032/0200/3140. Unpublished study prepared by Cheminova Agro A/S. 179 p.	60 FYF Amdt-1	Cheminova	4/26/1994	43213201	Yes	Acceptable after considering 43213201

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
[14C]Malathion	Kammerer, R.; Robinson, R. (1994) Procedure for Flow-Through Bluegill Bioconcentration Studies with Radiolabeled Test Substances (Test Substance: (carbon 14)-Malathion): Lab Project Number: XBL92151: RPT00179: 40542. Unpublished study prepared by ABC Laboratories, Inc. and XenoBiotic Laboratories, Inc. 313 p. 76 FYF Amdt-1	76 FYF Amdt-1	Cheminova	8/17/1994	43340301	Yes	Acceptable
Malathion Technical	Beavers, J.; Haberlein, D.; Mitchell, L.; et al. (1995). Malathion: A One-generation Reproduction Study with the Northern Bobwhite (Colinus virginianus): Lab Project Number: 232-112A. Unpublished study prepared by Wildlife International, Ltd. 220 p.	103 FYF 103 FYF, amdt 1	Cheminova	1/5/1995	43501501	Yes	Acceptable
Malathion 440 g/l EW (CHA 3110)	Ebke, K. (2002). Evaluation of DIrect and Indirect Effects of a 440 g/L EW Formulation of Malathion on Aquatic Organisms in Outdoor Ponds. Project Number: 0676/019/1754/0676/019, 0676/019, 676/19. Unpublished study prepared by Covance Laboratories, Gmbh and Covance Laboratories, Ltd. 309 p.	379 FYF	Cheminova	4/15/2005	46525901	No	
Malathion Technical	Mattock, S. (2002). Malathion: Screening Toxicity Tests to Daphnia magna. Project Number: 676/20. CHA Doc. No. 369 FYF. Unpublished study prepared by Covance Laboratories, Ltd. 7 p.	369 FYF	Cheminova	9/16/2008	47540301	No	
Malathion Technical	Gries, T.; Purghart, V. (2001). Malathion Technical: Acute Toxicity Test with Rainbow Trout (Oncorhynchus mykiss) Under Flow-Through Conditions. Project Number: 1005/018/108, 306/FYF. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 54 p. (306 FYF)	306 FYF	Cheminova	9/16/2008	47540302	No	

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion Technical	Gries, T.; Purghart, V. (2001). Malathion Technical: Acute Immobilisation Test with Daphnids (Daphnia magna) under Flow-Through Conditions: Final Report. Project Number: 1005/018/115. CHA Doc. No.: 310/FYF. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 62 p.	310 FYF	Cheminova	9/16/2008	47540303	No	
Malathion Technical	Gries, T.; Purghart, V. (2001). Malathion Technical: Acute Toxicity Test with Bluegill Sunfish (Lepomis macrochirus) Under Flow-Through Conditions: Final Report. Project Number: 314/FYF, 1005/018/105. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 58 p.	314 FYF	Cheminova	09/16/2008	47540304	No	
MDCA	Gries, T.; Purghart, V. (2001). Malathion Dicarboxylic Acid: Acute Immobilisation Test with Daphnids (Daphnia magna) Under Flow-Through Conditions: Final Report. Project Number: 1005/020/115. CHA Doc. No.: 313 FYF. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 58 p.	313 FYF	Cheminova	09/16/2008	47540305	Yes	
MDCA	Gries, T.; Purghart, V. (2001). Malathion Dicarboxylic Acid: Acute Toxicity Test with BlueGill Sunfish (Lepomis macrochirus) Under Flow-Through Conditions: Final Report. Project Number: 315/FYF, 1005/020/105. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 55 p. (315 FYF)	315 FYF	Cheminova	09/16/2008	47540306	Yes	
	Purghart, V. (2001) Malathion Dicarboxylic Acid: Validation of an Analytical Method for Determination of Malathion Dicarboxylic Acid in Aqueous Solutions (fish and daphnia medium): Final Report. Project Number: 1005/020/250, 323/FYF. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 31 p.	323 FYF	Cheminova	09/16/2008	47540307	No	

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion 440 g/L EW (CHA 3110)	Gries, T.; Purghart, V. (2001). CHA 3110: Acute Toxicity Test with Rainbow Trout (Oncorhynchus mykiss) Under Flow-Through Conditions: Final Report. Project Number: 316/FYF, 1005/021/108. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 60 p.	316 FYF	Cheminova	09/16/2008	47540308	No	
MMCA	Gries, T.; Purghart, V. (2001). Malathion Monocarboxylic Acid (alpha and beta mixture): Acute Toxicity Test with Bluegill Sunfish (Lepomis macrochirus) under Flow-Through Conditions: Final Report. Project Number: 1005/019/105, 317/FYF. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 57 p.	317 FYF	Cheminova	09/16/2008	47540309	Yes	
MMCA	Gries, T.; Purghart, V. (2001). Malathion Monocarboxylic Acid (alpha and beta mixture): Acute Immobilisation Test with daphnids (Daphnia magna) Under Flow-Through Conditions: Final Report. Project Number: 1005/019/115. CHA Doc. No.: 318 FYF. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 59 p.	318 FYF	Cheminova	09/16/2008	47540310	Yes	
	Purghart, V. (2001). Malathion Monocarboxylic Acid: Validation of an Analytical Method for Determination of Malathion Monocarboxylic Acid in Aqueous Solutions (Fish and daphnia medium): Final Report. P roject Number: 322/FYF, 1005/019/250. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 31 p.	322 FYF	Cheminova	09/16/2008	47540311	No	
Fyfanon ULV	Kuhajda, B.; Blanco, C.; Green, M.; et al. (1996). Impact of Malathion on Fish and Aquatic Invertebrate Communities and On Acetylcholinesterase Activity in Fishes within Stewart Creek, Fayette County, Alabama: Final Report. Unpublished study prepared by University of Alabama. 129 p.	?	EPA at the request of Cheminova	07/16/2010	47587601	No	

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malaoxon	Gallagher, S.; Grimes, J. ; Beavers, J. (2002). Malaoxon: An Acute Oral Toxicity Study With the Northern Bobwhite. Project Number: 37 4/FYF, 232/120. Unpublished study prepared by Wildlife International, Ltd. 52 p.	374 FYF	Cheminova	7/16/2010	48153104	Yes (June 9, 2016)	Acceptable
Malaoxon	Gallagher, S.; Grimes, J.; Beavers, J.; et al. (2002). Malaoxon: A Dietary LC50 Study with the Northern Bobwhite. Project Number: 383/FYF, 232/119A. Unpublished study prepared by Wildlife International, Ltd. 74 p.	383 FYF	Cheminova	7/16/2010	48153105	Yes (June 9, 2016)	Acceptable
Malathion Technical	Gallagher, S.; Grimes, J.; Beavers, J.; et al. (2003). Fyfanon Technical: A Dietary LC50 Study with the Northern Bobwhite. Project Number: 434/FYF, 232/121. Unpublished study prepared by Wildlife International, Ltd. 91 p	434 FYF	Cheminova	7/16/2010	48153106	No	
Malathion Technical	Rodgers, M. (2002). Malathion Technical: Acute Oral Toxicity (LD50) to the Bobwhite Quail (Colinus virginianus): Amended Final Report. Project Number: 338/FYF, CHV/075/012502. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 62 p.	338 FYF	Cheminova	7/16/2010	48153114	Yes (June 9, 2016)	Acceptable
Malathion Technical	Palmer, S.; Schneider, S.; Kendall, T.; et al. (2011) Malathion: A 96-Hour Flow-Through Acute Toxicity Test with Tadpoles of the African Clawed Frog (Xenopus laevis): Final Report. Project Number: 232 A/123. Unpublished study prepared by Wildlife International, Ltd. 77 p.	1391 FYF	Cheminova	3/4/2011	48409302	No	
Malaoxon	Gerke, A. (2011) Malaoxon: Acute Toxicity Test to the Bluegill Sunfish, *Lepomis macrochirus*, Determined Under Flow-through Test Conditions. Project Number: 66931. Unpublished study prepared by ABC Laboratories, Inc. 49p. (950 FYF).	950 FYF	Cheminova	8/12/2011	48571801	Yes (June 9, 2016)	Acceptable

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malaoxon	Gerke, A. (2011). Malaoxon: Acute Toxicity Test to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Flow-through Test Conditions. Project Number: 66932. Unpublished study prepared by ABC Laboratories, Inc. 50p. (? FYF)	951 FYF	Cheminova	8/12/2011	48571802	No	
Malaoxon	Bergfield, A. (2011). Malaoxon: Acute Toxicity to the Water Flea, Daphnia magna, Determined Under Flow-through Test Conditions. Project Number: 66930. Unpublished study prepared by ABC Laboratories, Inc. 48p.	949 FYF	Cheminova	8/12/2011	48571803	No	
Malathion Technical	Stafford, J. (2011). Malathion Technical: Acute Oral Toxicity Test (LD50) with the Canary (Serinus canaria). Project Number: 11704/4101. Unpublished study prepared by Smithers Viscient Laboratories. 121 p.	945 FYF	Cheminova	8/12/2011	48571805	No	
Malaoxon	Stafford, J. (2011). Malaoxon: Acute Oral Toxicity Test (LD50) with the Canary (Serinus canaria). Project Number: 11704/4102. Unpublished study prepared by Smithers Viscient Laboratories. 84p.	946 FYF	Cheminova	8/12/2011	48571806	No	
Malaoxon	Leak, T. (2011). Validation of Test Solution Preparations and Analytical Methods for Use in the Determination of Malaoxon in Various Media Used in Environmental Toxicity Studies. Project Number: 66934. Unpublished study prepared by ABC Laboratories, Inc. 38p.		Cheminova	08/12/2011	48571807	No	
Malathion Technical	Palmer, S.; Schneider, S.; Kendall, T. et al. (2011). Malathion: Amphibian Metamorphosis Assay: (African Clawed Frog (Xenopus laevis)): Final Report. Project Number: 232A/124. Unpublished study prepared by Wildlife International, Ltd. 136p.	1060 FYF	Cheminova	11/4/2011	48617501	Yes (8/3/2015)	Acceptable

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion Technical	Palmer, S.; Schneider, S.; Kendall, T. et al. (2011) Malathion: Fish Short-term Reproduction Assay with the Fathead Minnow (Pimephales promelas): Final Report. Project Number: 232A/121. Unpublished study prepared by Wildlife International, Ltd. 174p.	1058 FYF	Cheminova	11/4/2011	48617506	Yes (8/3/2015)	Acceptable
Malathion Technical	Hurd, K.; Sharpe, A. (2011) Malathion Technical: Determination of the Effects on the Early-Life Stage of the Sheepshead Minnow (Cyprinodon variegatus). Project Number: BR0472/B, 11/0037/B. Unpublished study prepared by AstraZeneca UK, Ltd. 66p.	1072 FYF	Cheminova	12/22/2011	48705301	Yes (June 9, 2016)	Acceptable
Malathion Technical	Claude, M.; Kendall, T.; Gallagher, S.; et al. (2012). Malathion: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (*Americamysis bahia*): Final Report. Project Number: 232A/127. Unpublihed study prepared by Wildlife International, Ltd. 119p.	1095 FYF	Cheminova	02/24/2012	48752901	Yes (June 9, 2016)	Acceptable
	Brown, S. (2011) Validation of the Residue Analytical Method: "Determination of Malathion and Malaoxon in Water by LC-MS/MS". Project Number: 66799. Unpublished study prepared by Morse Laboratories, Inc. 173p.		Cheminova	04/12/2012	48800201	No	
	Brown, S. (2011) Validation of the Residue Analytical Method: "Determination of Malathion and Malaoxon in Soil by LC-MS/MS". Project Number: 66797. Unpublished study prepared by Morse Laboratories, Inc. 169p.		Cheminova	04/12/2012	48800202	No	
	Cremin, P. (2012) Independent Laboratory Validation of the Analytical Method for Malathion and Malaoxon in Water by LC-MS/MS. Project Number: 2221W. Unpublished study prepared by PTRL West, Inc. 119 p.		Cheminova	04/12/2012	48800203	No	
	Cremin, P. (2012) Independent Laboratory Validation of the Analytical Method for Malathion and Malaoxon in Soil by LC-MS/MS. Project Number: 2220W. Unpublished study prepared by PTRL West, Inc. 97p.		Cheminova	04/12/2012	48800204	No	

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion Technical	Hubbard, P.; Martin, K.; Beavers, J. (2012). Malathion: A Dietary LC(50) Study with the Ring-necked Pheasant. Project Number: 232/156. Unpublished study prepared by Wildlife International Ltd. 92p.	1249 FYF	Cheminova	10/5/2012	48963301	Yes (June 9, 2016)	Acceptable
Malaoxon	Hubbard, P.; Martin, K.; Beavers, J. (2012). Malaoxon: A Dietary LC(50) Study with the Ring-necked Pheasant. Project Number: 232/162. Unpublished study prepared by Wildlife International Ltd. 83p.	1250 FYF	Cheminova	10/5/2012	48963302	Yes (June 9, 2016)	Acceptable
Malathion Technical	Hubbard, P.; Martin, K.; Beavers, J. (2012). Malathion: A Dietary LC(50) Study with the Mallard. Project Number: 232/155. Unpublished study prepared by Wildlife International Ltd. 81p.	1241 FYF	Cheminova	10/5/2012	48963303	Yes (June 9, 2016)	Acceptable
Malaoxon	Hubbard, P.; Martin, K.; Beavers, J. (2012). Malaoxon: A Dietary LC(50) Study with the Mallard. Project Number: 232/153. Unpublished study prepared by Wildlife International Ltd. 82p.	1240 FYF	Cheminova	10/5/2012	48963304	Yes (June 9, 2016)	Acceptable
Malathion Technical	Hubbard, P.; Martin, K.; Beavers, J. (2012). Malathion: An Acute Oral Toxicity Study with the Ring-necked Pheasant. Project Number: 232/153. Unpublished study prepared by Wildlife International Ltd. 82 p.	1091 FYF	Cheminova	10/5/2012	48963305	Yes (June 9, 2016)	Acceptable
Malaoxon	Hubbard, P. M., and Beavers, J. B. (2012). Malaoxon: An Acute Oral Toxicity Study With the Mallard. Unpublished study performed by Wildlife International, Ltd., Project Number 232-154. (MRID 48963306)	1089 FYF	Cheminova	10/5/2012	48963306	Yes (June 9, 2016)	Acceptable
Malathion Technical	Hubbard, P.; Beavers, J. (2012). Malathion: An Acute Oral Toxicity Study with the Mallard. Project Number: 232/157, 1090/FYF. Unpublished study prepared by Wildlife International Ltd. 70p.	1090 FYF	Cheminova	10/5/2012	48963307	Yes (June 9, 2016)	Acceptable

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion Technical	Dobbins, L.; Kendall, T.; Porch, J. (2012). Malathion Technical: A 96-Hour Toxicity Test with the Freshwater Diatom: (Navicula pelliculosa): Final Report. Project Number: 232P/104. Unpublished study prepared by Wildlife Inte6rnational Ltd. 83p. (1245 FYF)	1245 FYF	Cheminova	10/5/2012	48963310	Yes (June 9, 2016)	Supplemental Quantitative
Malathion Technical	Dobbins, L. ; Kendall, T. ; Porch, J. (2012). Malathion Technical: A 96-Hour Toxicity Test with the Freshwater Alga (Pseudokirchneriella subcapitata): Final Report. Project Number: 232P/105. Unpublished study prepared by Wildlife International Ltd. 79p. (1246 FYF)	1246 FYF	Cheminova	10/5/2012	48963311	Yes (June 9, 2016)	Supplemental Quantitative
Malathion Technical	Dobbins, L.; Kendall, T.; Porch, J. (2012). Malathion Technical: A 96-Hour Toxicity Test with the Freshwater Alga (Anabaena flos-aquae): Final Report. Project Number: 232P/103. Unpublished study prepared by Wildlife International Ltd. 81p.	?	Cheminova	11/20/2012	48998001	No	
Malathion Technical	Dobbins, L.; Kendall, T.; Porch, J. (2012). Malathion Technical: A 96-Hour Toxicity Test with the Marine Diatom (Skeletonema costatum): Final Report. Project Number: 232P/106B. Unpublished study prepared by Wildlife International, Ltd. 77p.	?	Cheminova	11/20/2012	48998002	No	
Malathion Technical	Dobbins, L.; Kendall, T.; Porch, J. (2012). Malathion Technical: A 7-Day Static-renewal Toxicity Test with Duckweed (Lemna gibba G3): Final Report. Project Number: 232P/107. Unpublished study prepared by Wildlife International Ltd. 73p.	?	Cheminova	11/20/2012	48998003	No	
Malathion Technical	Gries, T. (2002). Malathion Technical: Acute Toxicity Test with Fathead Minnow (Pimephales promelas) Under Flow-Through Conditions: Final Report. Project Number: 1005/022/522, 382/FYF. Unpublished study prepared by Springborn Smithers Laboratories (Europe). 63p. (382 FYF)	382 FYF	Cheminova	11/20/2012	48998004	No	

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Malathion Technical	Gries, T.; Van der Kolk, J. (2002).Malathion Technical: Acute Toxicity Test with Common Carp (Cyprinus carpio) Under Flow-Through Conditions: Final Report. Project Number: 1005/022/174, 380/FYF. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 59p (380 FYF)	380 FYF	Cheminova	11/20/2012	48998005	No	
Malathion Technical	Gries, T.; Van Der Kolk, J.; Purghart, V. (2002). Malathion Technical: Acute Toxicity Test with Three-spined Stickleback (Gasterosteus aculeatus) Under Flow-through Conditions: Final Report. Project Number: 381/FYF, 1005/022/177. Unpublished study prepared by Springborn Smithers Laboratories (Europe). 62p. (381 FYF)	381 FYF	Cheminova	11/20/2012	48998006	No	
Malaoxon	Hubbard, P.; Beavers, J.; Martin, K. (2012). Malaoxon: An Acute Oral Toxicity Study with the Ring-Necked Pheasant. Project Number: 232/163. Unpublished study prepared by Wildlife International, Ltd. 90p.	?	Cheminova	12/20/2012	49024601	Yes (June 9, 2016)	Acceptable
Malathion 440 g/L EW (CHA 3110)	Zajak, A, (1996). Cage test of Fyfanon EW on bee colonies. Application on flowering Phacelia sp. In the evening after the daily bee flight. Guideline No. 170, EPPO-1992). Study conducted by Ecotoxicology Laboratory (Hungary). Lab Study No.: 3213/96. CHA Doc. No.: 172 FYF. Final report dated September 26, 1996. 25 p.	172 FYF	Cheminova	2/14/2013	49051201	No	
Malathion 440 g/L EW (CHA 3110)	Gries and Purghart (2001). CHA 3110: Acute toxicity test with bluegill sunfish (Lepomis macrochirus) under flow-through conditions. Springborn Laboratories (Europe) AG. Project No.: 1005.021.105. CHA Doc. No.: 312 FYF. Final report dated March 2, 2001. 58 p.	312 FYF	Cheminova	2/4/2013	49051202	No	

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Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion 440 g/L EW (CHA 3110)	Gries, T.; Purghart, V. (2001) CHA 3110: Acute Toxicity Test with Rainbow Trout (Oncorhynchus mykiss) Under Flow-Through Conditions: Final Report. Project Number: 1005/021/108. CHA Doc. No. 316 FYF. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 60 p.	316 FYF	Cheminova	02/04/2013	49051203	No	
	Gries, T.; Purghart, V. (2001. CHA 3110: Acute Toxicity Test with Rainbow Trout (Oncorhynchus mykiss) Under Flow-Through Conditions: Final Report. Project Number: 1005/021/108, 316/FYF. Unpublished study prepared by Springborn Laboratories (Europe) Ag. 58p.	316 FYF	Cheminova	02/04/2013	49051203	No	
Malathion Technical	Rogers, M. (2002). Formulated Malathion CHA 3110: Acute Oral Toxicity (LD50) to the Bobwhite Quail (Colius virgianus). Huntingdon Life Sciences, Ltd. Project No.: CHV076/012815. CHA Doc. No.: 339 FYF	339 FYF	Cheminova	2/4/2013	49051204	No	
Malathion 440 g/L EW (CHA 3110)	Szentes, C. (2003). Acute oral and contact toxicity of Fyfanon 440 g/l EW on honeybees (Apis mellifera). OECD Guideline No.: 314, 1998. Ecotoxicological Laboratory (Hungary) Project No.: 2916/03. Final report dated October 27, 2003. 28 p.	459 FYF	Cheminova	2/4/2013	49051205	No	
Malathion Technical	Burke, J. (2011). Malathion: Acute toxicity to Cyprinodon variegatus. Covance Laboratories, UK Covance Study No.: 8246917 Cheminova A/S Report No.: 1039	1039 FYF 880 FYF	Cheminova	2/12/2013	49055701	No	
57 EC	Sindermann, et al (2012). Malathion 57%: A Test to Determine the Effects on Vegetative Vigor of Ten Species of Plants. Wildlife International, Ltd., Project No.: 232P-102. Final report dated February 27, 2013.	?	Cheminova	3/8/2013	49076001	Yes (June 9, 2016)	Acceptable
57 EC	Sindermann, et al (2012). Malathion 57%: A Test to Determine the Effects on Seedling Emergence and Growth of Ten Species of Plants. Wildlife International, Ltd., Project No.: 232P-101. Final report dated February 27, 2013..	?	Cheminova	3/8/2013	49076002	Yes (June 9, 2016)	Acceptable

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion 440 g/L EW (CHA 3110)	Gries and Purghart (2001). CHA 3110: Acute Immobilisation Test with Daphnids (Daphnia magna) Under Flow-through Conditions. Springborn Laboratories, Inc. Project No.: 1005.021.115. CHA Doc. No.: 311 FYF. Final report dated March 2, 2001. 64 p.	311 FYF	Cheminova	3/22/2013	49086401	No	
440 g/L EW (3110)	Stäbler, D. (2001). Acute Toxicity of CHA 3110 on Earthworms, Eisenia Foetida using an artificial Soil Test GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH Project/Study No.: 20011194/01-NLEf Cheminova A/S Report No.: 328 FYF	328 FYF	Cheminova	3/22/2013	49086402	No	
Malathion Technical	Wüthrich, V. (1991) Acute toxicity (LC50) Study of Fyfanon Technical to Earthworms. Cheminova unpublished report No.: 49 FYF	49 FYF	Cheminova	3/22/2013	49086403	No	
MMCA	Friedrich, S (2012). Acute toxicity of Malathion monocarboxylic acid (α +β mixture) to the earthworm Eisenia fetida in artificial soil. Biochem agrar GmbH, Gerichshain, Germany. Project No.: 12 10 48 043 S.Cheminova A/S Project No: 1100 FYF	1100 FYF	Cheminova	3/22/2013	49086404	No	
MDCA	Friedrich, S (2012). Acute toxicity of Malathion dicarboxcylic acid (α +β mixture) to the earthworm Eisenia fetida in artificial soil. Biochem agrar GmbH, Gerichshain, Germany. Project No.: 12 10 48 044 S. Cheminova A/S Project No: 1101 FYF	1101 FYF	Cheminova	3/22/2013	49086405	No	
Malathion 440 g/L EW (3110)	Kovacs, 1997. Cage test of Fyfanon EW (Batch no. 438-Aki-17a) on bee colonies. Application of flowering Phacelia sp. in the evening after the daily bee flight Cheminova report No.: 178 FYF	178 FYF	Cheminova	3/22/2013	49086406	No	
440 g/L EW (CHA 3110)	Siemoneit, S. (2005). Test to Determine the effects of Malathion 440 g/L EW On Vegetative Vigour of Terrestrial Plants. Laboratory Project No.:CHE03. CHA Doc. No.: 512 FYF. Final report dated March 9, 2005.	512 FYF	Cheminova	3/22/2013	49086407	Yes (June 9, 2016)	Supplemental Qualitative/non-guideline

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
440 g/L EW (3110)	Knäbe, S. (2003). A field trial assessing the impact of multiple applications of CHE 3110 on the non-target arthropod fauna of an alfalfa (*Medicago sativa*) crop (Italy). GAB Project No.: 20021159/11-FNTO. Final report dated April 22, 2003. 239 p.	438 FYF	Cheminova	3/22/2013	49086408	No	
440 g/L EW (3110)	Müther, J. (2003). A field study to evaluate the effects of CHA 3110 on the Non-target arthropod fauna in an apple orchard in France. Lab project No.: 20021159/F1-FNTO. CHA Doc. No. 437 FYF. Final report dated April 14, 2003. 231 p.	437 FYF	Cheminova	3/22/2013	49086409	No	
440 g.L EW	Staedtler, T.; Henkes, K.; Hanebeck, I. (2011). Residues of Malathion and Malaoxon in Arthropods and Ground Vegetation after Spray Application of an EW Formulation Containing 440 g Malathion/L in an Oilseed Rape Field in Germany - Magnitude of Residues and Time Course of Residue Decline: Final Report. Project Number: 1052/FYF, R11093, 132. Unpublished study prepared by RIFCon GmbH. 189p.	1052 FYF	Cheminova	3/22/2013	49086410	No	
440 g/L EW	Hanebeck, I.; Staedtler, T. (2011). Residues of Malathion and Malaoxon in Arthropods and Ground Vegetation after Spray Application of an EW Formulation Containing 440 g Malathion/L in an Alfalfa Field in Spain - Magnitude of Residues and Time Course of Residue Decline: Final Report. Project Number: 1051/FYF, 127, R10327. Unpublished study prepared by RIFCon GmbH. 198p.	1051 FYF	Cheminova	3/22/2013	49086411	No	
Malathion 440 g/L EW (CHA 3110)	Szentes, C. (1998). Field test of Fyfanon 440 EW on Bee Colonies in Flowering Sunfoower. USEPA Guideine 141-5, 1982, and EPPO No. 170, 1992. Ecotoxicological Laboratory (Hungary) Study No.: 3103-1/97. Final report dated January 19, 1998. 24 p.	223 FYF	Cheminova	3/22/2013	49127001	No	

Table C-1 Malathion and Metabolites – Ecological Effects

Test Substance	*Reference*	*CHA Doc. No.*	*Submitter*	*Date Submitted*	*EPA MRID*	*EPA DER Received? (Yes or No)*	*EPA Acceptability Rating*
Malathion Technical (spiked)	Claude, M. B.; Gallagher, S. P.; Krueger, H. O. (2013). Pure Technic al Malathion Spiked with 0.8% Iso-Malathion: A 96-Hour Flow-Through Acute Toxicity Screening Test with the Fathead Minnow (Pimephales promelas): Final Report. Project Number: 232A/143. Unpublished study prepared by Wildlife International, Ltd. 26p.		Cheminova	11/12/2013	49252801	No	
Malathion Technical (spiked at 0.8% iso-malathion)	Claude, et al, (2013). Pure Technical Malathion Spiked with 0.8% iso-malathion: A 96-hour Flow Through Acute Toxicity Screening Test with the Fathead Minnow (*Pimephales promelas*). Wildlife International Project Number 232A-142. Final report dated November 6, 2013. 26 p..	?	Cheminova	11/12/2013	49252801	No	
Malathion Technical (Pure)	Claude, M. B. ; Gallagher, S. P. ; Krueger, H. O. (2013) Pure Technical Malathion: A 96-Hour Flow-Through Acute Toxicity Screening Test with the Fathead Minnow (Pimephales promelas): Final Report. Project Number: 232A/142. Unpublished study prepared by Wildlife International, Ltd. 25p.		Cheminova	11/12/2013	49252802	No	
Malathion Technical (pure)	Claude, et al, (2013). Pure Technical Malathion: A 96-hour Flow Through Acute Toxicity Screening Test with the Fathead Minnow (*Pimephales promelas*). Wildlife International Project Number 232A-143. Final report dated November 6, 2013. 25p.	?	Cheminova	11/12/2013	49252802	No	
MDCA (malathion metabolite)	Sanders, A. (2008). Malathion Dicarboxylic Acid: Acute Oral Toxicity in the Rat - Fixed Dose Method. Project Number: 054/0640, 0545/06 40/DOC/CST, 611/FYF. Unpublished study prepared by Safepharm Laboratories, Ltd. 37p.		Cheminova	11/12/2013	49252803	No	
MMCA (malathion metabolite)	Sanders, A. (2008). Malathion Monocarboxylic Acid: Acute Oral Toxicity in the Rat - Fixed Dose Method. Project Number: 0545/0639, 610/ FYF, 0545/0639/DOC/CST. Unpublished study prepared by Safepharm Laboratories, Ltd. 38p.		Cheminova	11/12/2013	49252804	No	

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion Technical	Sindermann, A, and John Porch, 2013. Fyfanon Technical: An acute contact l toxicity study with the honey bee. Wildlife International, Ltd., Project Number 232P-113. Final report dated December 2, 2013. 39 p.	?	Cheminova	12/11/2013	49270301	No	
Malathion Technical	Sindermann, A, and John Porch, 2013. Fyfanon Technical: An acute oral toxicity study with the honey bee. Wildlife International, Ltd., Project Number 232P-114. Final report dated December 2, 2013. 41 p.	?	Cheminova	12/11/2013	49270302	No	
Malathion Technical	Brougher, et al, 2014. Malathion: A 96-hour Flow-through Acute Toxicity Test with the Green Sunfish (Lepomis cyanellus). Wildlife International Project No.: 232A-146; CHHA Doc. No. 1408. Final report dated April 2, 2014. 65 p.	?	Cheminova	04/22/2014	49364101	Yes (03/16/2016)	Acceptable
Malathion Technical	Brougher, et al, 2014. Malathion: A 96-hour Flow-through Acute Toxicity Test with the Medaka (Oryzias latipes). Wildlife International Project No.: 232A-144A; CHA Doc. No. 1407. Final report dated April 2, 2014. 64 p.	?	Cheminova	04/22/2014	49364102	Yes (03/16/2016)	Acceptable
Malathion Technical	Brougher, D.S., K.H, Martin, S.P. Gallagher, and E.S. Bodle, (2014). Malathion: A 96-Hour Flow-Through Acute Toxicity Test with the Saltwater Mysid (Americamysis bahia). Wildlife International Project Number: 232A-150A; Final Report dated May 15, 2014; (U.S. EPA OPPTS Guideline 850.1035); 68 p.	?	Cheminova	05/23/2014	49389401	Yes (03/16/2016)	Acceptable
Malathion Technical	Brougher, D.S., K.H, Martin, S.P. Gallagher, and E.S. Bodle, (2014). Malathion: A 96-Hour Flow-Through Acute Toxicity Test with the Freshwater Amphipod (Gammarus pseudolimnaeus). Wildlife International Project Number: 232A-148A; Final Report dated May 15, 2014; (U.S. EPA OPPTS Guideline 850.1020); 71 p.	?	Cheminova	05/23/2014	49389402	Yes (03/16/2016)	Acceptable

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion Technical	Brougher, D.S., K.H, Martin, S.P. Gallagher, and E.S. Bodle, (2014). Malathion: A 96-Hour Flow-Through Acute Toxicity Test with the Eastern Oyster (Crassostrea virginica). Wildlife International Project Number: 232A-149; CHA Doc. No. 14018. Final Report dated May 22, 2014; (U.S. EPA OPPTS Guideline 850.1025); 67 p.	1418	Cheminova	05/23/2014	49389403	Yes (03/16/2016)	Acceptable
Malathion Technical	Brougher, et al, 2014. Malathion: A 96-hour Flow-through Acute Toxicty Test with the Western Mosquitofish (Gambusia affinis). Wildlife International Project Number: 232A-147. 66 p.	?	Cheminova	07/02/2014	49422801	Yes (03/16/2016)	Acceptable
Malathion Technical	Brougher, D.; Keller, K.; Gallagher, S.; et al. (2014). Malathion: A 48-Hour Flow-Through Acute Toxicity Test with the Mayfly (Centroptilum triangulifer): Final Report. Project Number: 232A/152, 232/051414/MAY/48H2/OECD/OPPTS/SUB232. Unpublished study prepared by Wildlife International, Limited. 60p.		Cheminova	10/02/2014	49479001	Yes (03/16/2016)	Acceptable
Malathion Technical	Keller, K.; Gallagher, S.; Martin, K.; et al. (2014). Malathion: A 48-Hour Flow-Through Acute Toxicity Test with the Midge (Chironomus tentans): Final Report. Project Number: 232A/153, 232/091913/CHI/48H2/OECD/OPPTS/SUB232. Unpublished study prepared by Wildlife International, Ltd. 59p.		Cheminova	10/02/2014	49479002	Yes (03/16/2016)	Acceptable
Malathion Technical	Gallagher, S.; Martin, K.; Brougher, D.; et al. (2014). Malathion: A 96-Hour Flow-Through Acute Toxicity Test with the Coho Salmon (Oncorhynchus kisutch): Final Report. Project Number: 232A/145A, 232/082713/SAL/96H2/OECD/OPPTS/SUB232. Unpublished study prepared by Wildlife International, Ltd. 62p.		Cheminova	10/02/2014	49479003	Yes (03/16/2016)	Acceptable

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion Technical	Brougher, D.; Keller, K.; Gallagher, S.; et al. (2014). Malathion: A 96-Hour Flow-Through Acute Toxicity Test With the Crayfish (Procamarus clarkii). Project Number: 1481/FYF, 232A/155A. Unpublished study prepared by Wildlife International, Ltd. 63p.		Cheminova	12/19/2014	49534901	Yes (03/16/2016)	Acceptable
Malathion Technical	Brougher, D. ; Keller, K.; Gallagher, S.; et al. (2014). A 96-Hour Flow-Through Acute Toxicity Test with the Grass Shrimp (Palaemontetes pugio): Final Report. Project Number: 1482/FYF, 232A/156. Unpublished study prepared by Wildlife International Ltd. 62p.		Cheminova	12/19/2014	49534902	Yes (03/16/2016)	Acceptable
Fyfanon ULV (96.5%)	Sindermann, A.; Porch, J.; Schutt, W.; et al. (2015). Fyfanon ULV: A foliage Residue Toxicity Study with the Honeybee: Final Report. Project Number: 232P/115, 232/060614/BFR/SUB232. Unpublished study prepared by Wildlife International Limited. 69p.		Cheminova	02/23/2015	49574801	No	
TEPP	Fort, D. (2014). Acute Oral Toxicity of Fenthion and TEPP to Adult Bullfrog (Rana catesbeiana). Project Number: CHEM01/00340. Unpublished study prepared by Fort Environmental Laboratories, Inc. 88p.		Cheminova	08/10/2015	49693701	No	
Dimethoate Technical	Fort, D. (2015). Dose Range Finding Acute Oral Toxicity of Dimethoate to Bullfrog (Rana catesbeiana). Project Number: CHEM01/00347. Unpublished study prepared by Fort Environmental Laboratories, Inc. 78p		Cheminova	08/10/2015	49693702	No	
Dimethoate Technical	Fort, D. (2015). Definitive Acute Oral Toxicity of Dimethoate Technical to Adult Bullfrog (Lithobates catesbeianus). Project Number: CHEM01/00348. Unpublished study prepared by Fort Environmental Laboratories, Inc. 141p		Cheminova	08/10/2015	49693703	No	

Table C-1 Malathion and Metabolites – Ecological Effects							
Test Substance	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***	***EPA Acceptability Rating***
Malathion Technical	Fort, D. (2015). Dose Range Finding Acute Oral Toxicity of Fyfanon(R) Technical to Bullfrog (Lithobates catesbeianus). Project Number: CHEM01/00359. Unpublished study prepared by Fort Environmental Laboratories, Inc. 76p		Cheminova	08/10/2015	49693704	No	
Malathion Technical	Fort, D. (2015). Definitive Acute Oral Toxicity of Fyfanon(R) Technical to Adult Bullfrog (Lithobates catesbeianus). Project Number: CHEM01/00360. Unpublished study prepared by Fort Environmental Laboratories, Inc. 157p.		Cheminova	08/10/2015	49693705	No	
Malathion Technical	Rhodes, J.E., Leak, T. (1997). An Evaluation of Malathion in a Flow-through Diluter System Under Actual Full Life-Cycle Test Conditions. Data provided to EPA as an enclosure to a letter dated March 18, 1997.	175 FYF	Cheminova	03/18/1997 (resubmitted 09/22/2015 for MRID)	49723701	No	

Table C-2 Malathion and Metabolites – Environmental Fate

EPA Guideline	*Guideline Description*	*Reference*	*CHA Doc. No.*	*Submitter*	*Date Submitted*	*EPA MRID*	*EPA DER Received? (Yes or No)*
161-1	Hydrolysis (Malathion)	Teeter, D. (1988). Malathion (AC 6,601): Hydrolysis: Lab. Rept. No. PD-M 25-59. Unpublished study prepared by American Cyanamid Co. 66 p.		MALATHION REREGISTRATION TASK FORCE	12/22/1988	40941201	Yes
161-1	Hydrolysis (Malathion)	Severn, D. (1993). Overview of the Environmental Behavior of Malathion and Response to EPA's Reviews of Malathion Environmental Fate Studies: Lab Project Number: 38003-29: 38003-28: 38003-27. Unpublished study prepared by Jellinek, Schwartz & Connolly, Inc. 109 p.		Cheminova	12/07/1993	43166301	Yes
161-1	Hydrolysis (Malaoxon)	Shepler, K. (2004) Hydrolysis of [(Carbon 14)] Malaoxon at pH 5, 7 and 9. Project Number: 1258W, 1258W/1. Unpublished study prepared by PTRL West, Inc. 111 p.		Cheminova	11/3/2004	46396601	Yes
161-2	Photo degradation in water (malathion)	Carpenter, M. (1990). Determination of the Photolysis Rate of (Carbon 14)-Malathion in pH-4 Aqueous Solution: Lab Project No.: 37574. Unpublished study prepared by Analytical Bio-Chemistry Laboratories, Inc. 535 p.		MALATHION REREGISTRATION TASK FORCE	10/29/1990	41673001	Yes
161-2	Photo degradation in water (malathion)	Severn, D. (1993). Overview of the Environmental Behavior of Malathion and Response to EPA's Reviews of Malathion Environmental Fate Studies: Lab Project Number: 38003-29: 38003-28: 38003-27. Unpublished study prepared by Jellinek, Schwartz & Connolly, Inc. 109 p.		Cheminova	12/07/1993	43166301	Yes
161-3	Photo degradation on soil (malathion)	Dykes, J.; Kabler, K.; Allen, B. (1990). Determination of the Photolysis Rate on the Surface of Soil with Malathion: Lab Project Number: 37575. Unpublished study prepared by Analytical Bio-Chemistry Labs. 952 p.		BASF Corporation on behalf of the MALATHION REREGISTRATION TASK FORCE	11/19/1990	41695501	Yes
161-3	Photo degradation on	Ruzo, L. (1988) Soil Surface Photolysis of Carbon 14-Trichloro- methyl Captan in		CAPTAN TASK FORCE	06/14/1988	40658009	Yes

Table C-2 Malathion and Metabolites – Environmental Fate

EPA Guideline	*Guideline Description*	*Reference*	*CHA Doc. No.*	*Submitter*	*Date Submitted*	*EPA MRID*	*EPA DER Received? (Yes or No)*
	soil (malathion)	Natural Sunlight: Laboratory Project ID: 231. Unpublished study prepared by Pharmacology and Toxicology Research Laboratory. 117 p.					
161-3	Photo degradation on soil (malathion)	Severn, D. (1993). Overview of the Environmental Behavior of Malathion and Response to EPA's Reviews of Malathion Environmental Fate Studies: Lab Project Number: 38003-29: 38003-28: 38003-27. Unpublished study prepared by Jellinek, Schwartz & Connolly, Inc. 109 p.		Cheminova	12/07/1993	43166301	Yes
161-4	Photo degradation in air (malathion)	Miller, G. (1988). Vapor Phase Photolysis of Malathion: Laboratory Report No. PD-M-25-60. Unpublished study prepared by American Cyanamid Co. 17 p.		MALATHION REREGISTRATION TASK FORCE	01/19/1989	40969301	Yes
162-1	Aerobic soil metabolism (malathion)	Blumhorst, M. (1990) Aerobic Soil Metabolism Study of Malathion: Lab Project Number: 135-004. Unpublished study prepared by EPL Bio-Analytical Services, Inc. 211		MALATHION REREGISTRATION TASK FORCE	12/12/1990	41721701	Yes
162-1	Aerobic soil metabolism (malathion)	Severn, D. (1993). Overview of the Environmental Behavior of Malathion and Response to EPA's Reviews of Malathion Environmental Fate Studies: Lab Project Number: 38003-29: 38003-28: 38003-27. Unpublished study prepared by Jellinek, Schwartz & Connolly, Inc. 109 p.		Cheminova	12/07/1993	43166301	Yes
162-1	Aerobic soil metabolism (malathion)	Ma, Q.; Buonagurio, J.; Schocken, M.; et al. (2011). Determining the Acceptability of Foreign Soils Used in Malathion Aerobic Soil and Aerobic Aquatic Metabolism Studies. Project Number: VA10532/000/A0T0/0811/RR08. Unpublished study prepared by Exponent, Inc. 14p.		Cheminova	8/26/2011	48585102	Yes
162-1	Aerobic soil metabolism (malathion)	Knoch, E. (2001). Malathion: Aerobic Soil Metabolism. Project Number: IF/100/30745/00, 336/FYF. Unpublished study prepared by Institut Fresenius Chemische und		Cheminova	2/27/2006	46769501	Yes

Table C-2 Malathion and Metabolites – Environmental Fate

EPA Guideline	Guideline Description	Reference	CHA Doc. No.	Submitter	Date Submitted	EPA MRID	EPA DER Received? (Yes or No)
		Biologische. 240 p.					
162-1	Aerobic soil metabolism (malathion)	Saxena, A. (1998). Aerobic and Aerobic/Anaerobic Soil Metabolism of (Carbon 14)-Malathion on a Loamy Sand Soil: Final Report. Project Number: HLA/6123/153. Unpublished study prepared by Hazleton Laboratories America, Inc. 141 p.		Cheminova	8/21/2009	47834301	No
162-1	Aerobic soil metabolism (malaoxon)	Hiler, T. (2012) Aerobic Soil Metabolism of [Carbon 14]Malaoxon in Four Soils. Project Number: 2144W, 2144W/1. Unpublished study prepared by PTRL West, Inc. 254p.		Cheminova	9/9/2012	48903601	No
162-2	Aneerobic soil metabolism (malathion)	Saxena, A. (1998). Aerobic and Aerobic/Anaerobic Soil Metabolism of (Carbon 14)-Malathion on a Loamy Sand Soil: Final Report. Project Number: HLA/6123/153. Unpublished study prepared by Hazleton Laboratories America, Inc. 141 p.		Cheminova	8/21/2009	47834301	No
162-3	Anaerobic aquatic metabolism (malathion)	Blumhorst, M. (1991) Anaerobic Aquatic Metabolism Study of Malathion: Lab Project Number: 135-002. Unpublished study prepared by EPL Bio-Analytical Services, Inc. (EPL-BAS). 179 p.		Cheminova	1/8/1992	42216301	Yes
162-3	Anaerobic aquatic metabolism (malathion)	Severn, D. (1993). Overview of the Environmental Behavior of Malathion and Response to EPA's Reviews of Malathion Environmental Fate Studies: Lab Project Number: 38003-29: 38003-28: 38003-27. Unpublished study prepared by Jellinek, Schwartz & Connolly, Inc. 109 p.		Cheminova	12/07/1993	43166301	Yes
162-4	Aerobic aquatic metabolism (malathion)	Blumhorst, M. (1991). Aerobic Aquatic Metabolism Study of Malathion: Lab Project Number: 135-003. Unpublished study prepared by EPL Bio-Analytical Services, Inc. 155 p.		MALATHION REREGISTRATION TASK FORCE	1/8/1992	42271601	Yes
162-4	Aerobic aquatic metabolism (malathion)	Severn, D. (1993). Overview of the Environmental Behavior of Malathion and Response to EPA's Reviews of Malathion		Cheminova	12/07/1993	43166301	

Table C-2 Malathion and Metabolites – Environmental Fate

EPA Guideline	*Guideline Description*	*Reference*	*CHA Doc. No.*	*Submitter*	*Date Submitted*	*EPA MRID*	*EPA DER Received? (Yes or No)*
		Environmental Fate Studies: Lab Project Number: 38003-29: 38003-28: 38003-27. Unpublished study prepared by Jellinek, Schwartz & Connolly, Inc. 109 p.					
162-4	Aerobic aquatic metabolism (malathion)	Hiler, T.; Mannella, L. (2012). Aerobic Aquatic Metabolism of [Carbon 14]Malathion. Project Number: 2143W, 2143W/1. Unpublished study prepared by PTRL West, Inc. 343p.		Cheminova	9/13/2012	48906401	No
162-4	Aerobic aquatic metabolism (malathion)	Ma, Q.; Buonagurio, J.; Schocken, M.; et al. (2011). Determining the Acceptability of Foreign Soils Used in Malathion Aerobic Soil and Aerobic Aquatic Metabolism Studies. Project Number: VA10532/000/A0T0/0811/RR08. Unpublished study prepared by Exponent, Inc. 14p.		Cheminova	8/26/2011	48585102	Yes
162-4	Aerobic aquatic metabolism (malathion)	Knoch, E. (2001). Degradability and Fate of Malathion in the Aquatic Environment (Water/Sediment System). Project Number: IF/100/30746/00, 337/FYF. Unpublished study prepared by Institut Fresenius Chemische und Biologische. 241 p.02		Cheminova	2/27/2006	46769502	Yes
162-4	Aerobic aquatic metabolism (malaoxon)	Hiler, T. (2011). Aerobic Aquatic Metabolism of [Carbon 14]Malaoxon. Project Number: 889/FYF, 1980W, 1980W/1. Unpublished study prepared by PTRL West, Inc. 180 p.		Cheminova	6/15/2011	48508601	No
163-1	Mobility (leaching/ adsorption/ desorpotion) (malathion)	Blumhorst, M. (1989). Adsorption/Desorption Studies-Batch Equilibrum for Malathion: Lab Project Number: 135-001. Unpublished study prepared by EPL Bio-Analytical Services, Inc. 145 p.		MALATHION REREGISTRATION TASK FORCE	01/05/1990	41345201	Yes
163-1	Mobility (leaching/ adsorption/ desorpotion) (malathion)	Severn, D. (1993). Overview of the Environmental Behavior of Malathion and Response to EPA's Reviews of Malathion Environmental Fate Studies: Lab Project Number: 38003-29: 38003-28: 38003-27. Unpublished study prepared by Jellinek, Schwartz & Connolly, Inc. 109 p.		Cheminova	12/07/1993	43166301	Yes

Table C-2 Malathion and Metabolites – Environmental Fate

EPA Guideline	Guideline Description	Reference	CHA Doc. No.	Submitter	Date Submitted	EPA MRID	EPA DER Received? (Yes or No)
163-1	Mobility (leaching/ adsorption/ desorpotion) (malathion)	Nixon, W. (1995). Column Leaching of (carbon 14)-Malathion in Four Soil Types Following Aerobic Aging: Lab Project Number: 951: 1842. Unpublished study prepared by PTRL East, Inc. 163 p.		Cheminova	12/7/1995	43868601	Yes
163-1	Mobility (leaching/ adsorption/ desorpotion) (malaoxon)	Hiler, T. (2011). Soil Adsorption/Desorption of [(Carbon 14)]Malaoxon by the Batch Equilibrium Method. Project Number: 2145W 2145W/1. Unpublished study prepared by PTRL West, Inc. 238p.		Cheminova	08/12/2011	48571804	No
163-2	Volatility-lab (malathion)	Spare, W.; Cutchin, W. (1991). The Volatilization of Malathion from Soil (A Laboratory Study): Lab Project Number: 2901. Unpublished study prepared by Agrisearch, Inc. 163 p.		MALATHION REREGISTRATION TASK FORCE	09/12/1991	42015201	Yes
163-3	Volatility - Field						No required
164-1	Soil Field Dissipation	Rice, F.; Jacobson, B.; Lochhaas, C. (1990). Terrestrial Field Dissipation for Malathion in Cotton (Georgia): Lab Project Number 38005. Unpublished study prepared by Analytical Bio-chemistry Laboratories, Inc. 259 p		MALATHION REREGISTRATION TASK FORCE	01/16/91	41748901	Yes
164-1	Soil Field Dissipation	Jacobson, B. (1992). Supplement to: Terrestrial Field Dissipation for Malathion in Cotton: Final Report: Lab Project Number: 38005: FS-890106. Unpublished study prepared by ABC Labs, Inc. 11 p.		Cheminova	12/07/93	43042401	Yes
164-1	Soil Field Dissipation	Rice, F.; Jacobson, B.; Lochhaas, C. (1990). Terrestrial Field Dissipation For Malathion in Cotton (California): Lab Project Number: 38003. Unpublished study prepared by Analytical Bio-Chemistry Laboratories, Inc. 241 p		MALATHION REREGISTRATION TASK FORCE	12/19/1990	41727701	Yes
164-1	Soil Field Dissipation	Jacobson, B. (1992). Supplement to: Terrestrial Field Dissipation for Malathion in Cotton: Lab Project Number: 38003: FA-890210. Unpublished study prepared by ABC Labs, Inc. 124 p.		Cheminova	12/07/93	43042402	Yes

Table C-2 Malathion and Metabolites – Environmental Fate

EPA Guideline	*Guideline Description*	*Reference*	*CHA Doc. No.*	*Submitter*	*Date Submitted*	*EPA MRID*	*EPA DER Received? (Yes or No)*
164-1	Soil Field Dissipation	Severn, D. (1993). Overview of the Environmental Behavior of Malathion and Response to EPA's Reviews of Malathion Environmental Fate Studies: Lab Project Number: 38003-29: 38003-28: 38003-27. Unpublished study prepared by Jellinek, Schwartz & Connolly, Inc. 109 p.		Cheminova	12/07/1993	43166301	Yes
164-2	Aquatic Field Dissipation	Judy, D.; Jacobson, B.; Lochhaas, C. (1991). Combined Aquatic Sediment Field Dissipation and Irrigated Crop Accumulation Study with Malathion (CA): Lab Project Number: 38004. Unpublished study prepared by ABC Labs, Inc. and Pan-Agriculteral Labs. 368 p.		MALATHION REREGISTRATION TASK FORCE	10/18/91	42058401	Yes
164-2	Aquatic Field Dissipation	Judy, D.; Jacobson, B.; Lochhaas, C. (1991). Combined Aquatic Sediment Field Dissipation and Irrigated Crop Accumulation Study with Malathion (MO): Lab Project Number: 38006. Unpublished study prepared by ABC Labs, Inc. and Pan-Agriculteral Labs. 335 p.		MALATHION REREGISTRATION TASK FORCE	10/18/91	42058402	Yes
164-2	Aquatic Field Dissipation	Severn, D. (1993). Overview of the Environmental Behavior of Malathion and Response to EPA's Reviews of Malathion Environmental Fate Studies: Lab Project Number: 38003-29: 38003-28: 38003-27. Unpublished study prepared by Jellinek, Schwartz & Connolly, Inc. 109 p.		Cheminova	12/07/1993	43166301	Yes
Special study	Dissipation of residues on hard surfaces outdoors	Rice, F. (2011). Dissipation of Residues of Malathion and Malaoxon after Application of Fyfanon ULV AG Insecticide with and without the Use of NuLure Insect Bait to Hard Surfaces, Sand, and Soil. Project Number: 66208. Unpublished study prepared by ABC Laboratories, Inc. 1011p.		Cheminova	10/28/2011	48626701	No
Special study	Dissipation of residues on hard surfaces outdoors	Hiler, T.; Mannella, L. (2012). Environmental Fate of [Carbon 14]Malathion on Hard and Inert Surface Under Outdoor Conditions. Project Number: 2227W, 2227W/1.		Cheminova	11/05/2012	48986601	No

Table C-2 Malathion and Metabolites – Environmental Fate

EPA Guideline	*Guideline Description*	*Reference*	*CHA Doc. No.*	*Submitter*	*Date Submitted*	*EPA MRID*	*EPA DER Received? (Yes or No)*
		Unpublished study prepared by PTRL West, Inc. and Excel Research Services, Inc. 217p.					
164-3	Forest Field Dissipation						Not required
164-5	Long-term Soil Field Dissipation						Not required
165-2	Field rotational crop accumulation						Not required
165-3	Irrigated crop accumulation	Judy, D.; Jacobson, B.; Lochhaas, C. (1991). Combined Aquatic Sediment Field Dissipation and Irrigated Crop Accumulation Study with Malathion (CA): Lab Project Number: 38004. Unpublished study prepared by ABC Labs, Inc. and Pan-Agriculteral Labs. 368 p.		MALATHION REREGISTRATION TASK FORCE	10/18/91	42058401	Yes
165-3	Irrigated crop accumulation	Judy, D.; Jacobson, B.; Lochhaas, C. (1991). Combined Aquatic Sediment Field Dissipation and Irrigated Crop Accumulation Study with Malathion (MO): Lab Project Number: 38006. Unpublished study prepared by ABC Labs, Inc. and Pan-Agriculteral Labs. 335 p.		MALATHION REREGISTRATION TASK FORCE	10/18/91	42058402	Yes
165-3	Irrigated crop accumulation	Severn, D. (1993). Overview of the Environmental Behavior of Malathion and Response to EPA's Reviews of Malathion Environmental Fate Studies: Lab Project Number: 38003-29: 38003-28: 38003-27. Unpublished study prepared by Jellinek, Schwartz & Connolly, Inc. 109 p.		Cheminova	12/07/1993	43166301	Yes
165-4	Fish Bioaccumulation	Forbis, A.; Leak, T. (1994). Uptake Depuration, and Bioconcentration of (carbon 14)-Malathion by Bluegill Sunfish (Lepomis macrochirus) under Flow-Through Test Conditions: Lab Project Number: 40542: XBL-92151. Unpublished study prepared by ABC Labs, Inc. and XenoBiotic Labs, Inc. 96 p.		Cheminova	02/01/94	43106401	Yes
165-4	Fish	Forbis, A.; Leak, T. (1994). Raw Data Report		Cheminova	02/01/94	43106402	Yes

Table C-2 Malathion and Metabolites – Environmental Fate							
EPA Guideline	***Guideline Description***	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received? (Yes or No)***
	Bioaccumulation	for: Uptake Depuration, and Bioconcentration of (carbon 14)-Malathion by Bluegill Sunfish (Lepomis macrochirus) under Flow-Through Test Conditions: Lab Project Number: 40542R: XBL-92151. Unpublished study prepared by ABC Labs, Inc. and XenoBiotic Labs, Inc. 1096 p.					
165-4	Fish Bioaccumulation	Kammerer, R.; Robinson, R. (1994) Procedure for Flow-Through Bluegill Bioconcentration Studies with Radiolabeled Test Substances (Test Substance: (carbon 14)-Malathion): Lab Project Number: XBL92151: RPT00179: 40542. Unpublished study prepared by ABC Laboratories, Inc. and XenoBiotic Laboratories, Inc.313 p.		Cheminova	08/17/94	43340301	Yes

Table C-3 Malathion – Residue Chemistry								
EPA Guideline	***Guideline Description***	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received ? (Yes or No)***	***EPA Acceptability Rating***
860.1500	Magnitude of the Residues in Raw Agricultural Commodities	Rice, F. (2012). Magnitude of Malathion and Malaoxon Residues in/on Wheat Hay following Three Applications of Fyfanon ULV AG or Malathion 5EC with a 7-Day Retreatment Interval and a 7-Day PHI. Project Number: 66906. Unpublished study prepared by ABC Laboratories, Inc. 315p.		Cheminova	8/8/2012	48903001	No	
860.1500	Magnitude of the Residues in Raw Agricultural Commodities	Rice, F. (2012). Magnitude of Malathion and Malaoxon Residues in/on Grain Sorghum Forage and Stover Following Three Applications of Fyfanon ULV AG or Malathion 5EC with a 7-Day Retreatment Interval and a 7-Day PHI. Project Number: 66905, 66906. Unpublished study prepared by ABC Laboratories, Inc. 309p		Cheminova	9/21/2012	48945701	No	
860.1500	Magnitude of the Residues in Raw Agricultural Commodities	Vincent, T. (2014). Magnitude of Malathion and Malaoxon Residues in/on Grain Sorghum Forage and Stover Following Three Applications of Fyfanon ULV AG or Malathion 5EC with a 7-day Retreatment Interval and a 7-day PHI: Final Amended Report. Project Number: 66905. Unpublished study prepared by ABC Laboratories, Inc. 353p.		Cheminova	7/16/2014	49430001	No	
860.1500	Magnitude of the Residues in Raw Agricultural Commodities	Rice, F. (2012). Magnitude of Malathion and Malaoxon Residues in/on Cotton Gin By-Products Following Multiple Applications of Fyfanon ULV AG or Malathion 5EC. Project Number: 66904. Unpublished study prepared by ABC Laboratories, Inc. 205p.		Cheminova	10/9/2012	48964801	No	
860.1520	Magnitude of the Residues in Processed	Samoil, K. (2012). Malathion: Magnitude of the Residue on Flax Processed Commodities. Project Number: 10082,		Cheminova	4/12/2012	48791901	No	

Table C-3 Malathion – Residue Chemistry								
EPA Guideline	***Guideline Description***	***Reference***	***CHA Doc. No.***	***Submitter***	***Date Submitted***	***EPA MRID***	***EPA DER Received ? (Yes or No)***	***EPA Acceptability Rating***
	Commodities	10082/10/TIR04, 10082/10/IDP01. Unpublished study prepared by Interregional Research Project No. 4, USDA-ARS and University of Idaho. 180p.						

Appendix D
Study Evaluations Completed in Support of Cheminova's Response to EPA's Draft Biological Evaluation for Malathion

Aquatic Receptor Studies

Study Quality and Acceptability Evaluation

Compound: Malathion

Study reference (Primary): Parrish, P.R., E.E. Dyar, M.A. Lindberg, C.M. Shanika, and J.M. Enos. 1977. Chronic toxicity of methoxychlor, malathion, and carbofuran to sheepshead minnows (*Cyprinodon variegatus*). US EPA 68-03-0264.

Secondary reference: Not applicable.

Is the primary reference study a GLP study?: No

Species tested: Sheepshead minnow (*Cyprinodon variegatus*)

GENERIC CRITERIA		Y/N	COMMENTS
1	Single chemical exposure?	Y	
2	Was the study conducted according to a recognized international standard (OPPTS, OECD, ASTM, ISO, ETC.)?	Y	Guideline: EPA-600/3-75-009
3	If NO to 2, was a complete description given of the test system and methods? Were the methods used acceptable laboratory practices?	N/A	
4	Were the identification, purity and source of test substance given and comparable to the current technical material and formulation?	Y/N	Test substance: Malathion Source: American Cyanamid Company, Agricultural Division, Princeton, NJ Purity: 95% Batch: Not reported
5	Were effects endpoints ecologically relevant?	Y	LC50
6	Was an appropriate exposure duration used and reported?	Y	96-h, 140-d
7	Were appropriate controls included, reported and results adequate?	Y	
8	Were the statistical procedures reported and appropriate?	Y	Probit

ADDITIONALCRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
9	Were the concentrations provided?	Y	Nominal concentrations: 22, 30, 40, 53, 70, 94, 125 µg a.i./L (acute), 4, 8, 15, 30, 60 µg a.i./L (chronic)
10	Were the test concentrations measured / maintained?	Y	Renewal method: Intermittent flow-through Measured concentrations: 16, 26, 31, 46, 64, 88, 109 µg a.i./L (acute), 4, 9, 18, 37, 86 µg a.i./L (chronic)

ADDITIONALCRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
11	**Was the test species acclimated and characteristics of test species reported (e.g., strain, sex, age, life stage, length, weight)?**	Y	Organism: Sheepshead minnow (*Cyprinodon variegatus*) Source: Big Lagoon Acclimation: 14-d Age/lifestage: Size: 0.8-1.8 cm SL
12	**Were appropriate test conditions (e.g., pH, conductivity, salinity, light intensity, temperature, DO, hardness of water, feeding, photoperiod, loading density, etc.) reported and within acceptable ranges?**	Y/N	pH: Not reported Conductivity: Not reported Salinity: Not reported Light intensity: 1100 lux Temperature: 30 ± 1 ºC Dissolved oxygen: Not reported Hardness: Not reported Photoperiod: 16:8 l:d Feeding: Fish food daily Loading density: 20
13	**Was a concentration- or dose-response relationship demonstrated?**	Y	

EVALUATION: () ACCEPTABLE () SUPPLEMENTAL (X) UNACCEPTABLE

Comments: Insufficient information regarding test substance. As per the National Academy of Sciences (NAS, 2013), the test material must be determined to be relevant to the United States. For malathion, the relevance of the study can only be determined by knowing the composition of the product and demonstrating that the composition is similar to a currently registered product. For these reasons, a study is classified as acceptable when the composition of the impurities and/or inerts is known and is verifiable as being relevant to the United States, and meets all other generic and acceptable criteria. Studies conducted by American Cyanamid can be classified as supplemental if the batch number is available. If the batch number for an American Cyanamid study is not available, the study is unacceptable. All other studies are classified as unacceptable.

Endpoint(s):

Common Name	*Scientific Name*	*Purity (%)*	*Exposure Duration*	*Effect*	*Endpoint*	*Endpoint Conc.*	*Units*	*Study Classification*
Sheepshead minnow	*Cyprinodon variegatus*	95	96-h	Mortality	LC50	51	µg a.i./L	Unacceptable
Sheepshead minnow	*Cyprinodon variegatus*	95	140-d	Mortality	NOAEC	≤18	µg a.i./L	Unacceptable
Sheepshead minnow	*Cyprinodon variegatus*	95	140-d	Mortality	LOAEC	≥18	µg a.i./L	Unacceptable
Sheepshead minnow	*Cyprinodon variegatus*	95	140-d	Fry mortality	NOAEC	4	µg a.i./L	Unacceptable
Sheepshead minnow	*Cyprinodon variegatus*	95	140-d	Fry mortality	LOAEC	9	µg a.i./L	Unacceptable

Study Quality and Acceptability Evaluation

Compound: Malathion

Study reference (Primary): Tessier, L., J.L. Boisvert, L. B.-M. Vought, and J. O. Lacoursière. 2000. Anomalies on capture nets of *Hydropsyche slossonae* larvae (Trichoptera; Hydropsychidae), a potential indicator of chronic toxicity of malathion (organophosphate insecticide). Aqua. Tox. 50:125-139. [E65789]

Secondary reference: Tessier, L., J.L. Boisvert, L. B.-M. Vought, and J. O. Lacoursière. 1999. Anomalies on capture nets of *Hydropsyche slossonae* larvae (Trichoptera; Hydropsychidae) following a sublethal chronic exposure to cadmium. Environ. Pollut. 108:425-438.

Is the primary reference study a GLP study?: No

Species tested: Caddisfly (*Hydropsyche slossonae*)

GENERIC CRITERIA		Y/N	COMMENTS
1	**Single chemical exposure?**	Y	
2	**Was the study conducted according to a recognized international standard (OPPTS, OECD, ASTM, ISO, ETC.)?**	N	Guideline:
3	**If NO to 2, was a complete description given of the test system and methods? Were the methods used acceptable laboratory practices?**	Y	
4	**Were the identification, purity and source of test substance given and comparable to the current technical material and formulation?**	N	Test substance: Malathion Source: American Cyanamid Co. Purity: 96.7% Batch: Not reported
5	**Were effects endpoints ecologically relevant?**	N	EC50 net deformities
6	**Was an appropriate exposure duration used and reported?**	Y	20-d
7	**Were appropriate controls included, reported and results adequate?**	Y	
8	**Were the statistical procedures reported and appropriate?**	Y	Probit, ANOVA

ADDITIONALCRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
9	**Were the concentrations provided?**	Y	Nominal concentrations: 0.01, 0.05, 0.1, 0.5, 1.0 µg/L
10	**Were the test concentrations measured / maintained?**	N	Renewal method: Static Measured concentrations:
11	**Was the test species acclimated and characteristics of test species reported (e.g., strain, sex, age, life stage, length,**	Y	Organism: Caddisfly (*Hydropsyche slossonae*) Source: Becancour River, QC, CA

ADDITIONALCRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
	weight)?		Acclimation: 2-weeks Age/lifestage: 4th instar Size: Not reported
12	**Were appropriate test conditions (e.g., pH, conductivity, salinity, light intensity, temperature, DO, hardness of water, feeding, photoperiod, loading density, etc.) reported and within acceptable ranges?**	**Y**	pH: 7 ± 0.2 Conductivity: 124.2 ± 12.3 µS/cm Salinity: Not reported Light intensity: Not reported Temperature: 15.6 ± 0.2 ºC Dissolved oxygen: 98.0 ± 1.8% Hardness: Not reported Photoperiod: 16:8 l:d Feeding: *Artemia sp.* twice weekly Loading density: 70
13	**Was a concentration- or dose-response relationship demonstrated?**	**Y**	

EVALUATION: () ACCEPTABLE () SUPPLEMENTAL (X) UNACCEPTABLE

Comments: Endpoints not ecologically relevant and not enough information provided about test substance. As per the National Academy of Sciences (NAS, 2013), the test material must be determined to be relevant to the United States. For malathion, the relevance of the study can only be determined by knowing the composition of the product and demonstrating that the composition is similar to a currently registered product. For these reasons, a study is classified as acceptable when the composition of the impurities and/or inerts is known and is verifiable as being relevant to the United States, and meets all other generic and acceptable criteria. Studies conducted by American Cyanamid can be classified as supplemental if the batch number is available. If the batch number for an American Cyanamid study is not available, the study is unacceptable. All other studies are classified as unacceptable.

Secondary reference contains more information about experimental setup.

Endpoint(s):

Common Name	*Scientific Name*	*Purity (%)*	*Exposure Duration*	*Effect*	*Endpoint*	*Endpoint Conc.*	*Units*	*Study Classification*
Caddisfly	*Hydropsyche slossonae*	96.7	10-d	Net midline deformity	EC50	1.19 (1.15)	µg/L (µg a.i./L)	Unacceptable
Caddisfly	*Hydropsyche slossonae*	96.7	20-d	Net symmetry	NOAEC	0.1 (0.1)	µg/L (µg a.i./L)	Unacceptable
Caddisfly	*Hydropsyche slossonae*	96.7	20-d	Net symmetry	LOAEC	0.5 (0.5)	µg/L (µg a.i./L)	Unacceptable
Caddisfly	*Hydropsyche slossonae*	96.7	20-d	Net abnormalities / AChE activity	NOAEC	0.05 (0.05)	µg/L (µg a.i./L)	Unacceptable
Caddisfly	*Hydropsyche slossonae*	96.7	20-d	Net abnormalities / AChE activity	LOAEC	0.1 (0.1)	µg/L (µg a.i./L)	Unacceptable

Terrestrial Receptor Studies

Study Quality and Acceptability Evaluation

Compound: Malathion

Study reference (Primary): Acker, C.I., A.C.G. Souza, S. Pinton, J.T. da Rocha, C.A. Friggi, R. Zanella, and C.W. Nogueira. 2011. Repeated malathion exposure induces behavioral impairment and AChE activity inhibition in brains of rat pups. Ecotox Env Saf 74:2310-2315.

Secondary reference: Not applicable

Is the primary reference study a GLP study?: No

Species tested: Wistar rats (*Rattus norvegicus*)

GENERIC CRITERIA		Y/N	COMMENTS
1	**Single chemical exposure?**	**Y**	Malathion
2	**Was the study conducted according to a recognized international standard (OPPTS, OECD, ASTM, ISO, *etc.*)?**	**N**	
3	**If NO to 2, was a complete description given of the physical test system and methods? Were the methods used considered acceptable laboratory practices?**	**N**	
4	**Were the identification, purity and source of test substance given and comparable to the current technical material and formulation?**	**N**	Test substance: Malathion 500 CE Source: Indol do Brazil Agroquímica LTDA, Curitiba, PR, Brazil Purity: 49.7% Batch: Not reported
5	**Were the effects endpoints ecologically relevant?**	**Y**	Behavior, Bioactivity
6	**Were an appropriate exposure duration and post-exposure observation period used and reported?**	**Y**	4-d
7	**Were appropriate controls included, reported and the results adequate?**	**Y**	
8	**Were statistical procedures reported and appropriate?**	**Y**	ANOVA

ADDITIONAL CRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
9	**Were test diet concentrations provided?**	**Y**	Nominal concentrations: 100 or 200 mg a.i./kg

	ADDITIONAL CRITERIA FOR AN ACCEPTABLE STUDY	Y/N	COMMENTS
10	**Were test diet concentrations measured / maintained?**	**Y**	Measured concentrations: 99.3 and 198.6 mg a.i./kg
11	**Were the test species acclimated and characteristics of the test species reported (*e.g.*, source, sex, age, weight)?**	**Y**	Organism: Rat (*Rattus norvegicus*) Source: Wistar colony, maintained locally Acclimation: 12-14 d Age/lifestage: 12-14 d Size: Not reported
12	**Were appropriate test conditions (*e.g.*, temperature, light intensity, humidity, photoperiod, loading density, *etc.*) reported and within acceptable ranges?**	**Y/N**	Temperature: 22 ± 2 ºC Light intensity: Not reported Humidity: Not reported Photoperiod: 12:12 Loading density: Not reported Feeding: Commercial diet (GUABI, RS, Brazil)
13	**Were individual body weights measured during testing and observation periods?**	**N**	
14	**For dietary studies, was feeding consumption measured, or at least estimated, during the study?**	**N/A**	
15	**For reproduction tests, were the number of pups, development of the pups, viability of the pups, percent offspring survival and growth measured?**	**N/A**	
16	**Was a concentration-response relationship demonstrated?**	**Y**	

EVALUATION: ( ) ACCEPTABLE ( ) SUPPLEMENTAL (X) UNACCEPTABLE

Comments : As per the National Academy of Sciences (NAS, 2013), the test material must be determined to be relevant to the United States. For malathion, the relevance of the study can only be determined by knowing the composition of the product and demonstrating that the composition is similar to a currently registered product. For these reasons, a study is classified as acceptable when the composition of the impurities and/or inerts is known and is verifiable as being relevant to the United States, and meets all other generic and acceptable criteria. Studies conducted by American Cyanamid can be classified as supplemental if the batch number is available. If the batch number for an American Cyanamid study is not available, the study is unacceptable. All other studies are classified as unacceptable.

Common Name	Scientific Name	Purity (%)	Exposure Duration	Effect	Endpoint	Endpoint Concentration	Units	Study Classification
Rat	*Rattus norvegicus*	49.7	4-d	Behavior	LOAEC	≤100	mg a.i./kw bw	Unacceptable
Rat	*Rattus norvegicus*	49.7	4-d	Behavior	NOAEC	<100	mg a.i./kw bw	Unacceptable
Rat	*Rattus norvegicus*	49.7	4-d	Cholinesterase activity	LOAEC	≤100	mg a.i./kw bw	Unacceptable
Rat	*Rattus norvegicus*	49.7	4-d	Cholinesterase activity	NOAEC	<100	mg a.i./kw bw	Unacceptable

Study Quality and Acceptability Evaluation

Compound: Malathion

Study reference (Primary): Ahrens, W.H. 1990. Enhancement of Soybean (Glycine max) Injury and Weed Control by Thifensulfuron-Insecticide Mixtures. Weed Technology, 4(3):524-528. [E68422].

Secondary reference: Not applicable

Is the primary reference study a GLP study?: No

Species tested: Dicots – Soybean (*Glycine max*)

Endpoint(s): Not reported

GENERIC CRITERIA		Y/N	COMMENTS
1	**Single chemical exposure?**	**Y**	Malathion formulation
2	**Was the study conducted according to a recognized international standard (OPPTS, OECD, ASTM, ISO., *ETC.*)?**	**N**	
3	**If NO to 2, was a complete description given of the test system and methods? Were the methods used considered acceptable laboratory practices?**	**N**	
4	**Were the identification, purity and source of test substance given and comparable to the current technical material and formulation?**	**N**	Test substance, source and purity not reported.
5	**Were effects endpoints ecologically relevant?**	**Y**	Injury and fresh weight reduction
6	**Was an appropriate exposure duration used and reported?**	**N**	Soybeans were treated in the late unifoliolate stage and harvested 8 or 9 d after treatment
7	**Were appropriate controls included, reported, and the results adequate?**	**Y**	Control injury and fresh weight reduction of 0%
8	**Were the statistical procedures reported and appropriate?**	**N**	Experiments arranged as a randomized complete block design having 4 or 5 replications. Experimental runs were combined for analysis of variance. Differences between treatment means were determined using a protected LSD (0.05).

ADDITIONAL CRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
9	**Were the application rates and method of application provided and appropriate?**	**Y**	Application rate of 0 (control), 0.14, 0.28 or 0.56 kg/ha (0, 0.125, 0.250 or 0.500 lb/A). Treatments were applied using a moving nozzle pot sprayer. All treatments included nonionic surfactant (X-77).
10	**Were the characteristics of the test species reported and appropriate (i.e., species and source)?**	**Y**	'McCall' soybeans.
11	**Were the testing facilities, testing containers and support medium described and appropriate?**	**Y**	Soybeans were grown in plastic pots filled with sandy loam and peat (1:2 v/v) in a greenhouse. Soil organic matter content not reported.
12	**Were appropriate test conditions (*e.g.*, temperature, humidity, light intensity, and photoperiod) reported and within acceptable ranges?**	**Y**	Temperature of 22-26°C. Supplemental metal halide lighting for 15 h of light. Humidity and light intensity not reported.
13	**Was a rate-response relationship demonstrated?**	**Y**	Three test application rates and raw data provided.

EVALUATION: () ACCEPTABLE () SUPPLEMENTAL (X) UNACCEPTABLE

Comments: Lack of information on test substance purity and test substance source. No statistical comparison reported in the study that could be used to justify a NOEL/LOEL and no raw data reported to permit such a statistical comparison.

Study Quality and Acceptability Evaluation

Compound: Malathion

Study reference (Primary): Day, B.L, M.M. Walser, J.M. Sharma and D.E. Andersen. 1995. Immunopathology of 8-Week-old Ring-necked Pheasants (*Phasianus colchicus*) Exposed to Malathion. Environmental Toxicology and Chemistry 14(10):1719-1726. [E63276].

Secondary reference: Not applicable

Is the primary reference study a GLP study?: No

Species tested: Ring-necked pheasant (*Phasianus colchicus*)

GENERIC CRITERIA		Y/N	COMMENTS
1	**Single chemical exposure?**	**Y**	Malathion technical
2	**Was the study conducted according to a recognized international standard (OPPTS, OECD, ASTM, ISO, etc.)?**	**N**	
3	**If NO to 2, was a complete description given of the physical test system and methods? Were the methods used considered acceptable laboratory practices?**	**N**	
4	**Were the identification, purity and source of test substance given and comparable to the current technical material and formulation?**	**Y**	Test substance: Technical-grade malathion (Cythion® ULY) Source: American Cyanamid Company, Princeton, NJ Purity: 95%
5	**Were the effects endpoints ecologically relevant?**	**N**	LOEL (brain AChE activity)
6	**Were an appropriate exposure duration and post-exposure observation period used and reported?**	**Y/N**	Single oral dose followed by 3-d observation period
7	**Were appropriate controls included, reported and the results adequate?**	**N**	No interpretation of control results with respect to brain AChE activity. Control mortality unknown/not reported.
8	**Were statistical procedures reported and appropriate?**	**Y**	ANOVA, Bartlett's test, Kruskal-Wallis

ADDITIONAL CRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
9	**Were test concentrations/doses provided?**	**Y**	Nominal concentrations: 0 (control), 92 or 230 mg test substance/kg bw (0, 87.4 or 219 mg a.i./kg bw) in corn oil
10	**Were test concentrations/doses measured and maintained?**	**NA**	

ADDITIONAL CRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
11	**Were the test species acclimated and characteristics of the test species reported (*e.g.*, source, sex, age, weight)?**	Y	Organism: Ring-necked pheasant (*Phasianus colchicus*) Source: Local supplier (Oakwood Game Farm, Princeton, MN) Acclimation: 8 wk Age/lifestage: 1 d (at receipt), 8 wk (at initiation) Sex: Males and females Initial weight: Not reported
12	**Were appropriate test conditions (*e.g.*, temperature, light intensity, humidity, photoperiod, loading density, etc.) reported and within acceptable ranges?**	N	Light intensity: Not reported Temperature: Not reported Humidity: Not reported Photoperiod: Not reported Feeding: Food and water provided *ad libitum* Loading density: Not reported
13	**Were individual body weights measured during testing and observation periods?**	N	Body weights reported per dose group only
14	**For dietary studies, was feeding consumption measured, or at least estimated, during the study?**	NA	
15	**For reproduction tests, were the number of eggs laid, egg shell thickness, development of the eggs, viability of the embryos, percent hatchability and offspring survival and growth measured?**	NA	
16	**Was a concentration- or dose-response relationship demonstrated?**	N	Only two test dose concentrations. Raw data not provided.

EVALUATION: ( ) ACCEPTABLE () SUPPLEMENTAL (X) UNACCEPTABLE

Comments: Lack of details regarding study methods and lack of control results reported. Additionally, the endpoint reported is based on sublethal AChE inhibition and no explicit ink between this endpoint and apical endpoints (i.e., survival, growth and/or reproduction) is described.

Endpoint(s):

Common Name	*Scientific Name*	*Purity (%)*	*Exposure Duration (Observation Period)*	*Effect*	*Endpoint*	*Endpoint Conc./ Dose*	*Units*	*Study Classification*
Ring-necked pheasant	*Phasianus colchicus*	95	3 d	Brain AChE activity	LOEL	92[a]	mg test substance/ kg bw	Unacceptable

[a] Purity-corrected LOEL is 87.4 mg a.i./kg bw

Study Quality and Acceptability Evaluation

Compound: Malathion

Study reference (Primary): Geraldi, P.A., J.M. Delgado-Garcia and A. Gruart. 2008. Acute and Repeated Effects of Three Organophosphorus Pesticides on the Acquisition and Retention of an Instrumental Learning Task in Rats. Neurotoxicity Research 13(3,4):253-263.

Secondary reference: Not applicable

Is the primary reference study a GLP study?: No

Species tested: Rat (Wistar; male)

GENERIC CRITERIA		Y/N	COMMENTS
1	**Single chemical exposure?**	**Y**	Technical or formulation not reported
2	**Was the study conducted according to a recognized international standard (OPPTS, OECD, ASTM, ISO, *etc*.)?**	**N**	
3	**If NO to 2, was a complete description given of the physical test system and methods? Were the methods used considered acceptable laboratory practices?**	**N**	
4	**Were the identification, purity and source of test substance given and comparable to the current technical material and formulation?**	**N**	Test substance: Not reported Source: Not reported Purity: Not reported
5	**Were the effects endpoints ecologically relevant?**	**N**	Not reported
6	**Were an appropriate exposure duration and post-exposure observation period used and reported?**	**Y**	Acute (a single dose) and repeated (doses repeated for 5, 15, or 30 days). Observation period of 15 d.
7	**Were appropriate controls included, reported and the results adequate?**	**N**	Controls included. Control mortality not reported.
8	**Were statistical procedures reported and appropriate?**	**Y**	ANOVA, Holm-Sidak test, Pearson's correlation test

ADDITIONAL CRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
9	**Were test concentrations/doses provided?**	**N**	Total doses administered for acute (1 day): 240 mg/kg, 5-day: 280 mg/kg, 15-day 840 mg/kg and 30-day: 1,680 mg/kg
10	**Were test concentrations/doses measured and maintained?**	**N**	Not reported

ADDITIONAL CRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
11	**Were the test species acclimated and characteristics of the test species reported (*e.g.*, source, sex, age, weight)?**	Y	Organism: Wistar rat (male) Source: Universidade do Vale do Itajai-UNIVALI Animal House (Itajai, Santa Catarina, Brazil) Acclimation: 7 d Age/lifestage: 3 months Sex: Males Initial weight: 263-317 g
12	**Were appropriate test conditions (*e.g.*, temperature, light intensity, humidity, photoperiod, loading density, *etc.*) reported and within acceptable ranges?**	Y	Light intensity: Not reported Temperature: 18-20°C Humidity: 40-60% Photoperiod: 12 h light, 12 h dark Feeding: Animals deprived of water 24 h prior to dose administration Loading density: Not reported
13	**Were individual body weights measured during testing and observation periods?**	N	Body weights measured daily but not reported
14	**For dietary studies, was feeding consumption measured, or at least estimated, during the study?**	N	Total food intake measured daily. Mean total food intake 11.4-12.6 g.
15	**For reproduction tests, were the number of pups, development of the pups, viability of the pups, percent offspring survival and growth measured?**	NA	
16	**Was a concentration - or dose-response relationship demonstrated?**	N	Raw data not provided

EVALUATION: ( ) ACCEPTABLE ( ) SUPPLEMENTAL (X) UNACCEPTABLE

Comments:

Study Quality and Acceptability Evaluation

Compound: Malathion

Study reference (Primary): Lingappa, S., A. Jagadish, K. Shivaramu and H.P. Prabhuswamy. Relative Intrinsic Toxicity of Seven Insecticides to Foragers of the Indian Hive Bee, *Apis cerana indica* F. Insect Sci. Applic, 6(5):567- 568. [E94337]

Secondary reference: Not applicable

Is the primary reference study a GLP study?: No

Species tested: Indian hive bee (*Apis cerana indica*)

	GENERIC CRITERIA	Y/N	COMMENTS
1	**Single chemical exposure?**	**Y**	Malathion technical and malathion pure
2	**Was the study conducted according to a recognized international standard (OPPTS, OECD, ASTM, ISO, ETC.)?**	**N**	
3	**If NO to 2, was a complete description given of the test system and methods? Were the methods used considered acceptable laboratory practices?**	**N**	
4	**Were the identification, purity and source of test substance given and comparable to the current technical material and formulation?**	**N**	Test substance: Malathion technical and malathion pure Source: M/s. Shaw Wallace (India) Ltd. Purity: Not reported
5	**Were effects endpoints ecologically relevant?**	**Y**	LD50
6	**Was an appropriate exposure duration used and reported?**	**Y**	Mortality recorded 24 h following single topical application to abdominal sternum of each bee
7	**Were appropriate controls included, reported and results adequate?**	**Y**	Control bees treated with acetone. Experiments with control mortalities above 10% were rejected.
8	**Were the statistical procedures reported and appropriate?**	**N**	Mean mortality data subject to probit analysis

CRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
9	**Were the concentrations/doses provided?**	**N**	Concentrations giving mortality range from 10 to 90%
10	**Were test species acclimated and collection practices and characteristics of the test species reported (e.g., source, race, age, life stage, health)?**	**N**	Foraging worker bees were collected in the morning for each experiment from the same colony and acclimatized for 24 h in a mosquito-net cage. Age/lifestage and health not reported.
11	**Were appropriate test conditions (e.g., handling and feeding conditions, temperature, light conditions (dark), humidity, loading density, etc.) measured and within acceptable ranges?**	**N**	Treated bees were transferred to misquito-net cages and fed on honey solution. No other conditions reported.
12	**For oral studies, was feeding consumption measured, or at least estimated, during the study?**	**NA**	
13	**Was a dose-response relationship demonstrated?**	**N**	Raw data not provided
14	**Were metrics of disease measured, treated or discussed?**	**N**	

EVALUATION: ( ) ACCEPTABLE () SUPPLEMENTAL (X) UNACCEPTABLE

Comments: Study provides very little information about the study methods and provides no details regarding the test item source or purity (the two malathion test substances are identified only as 'malathion technical' and 'malathion pure').

Endpoint(s):

Common Name	*Scientific Name*	*Purity (%)*	*Exposure Duration (Observation Period)*	*Effect*	*Endpoint*	*Endpoint Conc./ Dose*	*Units*	*Study Classification*
Indian hive bee	*Apis cerana indica*	Malathion technical (Purity not reported)	24 h	Mortality	LD50	0.084	µg/bee	Unacceptable
Indian hive bee	*Apis cerana indica*	Malathion pure	24 h	Mortality	LD50	0.072	µg/bee	Unacceptable

Study Quality and Acceptability Evaluation

Compound: Malathion

Study reference (Primary): Mendoza, C.E. 1976. Toxicity and Effects of Malathion on Esterases of Suckling Albino Rats. Toxicology and Applied Pharmacology, 35:229-238. MRID 45046301

Secondary reference: Not applicable

Is the primary reference study a GLP study?: No

Species tested: Rat (Wistar)

GENERIC CRITERIA		Y/N	COMMENTS
1	**Single chemical exposure?**	Y	Malathion technical
2	**Was the study conducted according to a recognized international standard (OPPTS, OECD, ASTM, ISO, *etc.*)?**	N	
3	**If NO to 2, was a complete description given of the physical test system and methods? Were the methods used considered acceptable laboratory practices?**	N	
4	**Were the identification, purity and source of test substance given and comparable to the current technical material and formulation?**	Y	Test substance: Malathion Source: American Cyanamid Co., Wayne, NJ Purity: 99.3%
5	**Were the effects endpoints ecologically relevant?**	Y	LD50
6	**Were an appropriate exposure duration and post-exposure observation period used and reported?**	N	Observation period following single dose by oral gavage not reported
7	**Were appropriate controls included, reported and the results adequate?**	N	Control mortality not reported
8	**Were statistical procedures reported and appropriate?**	N	Not reported

ADDITIONAL CRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
9	**Were test concentrations/doses provided?**	Y	Nominal concentrations: 0 (control), 62.5, 125, 250 or 500 mg test substance/kg bw (0, 62.1, 124, 248 or 497 mg a.i./kg bw)
10	**Were test concentrations/doses measured and maintained?**	NA	
11	**Were the test species acclimated and characteristics of the test species reported (*e.g.*, source, sex, age, weight)?**	Y	Organism: Pups of virgin adult Wistar rats Source: Bio Breeding Laboratories, Ottawa, CA (adults) Acclimation: 1, 6, 12 or 17 d Age/lifestage: 1, 6, 12 or 17 d Sex: Males and females Initial weight: Not reported
12	**Were appropriate test conditions (*e.g.*, temperature, light intensity, humidity, photoperiod, loading density, *etc.*) reported and within acceptable ranges?**	N	Light intensity: Not reported Temperature: Not reported Humidity: Not reported Photoperiod: Not reported Feeding: Not reported Loading density: Not reported
13	**Were individual body weights measured during testing and observation periods?**	N	Not reported
14	**For dietary studies, was feeding consumption measured, or at least estimated, during the study?**	NA	
15	**For reproduction tests, were the number of pups, development of the pups, viability of the pups, percent offspring survival and growth measured?**	NA	
16	**Was a concentration - or dose-response relationship demonstrated?**	N	Raw data not provided

EVALUATION: () ACCEPTABLE ( ) SUPPLEMENTAL (X) UNACCEPTABLE

Comments: Control data not reported. Insufficient information provided regarding test methods. Author did not correct for purity. Intrinsik converted these endpoints using the reported purity of 99.3% assuming w/w.

Endpoint(s):

Common Name	*Scientific Name*	*Purity (%)*	*Exposure Duration (Observation Period)*	*Effect*	*Endpoint*	*Endpoint Conc./ Dose*	*Units*	*Study Classification*
Laboratory rat	Wistar, 1-d old	99.3	Single dose by oral gavage, observation period not reported	Mortality	LD50	209[a]	mg test substance/kg bw	Unacceptable
Laboratory rat	Wistar, 6-d old	99.3	Single dose by oral gavage, observation period not reported	Mortality	LD50	707[b]	mg test substance/kg bw	Unacceptable
Laboratory rat	Wistar, 6-d old	98.9	Single dose by oral gavage, observation period not reported	Mortality	LD50	469[c]	mg test substance/kg bw	Unacceptable
Laboratory rat	Wistar, 12-d old	99.3	Single dose by oral gavage, observation period not reported	Mortality	LD50	1,085[d]	mg test substance/kg bw	Unacceptable
Laboratory rat	Wistar, 17-d old	99.3	Single dose by oral gavage, observation period not reported	Mortality	LD50	1,806[e]	mg test substance/kg bw	Unacceptable

[a] Purity-corrected LD50 is 208 mg a.i./kg bw
[b] Purity-corrected LD50 is 702 mg a.i./kg bw
[c] Purity-corrected LD50 is 466 mg a.i./kg bw
[d] Purity-corrected LD50 is 1,077 mg a.i./kg bw
[e] Purity-corrected LD50 is 1,793 mg a.i./kg bw

Study Quality and Acceptability Evaluation

Compound: Malathion

Study reference (Primary): Panda, S and S.K. Sahu. 1999. Effects of malathion on the growth and reproduction of Drawida willsi (Oligochaeta) under laboratory conditions. Soil Biology and Biochemistry, 31:363-366. [E52962]

Secondary reference: Not applicable

Is the primary reference study a GLP study?: No

Species tested: Earthworm (*Drawida willsi*)

	GENERIC CRITERIA	Y/N	COMMENTS
1	**Single chemical exposure?**	Y	Malathion formulation
2	**Was the study conducted according to a recognized international standard (OPPTS, OECD, ASTM, ISO, *etc*.)?**	N	
3	**If NO to 2, was a complete description given of the physical test system and methods? Were the methods used considered acceptable laboratory practices?**	N	
4	**Were the identification, purity and source of test substance given and comparable to the current technical material and formulation?**	N	Test substance: Cythion 50% EC Source: Alkali and Chemical Corporation of India, New Delhi Purity: Not reported
5	**Were the effects endpoints ecologically relevant?**	Y	LD50
6	**Were an appropriate exposure duration and post-exposure observation period used and reported?**	Y	Mortality recorded at 96 h
7	**Were appropriate controls included, reported and the results adequate?**	Y	No control mortality
8	**Were statistical procedures reported and appropriate?**	N	Not reported

	CRITERIA FOR AN ACCEPTABLE STUDY	Y/N	COMMENTS
9	**Were the concentrations/doses provided?**	Y	Nominal concentrations: 0 (control), 2.2, or 4.4, 6.6, 8.8, 11.0, 13.2,15.4, 17.6, 19.8 or 22.0 mg a.i./kg soil dry weight
10	**Were test species acclimated and collection practices and characteristics of the test species reported (e.g., source, race, age, life stage, health)?**	Y	Organism: Earthworm (*Drawida willsi*) Source: Soil and earthworms were collected from an upland non-irrigated paddy field, which had no record of input of agrochemicals (fertilizers and pesticides) Acclimation: 1 month Age/lifestage: Juveniles (<2 cm),

CRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
			immatures (≥2 <4 cm) and adults (≥4 cm)
11	**Were appropriate test conditions (e.g., handling and feeding conditions, temperature, light conditions (dark), humidity, loading density, etc.) measured and within acceptable ranges?**	**Y**	Soil texture: Sandy loam Soil pH: 6.8 Soil organic matter: 2.7 mg/g soil Soil moisture: 200 mg/g soil Temperature: 23-25°C Feeding: During acclimatization, adequate provision of food (10% organic matter, cow dung+leaf litter) Loading density: 10 earthworms per beaker containing 500 g treated soil
12	**For oral studies, was feeding consumption measured, or at least estimated, during the study?**	**NA**	
13	**Was a dose-response relationship demonstrated?**	**Y**	Raw data provided

EVALUATION: () ACCEPTABLE ( ) SUPPLEMENTAL (X) UNACCEPTABLE

Comments: Control mortality not reported. Test material used had a purity of only 50%, which means that effects may have been influenced by the presence of impurities that are not relevant to current product formulations.

Endpoint(s):

Common Name	*Scientific Name*	*Purity (%)*	*Exposure Duration (Observation Period)*	*Effect*	*Endpoint*	*Endpoint Conc./ Dose*	*Units*	*Study Classification*
Earthworm	*Drawida willsi*, juvenile	Not reported	96 h	Mortality	LC50	15.07	mg a.i./kg soil dry weight	Unacceptable
Earthworm	*Drawida willsi*, immature	Not reported	96 h	Mortality	LC50	17.38	mg a.i./kg soil dry weight	Unacceptable
Earthworm	*Drawida willsi*, adult	Not reported	96 h	Mortality	LC50	18.81	mg a.i./kg soil dry weight	Unacceptable

Study Quality and Acceptability Evaluation

Compound: Malathion

Study reference (Primary): Robertson, J.L, R.L. Lyon and M. Page. 1975. Toxicity of Selected Insecticides Applied to Two Defoliators of Western Hemlock. Journal of Economic Entomology 68(2):193-196. [E89288]

Secondary reference: Not applicable

Is the primary reference study a GLP study?: No

Species tested: Hemlock sawfly (*Neodiprion tsugae*)

GENERIC CRITERIA		Y/N	COMMENTS
1	**Single chemical exposure?**	Y	Malathion technical
2	**Was the study conducted according to a recognized international standard (OPPTS, OECD, ASTM, ISO, *etc*.)?**	N	
3	**If NO to 2, was a complete description given of the physical test system and methods? Were the methods used considered acceptable laboratory practices?**	N	
4	**Were the identification, purity and source of test substance given and comparable to the current technical material and formulation?**	N	Test substance: Malathion Source: American Cyanamid Co. Purity: Not reported
5	**Were the effects endpoints ecologically relevant?**	Y	LD50
6	**Were an appropriate exposure duration and post-exposure observation period used and reported?**	Y	Spray was introduced into the aerosol chamber for 10 s and insects were exposed to the spray for 1 min. Mortalities recorded after 3 d.
7	**Were appropriate controls included, reported and the results adequate?**	N	Not reported
8	**Were statistical procedures reported and appropriate?**	Y/N	Probit analysis

CRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
9	**Were the concentrations/doses provided?**	N	Not reported
10	**Were test species acclimated and collection practices and characteristics of the test species reported (e.g., source, race, age, life stage, health)?**	Y	Organism: Hemlock sawfly (*Neodiprion tsugae*) Source: Collected from McKenzie Inlet, Alaska in July 1970 and shipped to Berkeley Acclimation: Refrigerated at 7°C until 1 day before their use in testing, when they were transferred to 20°C

			Age/lifestage: 4th and 5th instar
11	**Were appropriate test conditions (e.g., handling and feeding conditions, temperature, light conditions (dark), humidity, loading density, etc.) measured and within acceptable ranges?**	**N**	Feeding: Western hemlock foliage Insects were treated as described by Robertson (1972). No other conditions reported.
12	**For oral studies, was feeding consumption measured, or at least estimated, during the study?**	**NA**	
13	**Was a dose-response relationship demonstrated?**	**N**	Raw data not provided

EVALUATION: () ACCEPTABLE () SUPPLEMENTAL (X) UNACCEPTABLE

Comments: Control mortality not reported. Lack of information provided regarding the test substance. Author reported endpoints in units of oz a.i./A. Intrinsik converted these endpoints to lb a.i./A..

Endpoint(s):

Common Name	***Scientific Name***	***Purity (%)***	***Exposure Duration (Observation Period)***	***Effect***	***Endpoint***	***Endpoint Concentration/ Dose***	***Units***	***Study Classification***
Hemlock sawfly	*Neodiprion tsugae*, 4th instars	Not reported	72 h	Mortality	LD50	0.15[a]	oz a.i./A	Unacceptable
Hemlock sawfly	*Neodiprion tsugae*, 4th and 5th instars combined	Not reported	72 h	Mortality	LD50	0.14[b]	oz a.i./A	Unacceptable

[a] Converted LD50 is 0.00938 lb a.i./A
[b] Converted LD50 is 0.00875 lb a.i./A

Study Quality and Acceptability Evaluation

Compound: Malathion

Study reference (Primary): Samaan, H., M. Sadek, A. El-Garawany and O. Habib. 1989. Comparative Acute and Short-Term Chronic Toxicity Studies of Some Insecticides in Rats. Journal of Drug Research 18(1-2):145-152.

Secondary reference: Not applicable

Is the primary reference study a GLP study?: No

Species tested: Rat (albino)

	GENERIC CRITERIA	Y/N	COMMENTS
1	**Single chemical exposure?**	**Y**	Technical or formulation not reported
2	**Was the study conducted according to a recognized international standard (OPPTS, OECD, ASTM, ISO, *etc*.)?**	**N**	
3	**If NO to 2, was a complete description given of the physical test system and methods? Were the methods used considered acceptable laboratory practices?**	**N**	
4	**Were the identification, purity and source of test substance given and comparable to the current technical material and formulation?**	**N**	Test substance: Not reported Source: Not reported Purity: Not reported
5	**Were the effects endpoints ecologically relevant?**	**Y**	LD50
6	**Were an appropriate exposure duration and post-exposure observation period used and reported?**	**Y**	24-h test: single dose, 90-d test: orally administered daily
7	**Were appropriate controls included, reported and the results adequate?**	**N**	Controls included. Control mortality not reported.
8	**Were statistical procedures reported and appropriate?**	**Y**	Probit analysis by the method of Litchfield and Wilcoxon (1949)

	ADDITIONAL CRITERIA FOR AN ACCEPTABLE STUDY	Y/N	COMMENTS
9	**Were test concentrations/doses provided?**	**Y**	0 (control), 10, 20, 30, 50 or 80 mg/kg bw
10	**Were test concentrations/doses measured and maintained?**	**N**	Not reported

ADDITIONAL CRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
11	**Were the test species acclimated and characteristics of the test species reported (*e.g.*, source, sex, age, weight)?**	**Y**	Organism: Rat (albino) Source: Ministry of Agriculture, Egypt Acclimation: Not reported Age/lifestage: Not reported Sex: Males and females Initial weight: Average weights of 100 g for 24-h and 50 g for 90-d tests
12	**Were appropriate test conditions (*e.g.*, temperature, light intensity, humidity, photoperiod, loading density, *etc.*) reported and within acceptable ranges?**	**N**	Light intensity: Not reported Temperature: Not reported Humidity: Not reported Photoperiod: Not reported Feeding: Not reported Loading density: Not reported
13	**Were individual body weights measured during testing and observation periods?**	**N**	Rats were weighed every 21 days. Body weights not reported.
14	**For dietary studies, was feeding consumption measured, or at least estimated, during the study?**	**N**	Food consumption measured but not reported
15	**For reproduction tests, were the number of pups, development of the pups, viability of the pups, percent offspring survival and growth measured?**	**NA**	
16	**Was a concentration - or dose-response relationship demonstrated?**	**N**	Raw data not provided

EVALUATION: ( ) ACCEPTABLE ( ) SUPPLEMENTAL (X) UNACCEPTABLE

Comments:

Endpoint(s):

Common Name	*Scientific Name*	*Purity (%)*	*Exposure Duration (Observation Period)*	*Effect*	*Endpoint*	*Endpoint Concentration/ Dose*	*Units*	*Study Classification*
Rat (albino)	Not reported	Not reported	24 h	Mortality	LD50	500	mg/kg bw	Unacceptable
Rat (albino)	Not reported	Not reported	90 d	Mortality	LD50	130	mg/kg bw	Unacceptable

Study Quality and Acceptability Evaluation

Compound: Malathion

Study reference (Primary): Wuthrich, V. 1991. Acute Toxicity (LC50) Study of Fyfanon Technical to Earthworms. Unpublished study performed by RCC Umweltchemie Ag, Switzerland for Cheminova Agro A/S, Denmark. RCC Project 278932.

Secondary reference: Not applicable

Is the primary reference study a GLP study?: Yes

Species tested: Earthworm (*Eisenia foetida foetida*)

	GENERIC CRITERIA	Y/N	COMMENTS
1	**Single chemical exposure?**	**Y**	Malathion technical
2	**Was the study conducted according to a recognized international standard (OPPTS, OECD, ASTM, ISO, *etc.*)?**	**Y**	OECD Guideline for Testing of Chemicals No. 207, Section 2: "Earthworm, Acute Toxicity Tests", adopted April 4, 1984
3	**If NO to 2, was a complete description given of the physical test system and methods? Were the methods used considered acceptable laboratory practices?**	**Y**	
4	**Were the identification, purity and source of test substance given and comparable to the current technical material and formulation?**	**Y**	Test substance: Fyfanon technical Source: Not reported. Batch 00629-13. Purity: 96.2%
5	**Were the effects endpoints ecologically relevant?**	**Y**	LC50
6	**Were an appropriate exposure duration and post-exposure observation period used and reported?**	**Y**	14-d exposure
7	**Were appropriate controls included, reported and the results adequate?**	**Y**	No control mortality
8	**Were statistical procedures reported and appropriate?**	**Y**	LOGIT-analysis

	CRITERIA FOR AN ACCEPTABLE STUDY	Y/N	COMMENTS
9	**Were the concentrations/doses provided?**	**Y**	0 (control), 61.4, 123, 246, 491 or 982 mg/kg soil dry weight (59.1, 118, 237, 472 or 945 mg a.i./kg soil dry weight)
10	**Were test species acclimated and collection practices and characteristics of the test species reported (e.g., source,**	**Y**	Organism: Earthworm (*Eisenia foetida foetida*) Source: Bred by Or. P. Wilde, FARM RESEARCH, 0-7880 Bad Saeckingen

CRITERIA FOR AN ACCEPTABLE STUDY		Y/N	COMMENTS
	race, age, life stage, health)?		Acclimation: 1 d Age/lifestage: Adult, with clitellum Body weight: 207-310 mg (average)
11	**Were appropriate test conditions (e.g., handling and feeding conditions, temperature, light conditions (dark), humidity, loading density, etc.) measured and within acceptable ranges?**	**Y**	Soil texture: Sand and artificial soil (23.4% clay, 52.2% silt and 24.4% sand) Soil pH: 7.5-7.8 Soil organic matter: Not reported Soil moisture: 28.8-48.5% Temperature: 18.5-22°C Feeding: Not reported Loading density: 10 earthworms per glass beaker
12	**For oral studies, was feeding consumption measured, or at least estimated, during the study?**	**NA**	
13	**Was a dose-response relationship demonstrated?**	**Y**	Raw data provided

EVALUATION: (X) ACCEPTABLE ( ) SUPPLEMENTAL ( ) UNACCEPTABLE

Comments: Author did not correct for purity. Intrinsik converted these endpoints using the reported purity of 96.2% assuming w/w.

Endpoint(s):

Common Name	*Scientific Name*	*Purity (%)*	*Exposure Duration (Observation Period)*	*Effect*	*Endpoint*	*Endpoint Concentration/ Dose*	*Units*	*Study Classification*
Earthworm	*Eisenia foetida foetida*	96.2	7 d	Mortality	LC50	666[a]	mg/kg soil (dry weight)	Acceptable
Earthworm	*Eisenia foetida foetida*	96.2	14 d	Mortality	LC50	613[b]	mg/kg soil (dry weight)	Acceptable

[a] Purity-corrected LC50 is 641 mg a.i./kg soil (dry weight)
[b] Purity-corrected LC50 is 590 mg a.i./kg soil (dry weight)

Appendix E
Rationale for Not Assessing Malathion Degradation Products

This appendix justifies why Cheminova believes that the risk of malathion degradates to non-target organisms is negligible.

The acceptable and supplemental data for malathion degradates described below suggest that malathion degradates have variable toxicity. The only degradate known to be more toxic compared to the parent, malathion, is its oxon, malaoxon. In some instances, malaoxon is less toxic than malathion to some species (i.e. bluegill), similar in toxicity to some (i.e. water flea, rainbow trout, ring-necked pheasant, and northern bobwhite) and more toxic to others (i.e. mallard duck, yellow canary and rats). Although malaoxon has higher toxicity for some species compared to malathion, it is not expected to be produced in amounts in the natural environment that would cause harm to animals. Studies show that malaoxon is either not formed or formed in very small amounts that degrade and dissipate rapidly. Thus, Cheminova, believes that a quantitative assessment of the risk of malathion degradates is not warranted. Evidence to support this assertion is provided below.

Malathion decomposition in the environment is dependent on the media and surrounding environmental conditions. In aerobic and anaerobic soil and aquatic degradation studies, the primary degradates of malathion have been identified as malathion monocarboxylic acid and malathion dicarboxylic acid. Malaoxon has been identified as a minor degradation product on plants and dry/inert surfaces.

Toxicity of degradates of malathion to non-target organisms is presented below. A discussion of the fate and behavior of degradates is then presented.

Degradate Toxicity to Non-Target Organisms

The toxicity of malathion monocarboxylic acid and malathion dicarboxylic acid has been studied in four species: water flea, *(Daphnia magna*), bluegill (*Lepomis macrochirus*), Sprague-Dawley rat, and the earthworm (*Eisenia fetida*).

Gries and Purghart (2001a [MRID 47540310]) exposed neonate (<24 hours old) *Daphnia magna* to five nominal concentrations of malathion monocarboxylic acid (α + β mixture) ranging from 0.5 to 8.0 mg malathion monocarboxylic acid/L. After 48 hours of exposure, there was no immobility (mortality) observed in the control groups. However, there was 65 and 100% immobilization observed in the 4.0 and 8.0 mg malathion monocarboxylic acid/L test groups respectively. As such, the calculated EC50 for malathion monocarboxylic acid reported by the authors was 3.5 mg malathion monocarboxylic acid/L (3.2 mg a.i./L). This concentration is 4571 times higher than the malathion acute effects metric for freshwater aquatic invertebrates (48-hour EC50 of 0.70 µg a.i./L for *Daphnia magna*; Gries and Purghart, 2001b [MRID 47540303]).

The toxicity of malathion dicarboxylic acid to *Daphnia magna* was investigated by Gries and Purghart (2001e [MRID 47540305]). *Daphnia magna* were exposed under flow-through conditions to nominal test concentrations of 6.25, 12.5, 25, 50 and 100 mg malathion dicarboxylic acid/L for 48 hours. The 48-hour EC50 (immobilization) reported from this study

was 71 mg malathion dicarboxylic acid/L (70 mg a.i./L). This concentration is 100,000 times higher than the malathion acute effects metric for freshwater aquatic invertebrates (48-hour EC50 of 0.70 µg a.i./L for *Daphnia magna*; Gries and Purghart, 2001b [MRID 47540303]).

The toxicity of malathion monocarboxylic acid (α + β mixture) to bluegill sunfish (*Lepomis macrochirus*) was studied by Gries and Purghart (2001d [MRID 47540309]). Bluegill sunfish were exposed under flow-through conditions to nominal test concentrations of 0 (control), 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg malathion monocarboxylic acid/L. There were seven fish per test concentration that were observed for mortality. After 96 hours of exposure, mortality was recorded. There was no mortality in the controls or test groups, except for the 50 and 100 mg monocarboxylic acid/L test groups that had 14 and 71% mortality, respectively. The 96-hour LC50 was calculated to be 79 mg monocarboxylic acid/L (72.8 mg a.i./L) from this study. This LC50 is 1,400 times higher than the malathion acute effects metric for freshwater fish (96-hour LC50 of 52 µg a.i./L for *Lepomis macrochirus*; Gries and Purghart (2001e [MRID 47540304]).

A 96-hour LC50 of ≥100 mg malathion dicarboxylic acid/L (98.8 mg a.i./L) was reported for bluegill sunfish (Lepomis macrochirus) exposed to malathion dicarboxylic acid (Gries and Purghart, 2001f [MRID 47540306]). No mortality was observed after 96 hours in the controls or the one tested concentration of 100 mg malathion dicarboxylic acid/L (98.8 mg a.i./L). These results indicate that malathion dicarboxylic acid is much less toxic than malathion.

Malathion monocarboxylic acid toxicity to rats (Sprague-Dawley) was studied by Sanders (2008a [MRID 49252804]) following the OECD guideline 420 “acute oral toxicity – fixed dose” method. In this study, no mortality was observed up to 2,000 mg test substance/kg bw (1,840 mg a.i./kg bw), and there were no treatment related effects on body weight or necropsy findings. Thus, the estimated oral LD50 for this degradate was estimated to be >2,000 mg test substance/kg bw (>1,840 mg a.i./kg bw) for the rat. In addition, the NOEL is >2,000 mg test substance/kg bw (>1,840 mg a.i./kg bw). This result indicates that malathion monocarboxylic acid is less toxic to rats than malathion since the mammalian acute oral LD50 is 2,010 mg a.i./kg bw for malathion (Moore, 2003 [MRID 48153112]) .

Sanders (2008b [MRID 49252803]) exposed Sprague-Dawley rats to malathion dicarboxylic acid using the OECD 420 “Acute Oral Toxicity – Fixed Dose Method”. There were no mortalities after oral exposure to 2,000 mg test substance/kg bw (1,980 mg a.i./kg bw). As such, the reported LD50 from the study was >2,000 mg/kg bw (>1,980 mg a.i./kg bw).

The toxicity of malathion monocarboxylic acid (α + β mixture) to the earthworm (Eisenia fetida, subspecies andrei), was investigated following OECD (1984) guideline 207 for earthworm acute toxicity tests (Friedrich, 2012a [MRID 49086404]). Adult earthworms (approx. three months old) were exposed to malathion monocarboxylic acid (α + β mixture) via alimentary and dermal uptake. The worms were exposed in groups of ten to malathion monocarboxylic acid (α + β mixture), obtained by the study sponsor (Cheminova A/S; 89.4% w/w purity), in one liter glass containers filled in artificial soil. Test concentrations (n = 7) ranged from 15.63 to 1000 mg a.i./kg soil dw. Worms were monitored for mortality after seven and 14 days, while fresh weight

was measured at day 14. Other behavioral (i.e. reaction to light and touch) and pathological symptoms (i.e. ulcers, blisters, twisted segments) were also noted if observed. After 14 days, there was no observed abnormal behavior noted in any treatment groups or control. Mortality in the control group was ≤10%, thereby meeting control mortality validity criteria. There was also no mortality after 14 days of exposure in the 15.63, 31.25, 62.5, 125, 250 and 500 mg a.i./kg dw test concentrations. Mortality of 2.5% was observed in the 250 and 1,000 mg a.i./kg dw test groups. However, the effects were not significantly different from controls. As such, the estimate 14-day LC50 is >1,000 mg a.i./kg dw and the corresponding NOEL (mortality) is 1, 000 mg a.i./kg dw. There were significant decreases in fresh weight biomass measured in worms exposed to 62.5, 125, 250, 500 and 1,000 mg a.i./kg dw. Therefore, the NOEL and LOEL for biomass are 31.25 and 62.5 mg a.i./kg dw, respectively.

Friedrich (2012b [MRID 49086405]) investigated the toxicity of malathion dicarboxylic acid via alimentary and dermal exposure to earthworms (*Eisenia fetida,* subspecies *andrei*) following the OECD (1984) guideline 207 for earthworm acute toxicity tests. Adults worms were exposed to seven test concentrations (15.63, 31.25, 62.5, 125, 250, 500 and 1,000 mg a.i./kg dw) of malathion dicarboxylic acid (Cheminova A/S, 98.8% w/w purity) in one liter glass containers filled with artificial soil. Mortality was monitored after seven and 14 days of exposure, and fresh weight was measured after 14 days only. Mortality in the control group after 14 days was ≤10% which met the validity criteria required according to test guidelines (OECD, 1984). There was 2.5% mortality in the 62.5 mg a.i./kg dw test group. However, this effect was not significant compared to controls. Therefore, the 14 day LC50 is >1000 mg a.i./kg dw and the NOEL is 1000 mg a.i./kg dw. There were significant decreases in fresh weight biomass measured in worms exposed to 250, 500 and 1000 mg a.i./kg dw. Therefore, the NOEL and LOEL for biomass are 125 and 250 mg a.i./kg dw, respectively.

Field studies have demonstrated that when applied at maximum registered label rates, concentrations of malathion degradates in soil are not expected to reach anywhere near levels required to observe effects on earthworms. Rice et al. (1990a [MRID 41748901]) applied 1.16 lb a.i./A Cythion ULV end-use product once a week for six weeks to a treated cotton plot and bare ground plot in Dooly County, Georgia. The highest measured concentration of malathion was 1.0 mg/kg dw in soil from the cotton plot and 0.24 mg/kg dw in bare ground. In all treated plots, malathion degraded to malathion dicarboxylic acid (MDCA). Maximum levels of MDCA were 0.18 mg/kg dw in treated cotton plot soil and 0.25 mg/kg dw in bare ground soil. Further analysis revealed that concentrations of MCDA measured in the top 6 inches of soil immediately following the last application were 0.010 and 0.028 mg/kg dw for bare ground and crop plots, respectively. Residues of MDCA dissipated to below the LOQ (0.010 mg/kg dw) by day one following the final application of both bare ground and crop plots (Jacobson and Lochhaas, 1992a [MRID 43042401]). A similar study, using the same test substance and application rate, was carried out in Madera County, California (Rice et al., 1990b [MRID 41727701]). The highest measured concentration of malathion was 0.20 mg/kg dw in cotton plot soil and 0.15 mg/kg dw in bare ground soil samples. In all treated plots, malathion degraded to malathion dicarboxylic acid. Maximum levels of MDCA were 0.10 mg/kg dw in treated cotton plot soil samples and 0.17 mg/kg dw in bare ground soil samples. Additional analysis revealed that the MDCA

concentrations in crop plots were 0.052 and 0.044 mg/kg dw within the top six inches and between six and 12 inches of soil, respectively, immediately following the final application of malathion. MDCA in the top six inches of soil dissipated to below the LOQ (0.010 mg/kg dw) by seven days after application. When all residues from all depths were summed, the MDCA half-life was estimated to be 1.7 days (Jacobson and Lochhaas, 1992b [MRID 43042402]).

In a laboratory study, Rice (2011 [MRID 48626701]) applied Fyfanon ULV AG to sand and soil at target rate of 1.22 lb a.i./A and monitored the formation of MMCA and MDCA. Maximum concentrations of malathion were 9.2 mg/kg in sand and 10 mg/kg in soil. The highest concentrations of MMCA measured in sand and soil were 0.78 mg/kg and 0.29 mg/kg, respectively. The highest concentrations of MDCA measured in sand and soil were 0.092 mg/kg and 0.37 mg/kg, respectively.

The results of these field and laboratory studies indicate that concentrations of MMCA and MDCA measured in soil are well below the biomass NOELs of 31.25 mg a.i./kg dw (MMCA) and 125 mg a.i./kg dw (MDCA) for earthworms (Friedrich, 2012a,b [MRID 49086404, 49086405]). Furthermore, even measured concentrations of the parent compound are below the NOELs for these degrades, indicating that no effects to earthworms are expected due to exposure to MMCA and MDCA in soil.

Both of the main malathion degradates (malathion monocarboxylic acid and malathion dicarboxylic acid) are considerably less toxic than the parent compound in all four species tested.

Malaoxon Toxicity

Cheminova does not agree with EPA's assumption that malaoxon is a degradate of concern in malathion risk assessments.

EPA is proposing to use the potency ratio of 92.9 to assess risk of malaoxon to all aquatic organisms. The evidence supporting this potency ratio is based on a single open literature study with the yellow-legged frog (*Rana boylii*) (Sparling and Fellers, 2007). This study was deemed as unacceptable based on Cheminova's study evaluation criteria (Breton et al., 2014 [MRID 49333901]). The study did not follow a standard test method. The authors did not report control mortality, measured test concentrations, characteristics of test species and test conditions. In addition, a concentration-response relationship was not demonstrated and the authors acknowledged that confidence limits could not be calculated because the proportion of individuals dying in the lower concentrations of malathion was relatively constant. This study is, therefore, inappropriate for deriving a potency ratio in the risk assessment.

The sensitivity of species exposed to stressors can be quite variable and is influenced by biological (toxicokinetic and toxicodynamic characteristics) and physical characteristics. It is, therefore, not scientifically appropriate to apply a potency ratio derived for one species to species from widely disparate taxonomic groups (e.g., aquatic invertebrates and fish),

particularly when data are available to derive taxon-specific potency ratios. Moreover, the use of potency ratios are not warranted when acceptable and relevant toxicity data are available for malaoxon itself. Cheminova has conducted and submitted a variety of GLP toxicity studies for malaoxon. For comparison purposes, Cheminova has derived malathion/malaoxon potency ratios for aquatic species using acceptable guideline GLP studies (Table E-1). These acute ratios range from 0.86 to 41.6 (aquatic and terrestrial species) and are much lower than the ratio of 92.9 for aquatic species proposed by EPA using the unacceptable literature study.

Table E-1 Potency ratios for malathion/malaoxon toxicity derived using acceptable studies for aquatic and terrestrial species.

Taxa	*Species*	*Chemical*	*Effects Metric*	*Reference [MRID]*	*Ratio*
Invertebrates (Freshwater) - Acute	Water flea (*Daphnia magna*)	Malathion	48 h EC50 0.70 a.i./L	Gries and Purghart, 2001b [47540303]	2.38
		Malaoxon	48 h EC50 0.294 µg a.i./L	Bergfield, 2011 [48571803]	
Fish (Freshwater) - Acute	Rainbow trout (*Oncorhynchus mykiss*)	Malathion	96 h LC50 174 µg a.i./L	Gries and Purghart, 2001g [47540302]	2.58
		Malaoxon	96 h LC50 67.4 µg a.i./L	Gerke, 2011a [48571802]	
	Bluegill (*Lepomis macrochirus*)	Malathion	96 h LC50 52 µg a.i./L	Gries and Purghart, 2001e [47540304]	0.83
		Malaoxon	96 h LC50 62.6 µg a.i./L	Gerke, 2011b [48571801]	
Birds - Acute	Northern bobwhite (*Colinus virginianus*)	Malathion	Oral LD50 345 mg a.i./kg bw	Rodgers, 2002 [48153114]	8.0
		Malaoxon	Oral LD50 43 mg a.i./kg bw	Gallagher et al., 2002a [48153104]	
	Yellow canary (*Serinus canaria*)	Malathion	Oral LD50 >2400 mg a.i./kg bw	Stafford, 2011a [48571805]	>36.9
		Malaoxon	Oral LD50 65 mg a.i./kg bw	Stafford, 2011b [48571806]	
	Mallard duck (*Anas platyrhynchos*)	Malathion	Oral LD50 >2250 mg a.i./kg bw	Hubbard and Beavers, 2012a [48963307]	>25.9
		Malaoxon	Oral LD50 87 mg a.i./kg bw	Hubbard and Beavers, 2012b [48963306]	
	Ring-necked pheasant (*Phasianus colchicus*)	Malathion	Oral LD50 136 mg a.i./kg bw	Hubbard and Beavers, 2012c [48963305]	6.48
		Malaoxon	Oral LD50 21 mg a.i./kg bw	Hubbard et al, 2012a [49024601]	
		Malathion	Dietary LC50 2514 mg a.i./kg diet	Hubbard et al., 2012b [48963301]	1.37
		Malaoxon	Dietary LC50 1832 mg a.i./kg diet	Hubbard et al., 2012c [48963302]	
	Northern bobwhite (*Colinus virginianus*)	Malathion	Dietary 2022 mg a.i./kg diet	Gallagher et al., 2003 [48153106]	2.38
		Malaoxon	Dietary LC50 848 mg a.i./kg diet	Gallagher et al., 2002b [48153105]	
	Mallard duck (*Anas platyrhynchos*)	Malathion	Dietary LC50 >5620 mg a.i./kg diet	Hubbard et al., 2012d [48963303]	NA
		Malaoxon	Dietary LC50 >3000 mg a.i./kg diet	Hubbard et al., 2012e [48963304]	
Mammal - Acute	Rat (Sprague-Dawley)	Malathion	Oral LD50 (female survival) 2010 mg a.i./kg bw	Moore, 2003 [48153112]	41

Table E-1 Potency ratios for malathion/malaoxon toxicity derived using acceptable studies for aquatic and terrestrial species.					
Taxa	***Species***	***Chemical***	***Effects Metric***	***Reference [MRID]***	***Ratio***
		Malaoxon	Oral LD50 (female survival) 49 mg a.i./kg bw	Lowe, 2011 [48571808]	

Non-Target Organism Exposure to Malathion and Malaoxon

As stated above, evidence suggests that although malaoxon may be more toxic than malathion for some species, exposure of non-target organisms to malaoxon is unlikely. Malaoxon is generally not produced or measured in soil, soil leachates or water. It is also infrequently detected in aquatic environments. Moreover, malaoxon degrades quickly and when it is detected under environmental conditions, concentrations are typically small percentages or applied malathion. Further details are provided below.

GLP studies investigating the fate and behavior of malathion and malaoxon suggest that malaoxon is generally not produced or measured in soil, soil leachates and water. Specifically, malaoxon has not been detected in laboratory adsorption and desorption studies (Nixon, 1995 [MRID 43868601]), aerobic and anaerobic soil metabolism studies (Knoch, 2001a [MRID 46769501]), except in one study where the applied product was contaminated (Blumhorst, 1990 [MRID 41721701]); hydrolysis and photolysis studies (Teeter, 1988 [MRID 40941201]; Miller, 1988 [MRID 40969301]; Carpenter, 1990 [MRID 41673001]; Sanders, 1988 [MRID 41039001]; Dykes et al., 1990 [MRID 41695501]; Spare and Cutchin, 1991 [MRID 42015201]); soil and water samples from two aquatic dissipation studies and two soil dissipation studies (Rice et al., 1990a,b [41748901, MRID 41727701]; Jacobson and Lochhaas, 1992a,b [MRID 43042401 and 43042402,]; Judy et al., 1991a,b [MRID 42058402, 42058401]); and five water-sediment systems treated with malathion (Knoch 2001b [MRID 46769502]; Hiler and Mannella, 2012a [MRID 48906401]).

Malaoxon is infrequently detected in aquatic environments. Malaoxon surface water and groundwater monitoring data from the U.S. Department of Agriculture (USDA) Pesticide Data Program (PDP), U.S. Geological Survey (USGS) Nation Water Quality Assessment (NAWQA) Program, USGS National Water Information System (NWIS), California Department of Pesticide Regulation (CDPR) Surface Water Database (SWD), Quality-Assessed Agrichemical Contaminant Database for Nebraska Ground Water (ACDNGW), Oregon Department of Environmental Quality (ODEQ) Laboratory Analytical Storage and Retrieval (LASAR) database, and the Texas Commission on Environmental Quality (TCEQ) Interagency Pesticide Database collected between 2001 and 2013 were examined. The monitoring programs targeted areas expected to be contaminated with pesticides. No malaoxon detections were reported in the USDA PDP, CDPR, ACDNGW, or TCEQ IPD databases. In surface water data collected from the NAWQA, NWIS and ODEQ LASAR databases, malaoxon was detected at concentrations ranging from 0.0209 to 0.27 µg/L (limits of detection (LODs) = 0.0025 – 0.7 µg/L). Overall, malaoxon was detected in less than 2.3% of surface water samples (Table E-2). Malaoxon was detected in 0.04% of groundwater samples from NWIS at concentrations ranging from 0.01 to

0.034 µg/L (LODs = 0.003 – 0.24 µg/L). The most sensitive aquatic endpoint for malaoxon was a 48-hour NOEL of 0.130 µg a.i./L for immobilization in *Daphnia magna* (Bergfield, 2011 [MRID 48571803]). Only 0.023%, 0.026% and 0.21% of malaoxon detections in surface water samples in the NAWQA, NWIS and ODEQ LASAR databases exceeded this NOEL. Furthermore, no malaoxon concentrations in surface water samples exceeded the 48-hour EC50 of 0.294 µg a.i./L for immobilization in *Daphnia magna* (Bergfield, 2011 [MRID 48571803]). The levels of malaoxon detected in groundwater samples did not exceed any aquatic endpoints for malaoxon. These results indicate that malaoxon is rarely observed in surface water and groundwater, and when detected, is reported at very low concentrations that are not likely to significantly affect aquatic organisms.

Table E-2 Summary of groundwater and surface water monitoring data for malaoxon from 2001 to 2013.

Database	*Date Range*	*Sample Medium*	*No. Samples*	*No. Detects*	*% Detects*	*Range of Detected Values (µg/L)*	*Range of Reporting Limits (µg/L)*
USDA PDP	2007-2011	Groundwater	1316	0	0.00%	ND	0.006 – 0.6
	2004-2011	Surface water	918	0	0.00%	ND	0.006 – 0.04
USGS NAWQA	2001-2012	Surface water	8729	9	0.10%	0.0209 – 0.204	0.0025 – 0.7
USGS NWIS	2001-2013	Groundwater	6867	3	0.04%	0.01 – 0.034	0.003 – 0.24
	2001-2013	Surface water	11,594	14	0.12%	0.021 – 0.204	0.003 – 0.7
CDPR SWD	2005-2010	Surface water	293	0	0.00%	ND	0.02 – 1
ACDNGW	2002-2005	Groundwater	90	0	0.00%	ND	0.008 – 0.08
ODEQ LASAR	2004	Groundwater	4	0	0.00%	ND	0.51 – 1
	2003-2008	Surface water	1399	32	2.29%	0.052 – 0.27	0.023 – 0.53
TCEQ IPD	2003-2007	Groundwater	376	0	0.00%	ND	0.008 – 0.08

Malaoxon degrades quickly in soil and aquatic environments. Malaoxon degradation was investigated in a recent aerobic soil metabolism study (Hiler, 2012 [MRID 48903601]). The study was conducted with radio-labeled malaoxon (99.2 purity) and four acidic soils (pH 5.8 to 6.3): sandy loam with low organic content (Hanford), sandy loam with high organic content (Vacaville), loamy sand (Speyer, Germany), and loam (Iowa). In addition to determining the degradation rate of the four soils, the Vacaville sandy loam was used to determine the degradation pathway of malaoxon. A dose rate of 1.02 ppm malaoxon was used, assuming the highest possible application rate of malathion (citrus, 4.5 lbs a.i./A) and a conservative rate of conversion of malathion to malaoxon (5%). This dose rate represented an application rate of 1.0 µg/g of dry soil. Malaoxon was applied to 50 g (dry weight) samples of each soil and the samples were stored in amber glass bottles and aerated for 120 days. A field moisture capacity of pF 2.0 to 2.5 and temperature of 20 ± 2°C were maintained throughout the testing period. Duplicate soil samples was analyzed for malaoxon three and six hours, and 1, 2, 3, 7, 13, 30, 62, 90 (92 for Iowa soil), and 120 days after dosing. High-performance liquid chromatography (HPLC) was used to quantify malaoxon in the soil samples. Average radiocarbon recoveries ranged from

92.6 to 93.9%. Malaoxon had degraded to ≤2.0% of the applied rate in all soils by the end of the study period (120 days). The major degradate was a polar product that consisted of the α/β monoacid and a dehydrogenated form of the diacid. Monoethyl maleate, diethyl maleate and diethylmethylthiosuccinate were minor degradation products. The half-lives were calculated using a Single First-Order (SFO) model. The half-lives for the Hanford, Speyer, Vacaville, and Iowa soils were 0.3, 0.2, 0.6 and 0.5 days, respectively. Their respective degradation rate constants were 2.1947, 3.9121, 1.1428 and 1.4848 d^{-1}. The DT_{90} values were ≤2.0 days for all soils. This study suggests that even if malaoxon is present in very small amounts after application of malathion (either formed after application of malathion or released as an impurity) it would degrade quickly in the environment. Shepler (2004 [MRID 46396601]) studied the rate of hydrolysis and hydrolytic products of [^{14}C]malaoxon. Concentrations of [^{14}C]malaoxon at varying pHs (5, 7 and 9) were maintained in the dark at 25^{o}C throughout the study period (31 days). Radioactive malaoxon and its degradates were quantified by high performance liquid chromatography (HPLC). Radiocarbon recoveries ranged between 92.1 and 110.5% of the applied dose for all samples. Half-life hydrolysis of malaoxon in aqueous buffer samples were 32.5, 8.8 and 0.18 days for pH 5, 7, and 9 respectively. DT90s were 108.1, 29.3, and 0.61 days for pHs 5, 7 and 9, respectively. Malaoxon is most stable under acidic conditions which are less likely to occur in nature. At neutral or slightly basic conditions, those representative of ecological relevant conditions, malaoxon degrades quickly.

As noted above, when malaoxon is found in the environment, it is only at very small percentages of applied malathion. This is further supported by Hiler (2011 [MRID 48508601]) who conducted an aerobic aquatic metabolism study using [^{14}C]malaoxon applied to sediment/water samples from two sites in the U.S. The samples were inoculated and incubated in the dark under aerobic conditions at 20^{o}C for 14 days (North Dakota sample) to one month (Georgia sample) to determine the breakdown and radiocarbon distribution of [^{14}C]malaoxon. The site in Georgia had sandy sediment with a 1:1 sediment/water ratio at pH of 4.7. The test system had a pH of 6.1. The North Dakota site had a more loamy sediment (greater humic and fulvic acid component) with a 1:1 sediment/water ratio at pH of 8.1. The test system had a pH of 8.4. Hiler (2011 [MRID 48508601]) calculated DT50s and DT90s for malaoxon using sediment/water samples from both sites. In sandy sediment (Georgia sample), the DT50 and DT90 of malaoxon were 5.1 and 17.1 days, respectively, and in the water, more than 90% of the malaoxon had degraded by day 14. In loamy sediment (North Dakota), the DT50 and DT90 of malaoxon were one and 3.3 days, respectively. Malaoxon in the North Dakota test system had degraded more than 90% in the water layer within four days. These studies also suggest that even if malaoxon would be present in very small amounts after application of malathion (either formed after application of malathion or released as an impurity) it would degrade quickly in the environment.

Malaoxon is found at small percentages of applied malathion under dry and microbially inactive environmental conditions. Malathion was applied in diluted slurry with a ratio of 10,000: 1 bait to malathion. Bait and malathion are typically mixed in a ratio of 4:1. According CaEPA (1993), all matrices were covered with a film of bait mixture following application, as opposed to the discrete droplets that would be expected with a field application. As indicated by the authors, the

influence of this extreme mixture on malathion degradation is unknown and requires further study. The conditions in the CaEPA (1993) study have no relevance to public health spraying programs or agricultural uses involving malathion. Cheminova submitted a study that was conducted in EPA Region 10 (California) and measured the formation of malathion degradates in soil and sand samples treated with malathion alone (target rate of 1.22 lb a.i./A) or in combination with NuLure® insect bait (ratios of 4:1 and 10,000:1 bait to malathion) (Rice, 2011 [MRID 48626701]). Malaoxon concentrations did not exceed 6% and 10% of the corresponding malathion levels in sand and soil, respectively (Rice, 2011 [MRID 48626701]). A similar study quantifying degradation products in sand and soil treated with an equivalent rate of 1.22 lb [^{14}C]malathion/A observed maximum malaoxon levels of 2% and 4% applied radiocarbon (AR) for sand and soil, respectively (Hiler and Mannella, 2012b [MRID 48986601]). By the end of the 30-day study period, malaoxon levels had declined to below 2% AR in both sand and soil.

Several GLP field residue and dissipation studies commissioned by Cheminova were reviewed and analyzed by Habig (2011). Malathion and malaoxon residues were measured in a variety of field crops, fruit and long grasses at numerous time points after application of malathion (i.e., 0, 1, 3, 4, 7, 10, 11, 14, and 21 days). Residue data were separated into categories to represent particular feed item groups (i.e. long/tall grass, forage and leafy crops, large fruit, and small fruit/seeds). Malathion residues peaked on the day of application for all feed item categories. The highest mean residue concentration of 43.8 mg/kg ww was measured on forage and leafy crops. Similarly, malaoxon residues peaked on the day of application on forage and leafy crops, long/tall grass and small fruit. However, on large fruit malaoxon peaked on the day after application. The highest mean measured malaoxon residue concentration from all feed item classes was 0.2 mg/kg ww, measured on long/tall grass (Habig, 2011).

In a field study conducted in southwest Germany Knäbe (2004 [MRID 46525902]) investigated malathion and malaoxon residues on plant material and arthropods in an apple orchard after airblast application of EW formulation containing 440 g/L malathion. The application rate was 1.61 lb a.i./A (1810.08 g a.i./ha). Ground vegetation between the apple trees was collected (weeds and grass) as well as canopy-dwelling (using inventory sampling) and ground-dwelling arthropods (using pitfall traps and D-vac sampling). Samples were collected using inventory sampling seven days before application, and then 0, 2, 4, 8, 16 and 27 days after application. Pitfall traps were collected six days prior to application, and then 1, 2, 3, 4, 8, 16 and 24 days after application. D-vac sampling was carried out on days 0, 1, 2 and 8 days after application.

Procedural recoveries for malathion and malaoxon measured on arthropod samples were 94% and 84%, respectively. The LOQ was 0.01 mg/kg ww. In canopy-dwelling arthropods the author reports dissipation half-lives of 11.69 hours and 0.13 hours, for malathion and malaoxon, respectively. On these arthropods, measured malaoxon residues peaked on the day of application (Day 0), at 0.09 mg/kg ww. Malathion residues were highest on the day of application, and peaked at 9.4 mg/kg ww. By mass, the highest measured malaoxon residues on canopy-dwelling arthropods was ~0.96% of peak measured malathion (Knäbe, 2004 [MRID 46525902]).

Recoveries for short vegetation (grass and weeds) were 95% and 94%, respectively, for malathion and malaoxon. The LOQ was 0.01 mg/kg ww. The author reported dissipation half-lives for malathion and malaoxon were 18.48 hours and 0.19 hours respectively. Malathion and malaoxon both peaked on the day of application (45.0 mg/kg ww and 0.09 mg/kg ww, respectively). By mass, the highest measured malaoxon residues on short vegetation was ~0.2% of peak measured malathion (Knäbe, 2004 [MRID 46525902]).

Discussion and Conclusion

Though malaoxon has been demonstrated to be more toxic than malathion to some species, the fate and behavior of malaoxon suggests that it is likely not produced in the aquatic environment. Moreover, malaoxon degrades rapidly in water, sediment and soil samples. On the rare occasion when it is detected, malaoxon is found only at small percentages of applied malathion or at small concentrations in aquatic environments. As such, malaoxon is unlikely to be transported at environmentally relevant concentrations in which exposure would cause significant effects on growth, reproduction and survival.

In the terrestrial environment, malaoxon has half-lives shorter then one day, indicating that malaoxon degrades quickly on arthropods and short vegetation, which are important feed items for terrestrial organisms. Given the above, risk associated with malaoxon exposure is likely negligible for birds and mammals.

Cheminova believes that an ecological risk assessment of malathion only is warranted, and would be protective of combined malathion and malaoxon exposure to declining malathion residues and their degradates in the environment.

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Appendix F
Cheminova's Aquatic Plant Effects Metric Calculations

Freshwater Non-vascular Plants

The most sensitive NOEL and LOEL values for freshwater non-vascular plants are the 72-hour NOELs and LOELs (biomass, cell density, yield) of <1,000 µg a.i./L and ≤1,000 µg a.i./L, respectively, for algae (*Pseudokirchneriella subcapitata*) (Dobbins et al., 2012a [MRID 48963311]). This is a GLP study conducted with Cheminova's technical grade malathion and was rated acceptable based on Cheminova's study evaluation criteria. The endpoint is supported by the results of other acceptable studies (Dobbins et al., 2012b,c [MRID 48963310, 48998001]), both of which report higher NOEL and LOEL values for freshwater non-vascular plants.

The lowest reported EC05 (biomass) was 3340 µg a.i./L for *Pseudokirchneriella subcapitata* (Dobbins et al., 2012a [MRID 48963311]). EC25 values were not reported in any studies for freshwater non-vascular plants (Dobbins et al., 2012a,b,c [MRID 48963311, 48963310, 48998001]). Furthermore, Intrinsik did not calculate EC25 values (Dobbins et al., 2012a,b,c [MRID 48963311, 48963310, 48998001]) since all EC20 values reported in these studies ranged from 3000 to 87,000 µg a.i./L and were therefore above the lowest LOEL of ≤1000 µg a.i./L.

Dobbins et al. (2012a ([MRID 48963311]) reported all endpoints as the day 0 measured concentrations malathion concentrations. However, measured malathion concentrations at the end of the test were below the LOQ of 500 µg a.i./L. Thus, the 72-hour NOEL and LOEL for algae (*Pseudokirchneriella subcapitata*) based on mean measured concentrations was calculated to be <635 and ≤635 µg a.i./L, respectively, assuming that the measured concentration below the LOQ was equal to 250 µg a.i./L (half the LOQ). Since the 72-hour endpoints are being used to assess the effects of freshwater non-vascular plants in this ESA, the mean measured concentrations are more representative of the malathion exposure than day 0 concentrations.

Since all EC05 and EC25 values were above the lowest NOEL and LOEL values from Dobbins et al. (2012a ([MRID 48963311]), the most appropriate effects metric for screening for direct effects to listed freshwater non-vascular plants is the NOEL of <635 µg a.i./L for biomass, cell density, and yield and the most appropriate effects metric for indirect effects (for listed species dependent on freshwater non-vascular plants) is the LOEL of ≤635 µg a.i./L for biomass, cell density, and yield (Agencies, 2013).

Freshwater Vascular Plants

The most sensitive NOEL and LOEL values for freshwater vascular plants are the seven-day NOELs and LOELs (biomass, biomass yield and biomass growth rate) based on mean measured concentrations of 8270 µg a.i./L and 17,270 µg a.i./L, respectively, for duckweed (*Lemna gibba*; Dobbins et al., 2012d [MRID 48998003]). This is a GLP study conducted with Cheminova's technical grade malathion and is the only freshwater vascular plant study rated acceptable based on Cheminova's study evaluation criteria.

Concentration-response models were fit to the following measure of effects versus mean measured malathion concentration: seven-day biomass (mg), seven-day biomass yield (mg), seven-day biomass growth rate (d^{-1}), seven-day frond number, seven-day frond yield and seven-day frond growth rate (d^{-1}). The purpose of these analyses was to determine the lowest EC05 and EC25, for comparison with NOEL and LOEL values in establishing screening-level effects metrics for direct and indirect effects resulting from freshwater vascular aquatic plant exposure to malathion. Based on the plotted data, plausible generalized linear models were attempted following the approach of Bailer and Oris (1997). The PROC GENMOD procedure in SAS Software® (SAS Version 9.3) was used in these analyses. Figures F-1 to F-6 below present the data with the selected best-fitting models. Best fitting models were selected based on lowest AICc (Akaike information criterion) and BIC (Bayesian information criterion), and visual inspection of model fit. All predictor variables had to be significant in the model based on Type III analyses. Table F-1 contains details of the selected models and associated EC05 and EC25 estimates.

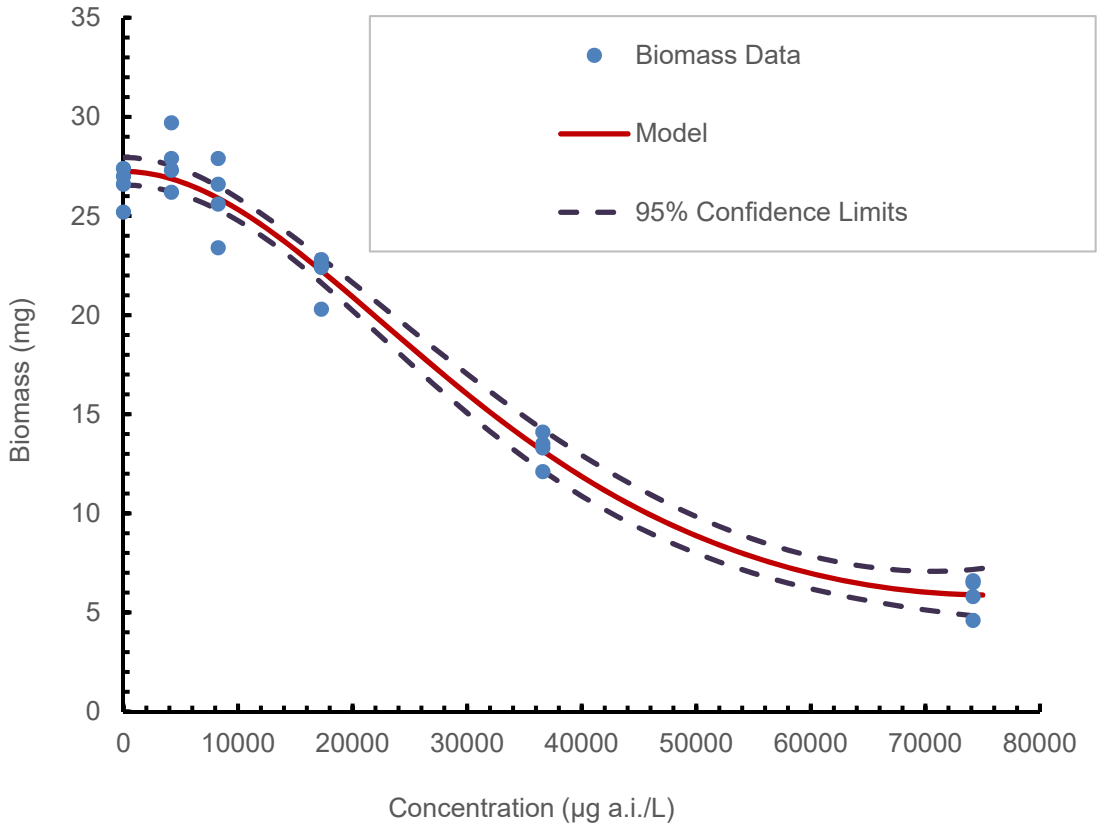

Figure F-1 **Generalized linear model fit to *Lemna gibba* biomass after 7 days versus mean measured malathion concentration (data from Dobbins et al. (2012d [MRID 48998003]))**

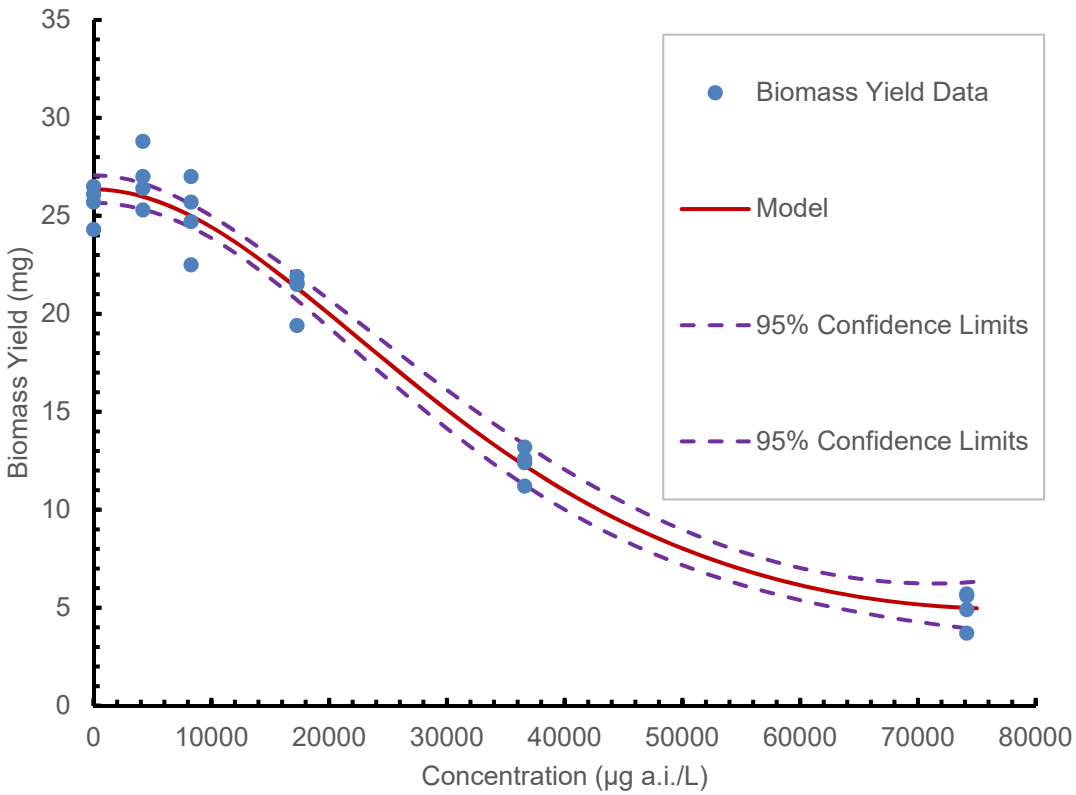

Figure F-2 **Generalized linear model fit to *Lemna gibba* biomass yield after 7 days versus mean measured malathion concentration (data from Dobbins et al. (2012d [MRID 48998003]))**

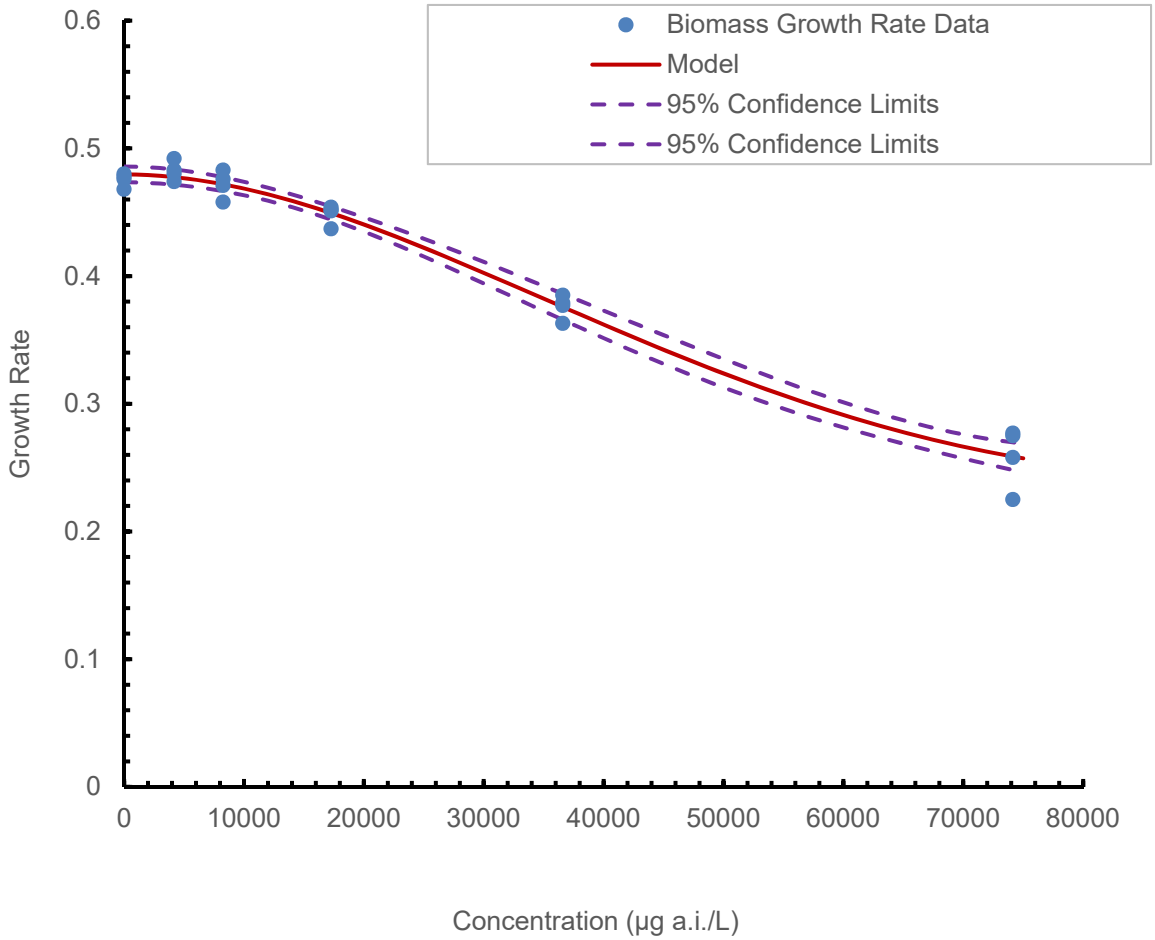

Figure F-3 **Generalized linear model fit to *Lemna gibba* growth rate after 7 days versus mean measured malathion concentration (data from Dobbins et al. (2012d [MRID 48998003]))**

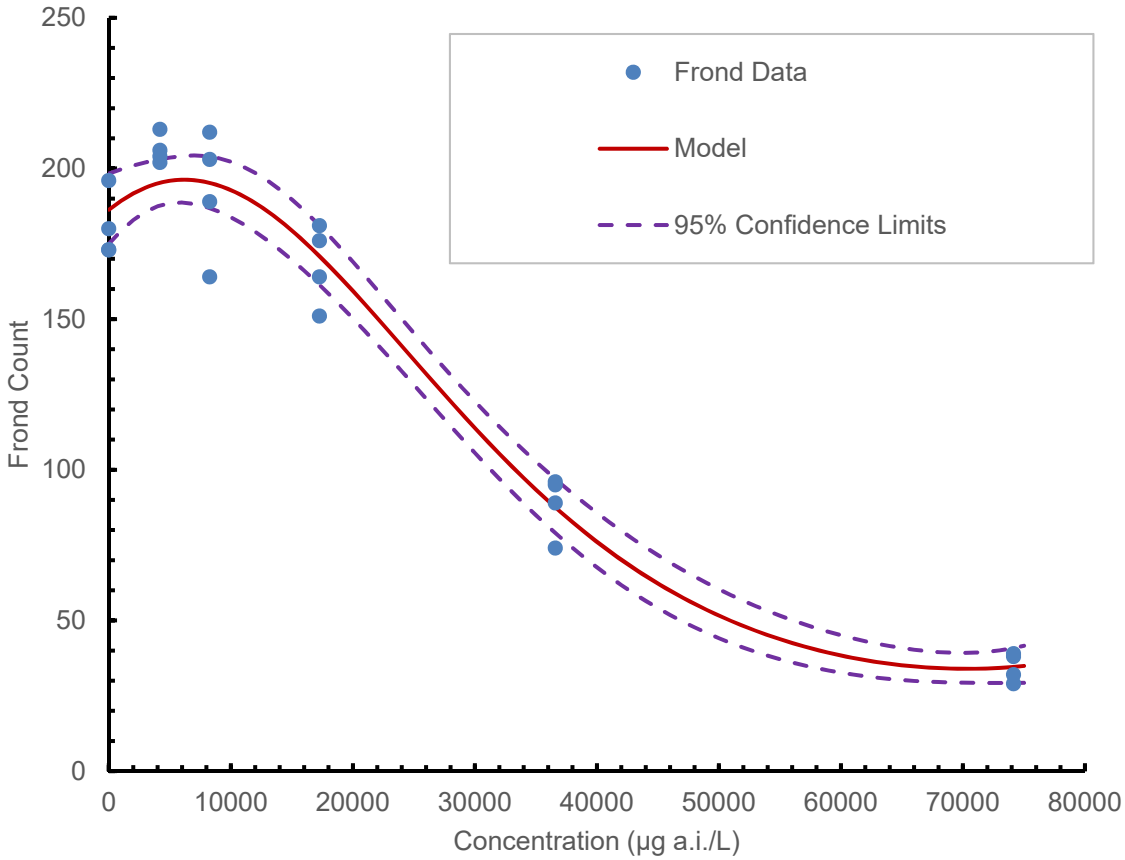

Figure F-4 **Generalized linear model fit to *Lemna gibba* frond count after 7 days versus mean measured malathion concentration (data from Dobbins et al. (2012d [MRID 48998003]))**

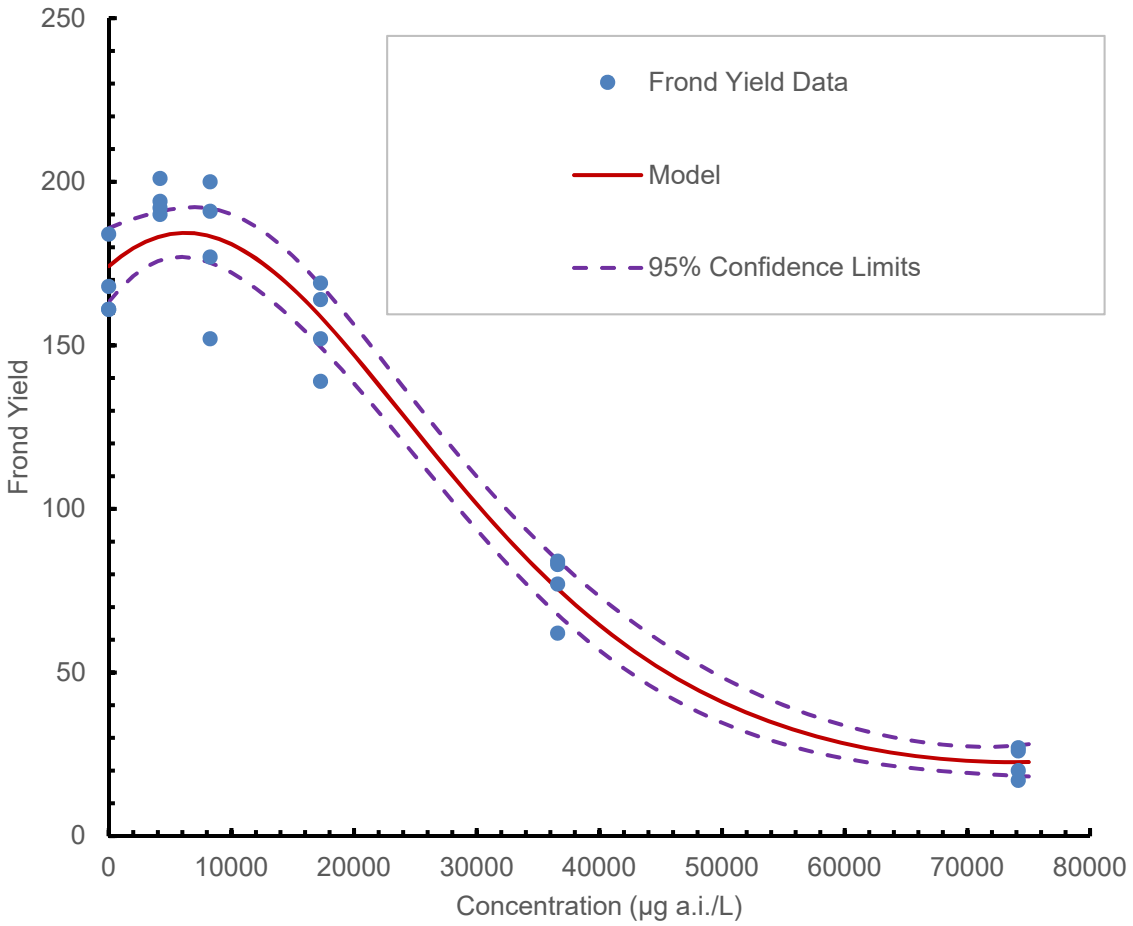

Figure F-5 **Generalized linear model fit to *Lemna gibba* frond yield after 7 days versus mean measured malathion concentration (data from Dobbins et al. (2012d [MRID 48998003]))**

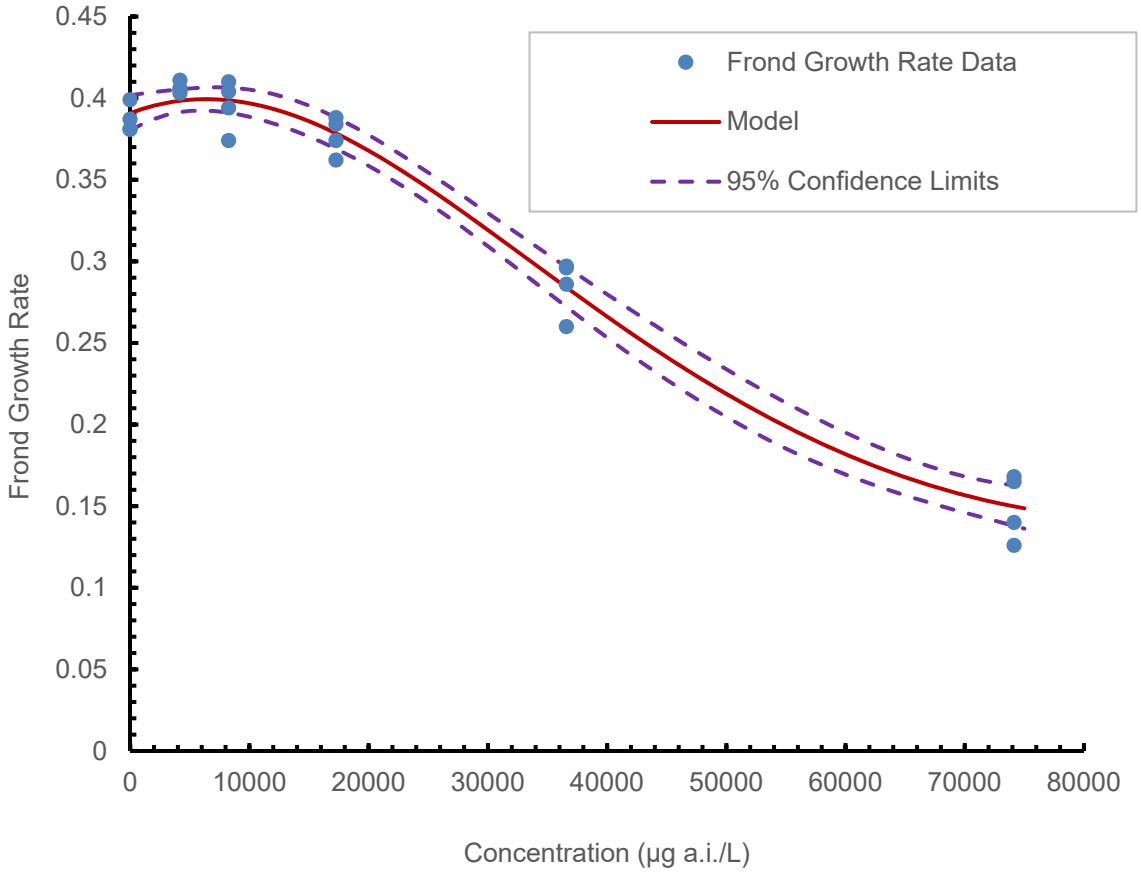

Figure F-6 **Generalized linear model fit to Lemna gibba frond growth rate after 7 days versus mean measured malathion concentration (data from Dobbins et al. (2012d [MRID 48998003]))**

Table F-1 Summary of best-fitting concentration-response models for *Lemna gibba* after 7 days versus mean measured malathion concentration (data from Dobbins et al. (2012d [MRID 48998003])

Measure of Effects	*Best-Fitting Generalized Linear Model*[a]	*Model Parameters*[b]				*EC05 (µg/L)*	*EC25 (µg/L)*
		B_0 (Intercept)	*β_1 (concentration)*	*β_2 (squared concentration)*	*β_3 (cubed concentration)*		
Biomass	Log link, normal response	3.31	NA	-8.03E-04	7.07E-06	8304	20967
Biomass yield	**Log link, normal response**	**3.27**	**NA**	**-8.34E-04**	**7.16E-06**	**8134**	**20463**
Biomass growth rate	Log link, normal response	-0.735	NA	-2.50E-04	1.86E-06	15207	40592
Frond count	Log link, Poisson response	5.23	0.0173	-0.00152	1.33E-05	15675	24299
Frond yield	Log link, Poisson response	5.16	0.0188	-0.00164	1.36E-05	15517	23578
Frond growth rate	Log link, normal response	-0.939	0.00693	-5.84E-04	4.26E-06	19134	34879

[a] Best-fitting models selected of models attempted based on lowest AICc and BIC, predictor significance and visual inspection of model fit. Where possible model assumptions were assessed, and met.

[b] Models were fit to the data with concentrations in units of milligrams due to software constraints.

Based on these analyses, and the Interagency Interim Approaches for National-Level Pesticide Endangered Species Act Assessments (Agencies, 2013) the appropriate effects metric for screening for direct effects to listed freshwater vascular plants is the EC05 of 8,134 µg a.i./L for biomass yield (Table E-1 [above]), which is lower than the NOEL of 9,680 µg a.i./L. The most appropriate effects metric for indirect effects (for listed species dependent on freshwater vascular plants) is the LOEL of 17,270 µg a.i./L which is lower than the lowest EC25 from the study (20,463 µg a.i./L, biomass yield).

Marine Non-vascular Plants

The most sensitive NOEL and LOEL values for estuarine/marine non-vascular plants are the 72-hour NOELs and LOELs (biomass, cell density, yield, growth rate) based on mean measured concentrations of 1506 µg a.i./L and 4432 µg a.i./L, respectively, for the marine diatom (*Skeletonema costatum*; Dobbins et al., 2012e [MRID 48998002]). Because the test concentrations were not maintained throughout the test and the test endpoints were calculated using the nominal test concentrations, this study was rated as supplemental based on Cheminova's study evaluation criteria.

Concentration-response models were fit to the following measure of effects versus mean measured malathion concentration: 72-hour cell density (cells/mL), 72-hour cell yield (cells/mL), 72-hour biomass (cells/mL•h^{-1} and 72-hour growth rate (h^{-1}). The purpose of these analyses was to determine the lowest EC05 and EC25, for comparison with NOEL and LOEL values in establishing screening-level effects metrics for direct and indirect effects resulting from marine non-vascular aquatic plant exposure to malathion (with the diatom as a surrogate species). Based on the plotted data, plausible generalized linear models were attempted following the approach of Bailer and Oris (1997). The PROC GENMOD procedure in SAS Software® (SAS Version 9.3) was used in these analyses. Figures F-7 to F-10 below present the data with the selected best-fitting models. Best-fitting models were selected based on lowest AICc (Akaike information criterion) and BIC (Bayesian information criterion), and visual inspection of model fit. All predictor variables had to be significant in the model based on Type III analyses. Table F-2 contains details of the selected models and associated EC05 and EC25 estimates.

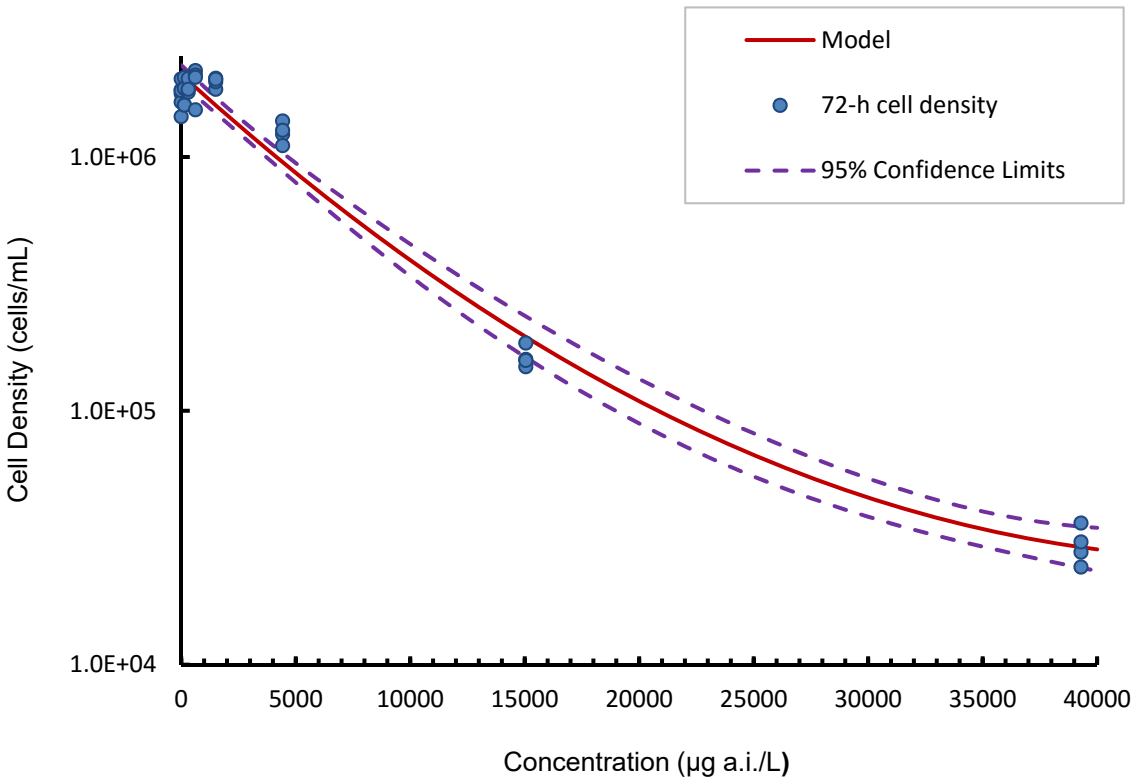

Figure F-7 **Generalized linear model fit to *Skeletonema costatum* cell density after 72 hours versus mean measured malathion concentration (data from Dobbins et al.(2012e [MRID 48998002]))**

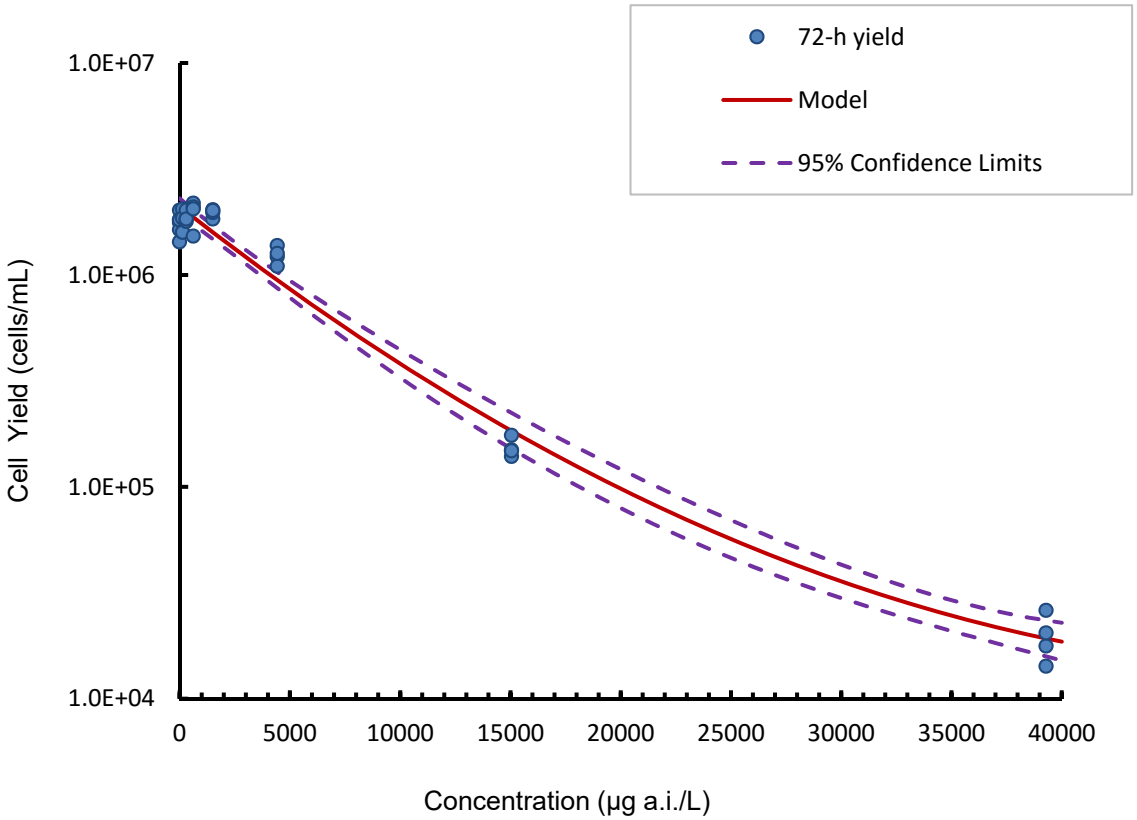

Figure E-8 Generalized linear model fit to *Skeletonema costatum* cell yield after 72 hours versus mean measured malathion concentration (data from Dobbins et al.(2012e [MRID 48998002]))

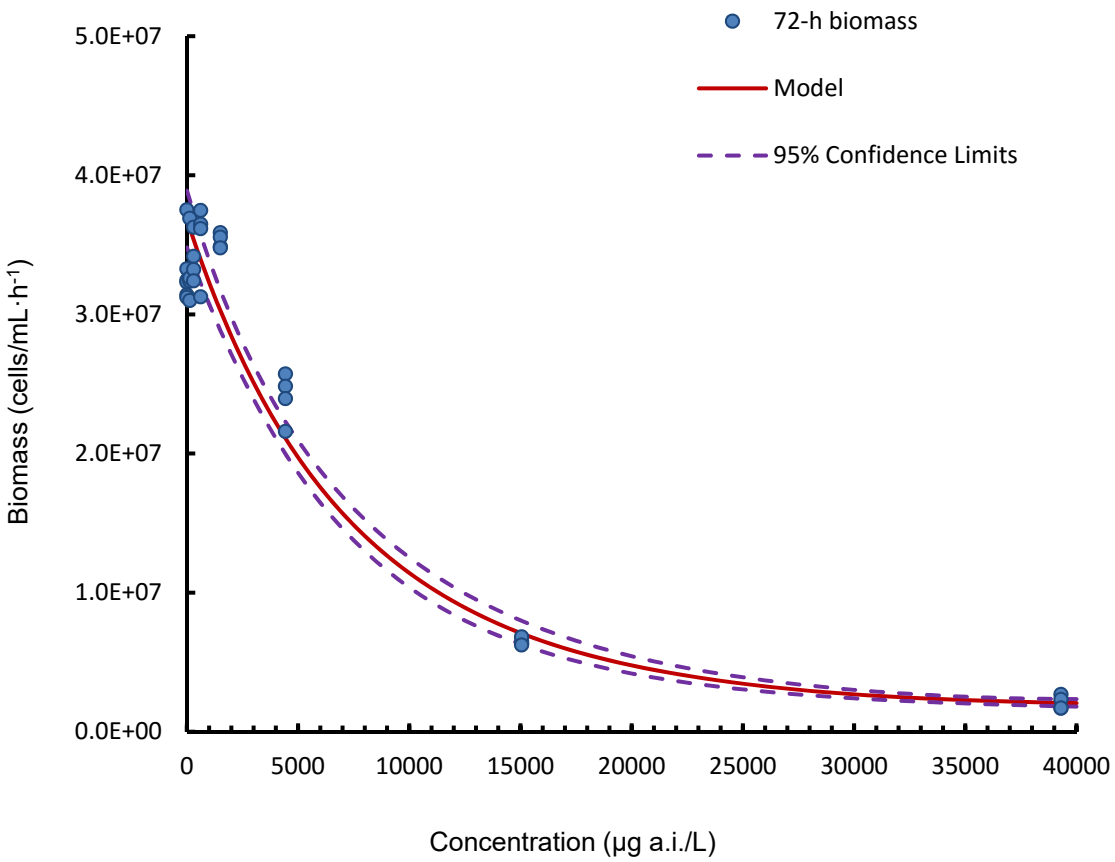

Figure F-9 **Generalized linear model fit to *Skeletonema costatum* biomass after 72 hours versus mean measured malathion concentration (data from Dobbins et al.(2012e [MRID 48998002]))**

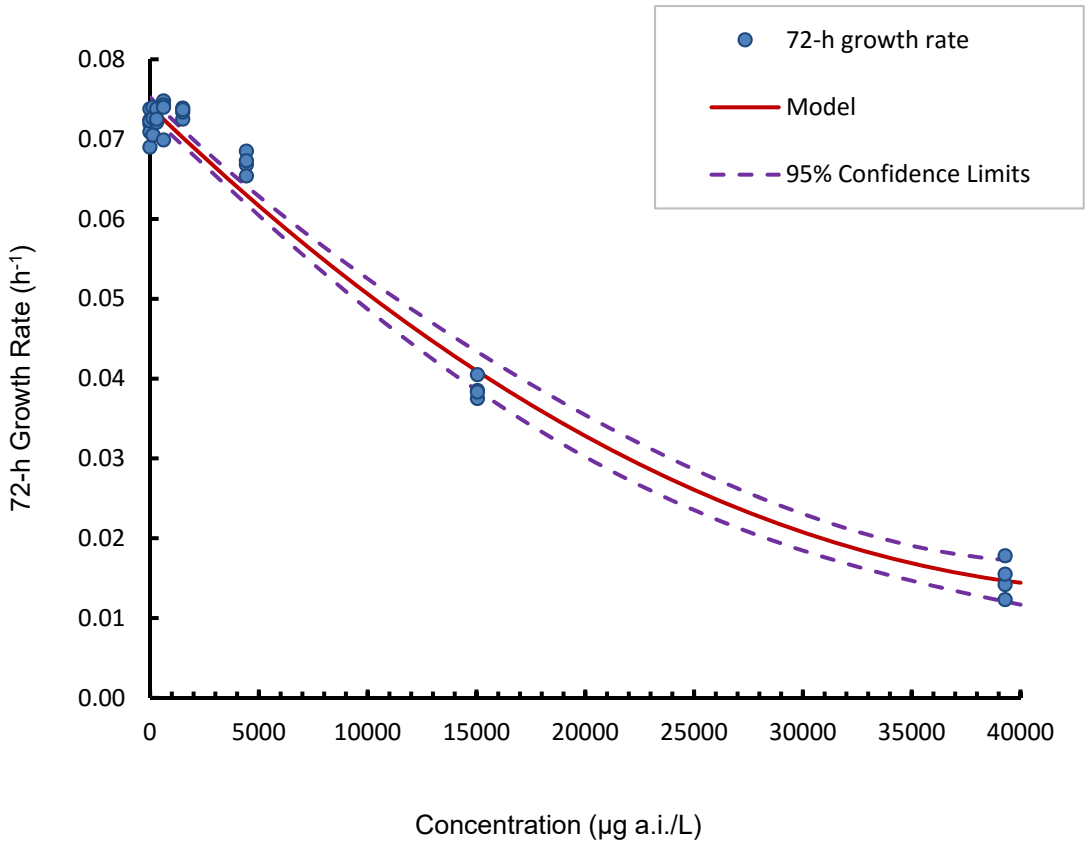

Figure F-10 **Generalized linear model fit to *Skeletonema costatum* growth rate after 72 hours versus mean measured malathion concentration (data from Dobbins et al.(2012e [MRID 48998002]))**

Table F-2 Summary of best-fitting concentration-response models for *Skeletonema costatum* after 72 hours versus mean measured malathion concentration (data from Dobbins et al. (2012e [MRID 48998002]))

Measure of Effects	*Best-Fitting Generalized Linear Model*[a]	*Model Parameters*[b]			*EC05 (µg a.i./L)*	*EC25(µg a.i./L)*
		Intercept	*β1 (concentration parameter)*	*β2 (squared concentration parameter)*		
Cell density	Log link, gamma response, polynomial	7.66	-0.189	0.00202	273	1551
Cell yield	Log link, gamma response, polynomial	7.65	-0.188	0.00175	273	1549
Biomass	Log link, gamma response, polynomial	10.51	-0.132	0.00150	390	2234
Growth rate	Identity link, normal response, polynomial	0.0741	-0.00264	0.00267	1427	7663

[a] Best-fitting models selected of models attempted based on lowest AICc and BIC, predictor significance and visual inspection of model fit. Where possible model assumptions were assessed, and met.
[b] Models were fit to the data with concentrations in units of milligrams due to software constraints.

Based on these analyses, and the Interim Approaches for National-Level Pesticide Endangered Species Act Assessments (Agencies, 2013) the appropriate effects metric for screening for direct effects to listed marine non-vascular plants is the EC05 of 273 µg a.i./L common to cell density and cell yield (Table F-2 [above]), which is lower than the NOEL of 1506 µg a.i./L. The most appropriate effects metric for indirect effects (for listed species dependent on marine non-vascular plants) is the EC25 for cell yield of 1549 µg a.i./L which is lower than the LOEL of 4432 µg a.i./L (Table F-2 [above]).

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Appendix G
Field and Mecocosm Studies for MAL

Other Lines of Evidence

Field Studies

Kuhajda et al. (1996 [MRID 47587601]) investigated effects to fish and aquatic invertebrates in Stewart Creek, Fayette County, Alabama following malathion application to adjacent cotton fields. Applications to cotton fields surrounding Stewart Creek were considered a worst-case scenario because there were no buffers between the Creek and treated fields and the banks of the Creek lacked riparian vegetation to intercept runoff or spray drift. The field study was carried out over a three-year period, with malathion being applied only in the first two years. There were three sampling locations along Stewart Creek: a malathion spray location ("Spray"), a site downstream of the spray location ("Downstream") and a site upstream of the spray location ("Control"). The Spray and Downstream locations were channelized habitats, whereas the Control location was a more natural and stable habitat for aquatic biota. During the course of the study, measurements of species diversity, quantity of species, quantity of individuals, brain acetylcholinesterase (AChE) activity in two fish species and malathion concentrations in water were taken.

Kuhajda et al. (1996 [MRID 47587601]) applied malathion to cotton fields via ground and aerial spraying at a rate of 1 lb/A (1.12 kg/ha). Over the course of the study, malathion applications to two cotton fields within 25 feet of Stewart Creek were made eight times in 1993 and fifteen times in 1994 as part of a boll weevil eradication effort. Multiple malathion applications were applied to both cotton fields adjacent to Stewart Creek. Application intervals ranged from four to 39-day interval between applications but were typically between five and eight days. This use pattern is representative of many worst-case scenarios on current malathion labels, such as application to strawberries (2 lb a.i./A applied four times per season with a seven-day retreatment interval), papaya (1.25 lb a.i./A applied eight times per season with a three-day retreatment interval), and guava (1.25 lb a.i./A applied 13 times per season with a three day retreatment interval; EPA, 2009). During the course of the study, an 11 acre cotton field located upstream of the spray location was also treated with 1 lb/A malathion nine times in 1993 and 14 times in 1994. Many other pesticides were applied in addition to malathion within the Stewart Creek area between 1993 and 1994. Collection of water samples to determine malathion concentrations in Stewart Creek were taken every 15 minutes, from one hour before to four hours following malathion application. To evaluate differences between the sample sites and changes between the sampling periods, biological samples were grouped into spray and no-spray periods and counts of individuals, numbers of species and diversity were determined. Two species of fish were analyzed for brain AChE activity, *Notropis baileyi* (rough shiner) and *Etheostoma rupestre* (rock darter).

Malathion concentrations in Stewart Creek ranged from below the detection limit (detection limit not reported) to a maximum of 31.1 µg/L in 1993 and 1994 (Kuhajda et al., 1996 [MRID 47587601]). The highest concentrations were observed at the spray location shortly after application of malathion in nearby fields. Aerial applications produced higher concentrations of malathion than ground applications. Concentrations of malathion also declined rapidly following

application, as four-hour average concentrations were approximately six- to nine-fold lower than peak concentrations, indicating that malathion dispersed rapidly in the flowing-water system. Malathion application to cotton fields bordering Stewart Creek significantly affected the brain AChE activity in one of the two species examined, *N. baileyi*. Percent reductions in brain AChE activity relative to *N. baileyi* from the control site were up to 26.0% in fish sampled at the spray site and up to 36.3% in fish sampled downstream of the spray site. A number of estuarine fish studies have suggested that brain AChE inhibition of >50% is associated with mortality and some species of fish appear capable of tolerating much higher (>90%) brain AChE inhibition (Fulton and Key, 2001; Sandahl et al., 2005). Furthermore, based upon the abundance data for this species over the course of the study, *N. baileyi* populations at the spray and downstream locations were not adversely affected by malathion application in nearby cotton fields. In conclusion, Kuhajda et al (1996 [MRID 47587601]) found that no adverse or long-term effects in the fish and aquatic invertebrates were apparent based on the number of individuals, number of taxa or diversity indices.

A field study conducted by Jensen et al. (1999) tested the effects of ultra-low volume malathion (Cythion™; emulsifiable concentrate) on wetland communities in the Central Valley of California. Two sets of paired treatment and control sites were studied, with malathion applied over seasonally impounded wetlands in the Colusa National Wildlife Refuge. Control wetlands were placed upwind from treated sites to prevent spray drift. Malathion was applied at dusk on September 30, 1996 at a rate of 0.236 L/min and using a truck speed of 16 km/h. The applications were identical to operational pest control in the area. Water samples were taken before and after application. Approximately 6 µg/L malathion was detected in post-treatment water samples from both treated wetlands, but not detected in control wetlands or pre-treatment samples. Adult female mosquitoes (*Aedes melanimon*), larval mosquitoes (*Culex tarsalis*) and mosquitofish (*Gambusia affinis*) were pre-counted and placed into separate appropriate enclosures to monitor survivorship in control and treatment wetlands after malathion exposure. Aquatic macroinvertebrates were collected using D-ring aquatic sweep nets from each wetland before and after application of malathion and analyzed for effects on abundance and biomass. Flying insect abundance was also monitored before and after malathion application in each wetland using ultraviolet light traps. Results found no detectable effects of malathion on survival of mosquito larvae or on the abundance or biomass of any other aquatic macroinvertebrates or on mosquitofish. However, all adult mosquitoes died in treated wetlands. Adult mosquitos did survive in control wetlands, indicating that drift did not occur. Flying insect abundance decreased on the day of malathion application in both treated and control wetlands, but within 24 hours abundance rebounded to pre-treatment levels. This suggests that flying insect populations are resilient to malathion exposure due to renewed activity or immigration and continued emergence of adults. The authors concluded that ultra-low volume applications of malathion used to control adult mosquitoes are unlikely to substantially affect aquatic invertebrate or fish populations in seasonal wetlands.

Tagatz et al. (1974) applied malathion as an ULV aerosol spray to a salt-marsh wetland in northwestern Florida. Three applications of 57 g/ha (0.051 lb/A) ULV malathion (Cythion Technical, 95% purity) were made at low tide to maximize settling and retention and at sunset,

when mosquito activity was greatest. Grass shrimp (*Palaemonetes vulgaris*), blue crabs (*Callinectes sapidus*) and sheepshead minnow (*Cyprinodon variegatus*) were placed in containers of water throughout the treated and untreated marshes. No negative effects on the shrimp, crabs and minnows were observed and no organisms contained detectable levels of malathion. The highest water concentrations, up to 0.34 µg/L, were measured in the containers within three days of application.

Giles (1970 [MRID 00058820]) studied the effects of malathion on aquatic organisms in a small forested watershed over a two-year period. In 1961, tracer plot studies were conducted to develop techniques for use of the radionuclide tracer method. Malathion-S^{35} was applied using a backpack ground sprayer to two 0.04 ha (0.1 A) plots on August 29th, 1961 at a rate of 0.81 kg/ha (0.73 lb/A) and two other plots were included as carrier and untreated controls. In 1962, malathion-S^{35} was aerially applied (0.81 kg/ha or 0.73 lb/A) to one of two adjoining eight hectare (roughly 20 acres) forested watersheds. On May 15th, 1962 the plane crashed at the end of the first swath and most of the malathion in the tank was confined to the airplane and the crash site. A second flight occurred on May 25th, 1962 and malathion application was applied as planned.

In 1962, crayfish (*Cambarus bartoni sciotensis*) were caught from 500 m below the watersheds and placed in ponds within the treated and untreated areas. Crayfish were observed daily before and after malathion was applied. Concentrations of malathion in the pond on the day of malathion application (May 25th, 1962) ranged from 1500 to 8500 µg/L. The stream in the treated area was strained for three hours after spraying to collect dead and moribund animals. A large amount of dead organisms was collected within the first hour after spraying (n=1274), but this number was reduced to 640 organisms in the second hour and 598 organisms in the third hour. The majority of collected organisms belonged to the Chironomidae, Ceratopogonidae, Sciaridae and Empididae families. Measured malathion concentrations in the stream were not reported for the May 25th, 1962 application and effects on aquatic invertebrate abundance and diversity were not characterized in this study. Thus, the reported effects to aquatic invertebrates in the stream are of little use for assessing malathion risk to aquatic invertebrate populations and communities.

In the 1961 tracer plot studies, fish captured from streams were placed in buckets of water on each of the plot areas. Twenty four hours after malathion application, all but one of the brook sticklebacks (*Eucalia inconstans*) on the treated plots were dead. However, brook sticklebacks on untreated plots all survived. All blacknose dace (*Rhinichthys atratulus meleagris*) and a mud minnow (*Umbra limi*) survived and appeared to be healthy. Measurements for the buckets used to house fish in the tracer plot study were not reported, nor were the malathion concentrations measured following application. As such, the results of the tracer plot study are not useful for characterizing risk to fish. In the 1962 watershed study, four southern red-bellied dace (*Chrosomus erythrogaster*) were held in cages within the treated pond and observed for four days before and seven days after the malathion application. Blacknose dace were also observed in the ponds for two weeks after treatment. Concentrations of malathion in the pond on the day of malathion application (May 25th, 1962) ranged from 1500 to 8500 µg/L. No effects to fish were observed and above-background radiation was not detected in either fish species in

the treated and untreated ponds. Thus, southern red-bellied dace, blacknose dace and crayfish in the treated pond were not adversely affected by malathion (Giles, 1970 [MRID 00058820]).

Crane et al. (1995) examined the effects of Malathion 60 applied on watercress crops to amphipods, *Gammarus pulex*, living below the watercress beds. Two watercress beds were chosen; one large bed that required spray applications on two consecutive days to ensure coverage of the entire crop and one small bed that only required one application. Wild-caught amphipods were placed in cages below the watercress crops before (control) and after spraying. Additional cages with amphipods were placed below settling pools that received water from the watercress beds and were used to minimize the release of suspended sediment to the adjacent chalk streams. Cages were collected two hours to seven days after deployment. Malathion 60 was applied at a rate of 10 mL Malathion 60/ha. Heavy rain and a high water table resulted in an additional application being made to the large watercress bed; applications occurred on days one, three and four. No significant mortality was observed pre- or post-spray except for below the small bed, where mortality was significantly higher than the corresponding settling pond at both sampling times. There were no significant decreases in feeding rates at any of the sampling times or locations. The field observations indicate that Malathion 60 applied to watercress beds may affect survival of amphipods below the beds, but not in the settling pools.

The American lobster (*Homarus americanus*) population in Long Island Sound suffered a significant mortality event in the fall of 1999, which was followed by two years of smaller, more localized die-offs (Pearce and Balcom, 2005). In comparison to the previous year, lobster landings in western Long Island Sound declined by 99% and landings in the eastern portion of Long Island Sound decreased by 64 to 91% (CTDEP, 2000). To investigate the potential causes of these die-offs (e.g., disease, anthropogenic contaminants and other environmental stressors), the Long Island Sound Lobster Research Initiative (LRI) was put in place and managed by the Steering Committee for Lobster Disease Research (SCLDR), a subcommittee of the Atlantic States Marine Fisheries Commission (ASMFC). Under this initiative, researchers were asked to submit proposals related to the physical/chemical environment, disease, pesticides and immunological responses to stress (Pearce and Balcom, 2005) and 17 projects were selected by the SCLDR (Balcom and Clemetson, 2006). Pearce and Balcom (2005) summarized the collective results of the LRI that were presented and discussed during the Fourth Long Island Sound Lobster Health Symposium, held in Stony Brook, NY in October, 2004.

A number of potential causes of the lobster die-offs, including increased water temperature, low dissolved oxygen levels, exposure to sulfides and ammonia, infection by parasitic amoebae (*Neoparamoeba pemaquidensis*) and exposure to pesticides were investigated under the LRI. Mosquito control programs were undertaken in New York and Connecticut in response to the appearance of mosquito-borne West Nile Virus in the summer and fall of 1999 and there was a belief among lobstermen that the lobster die-off was linked to pesticide applications used to control mosquitoes (Balcom and Clemetson, 2006). Malathion, resmethrin, sumithrin and methoprene were applied in New York and/or Connecticut between August and October of 1999 and application data for these pesticides were collected from the New York State Department of

Environmental Conservation and the Connecticut Department of Environmental Protection (Miller et al., 2005; Wilson et al., 2005).

Laboratory toxicity studies investigating the lethal and immunotoxic effects of malathion, resmethrin and methoprene to lobsters were conducted under the research initiative (Pearce and Balcom, 2005). In addition, two independent modeling efforts were conducted using hydrodynamic models and the aforementioned pesticide application data to determine whether individual pesticides applied for mosquito control could have been responsible for the lobster mortalities (Miller et al., 2005; Wilson et al., 2005). Miller et al. (2005) modeled two extremely conservative exposure scenarios. The first scenario assumed that the entire amount of a pesticide applied within the Long Island Sound drainage area between June and November 1999 reached Long Island Sound and did not degrade over time. The second scenario assumed that all of the applied pesticide reached Long Island Sound and then degraded. The maximum malathion concentration calculated in the first scenario was 10.3 µg/L. This concentration exceeds the most sensitive malathion LC50 of 3.7 µg/L for effects to larvae (Zulkosky et al., 2005), but is well below the adult lobster LC50 of 38 µg/L (De Guise et al., 2004). Assuming a decay rate of 0.88 d^{-1} (or a half-life of approximately 19 h), the highest malathion concentration predicted in the second scenario was 5.4 µg/L and occurred in the surface waters of Flushing Bay (Miller et al., 2005). However, the predicted concentrations of malathion near bottom waters (areas inhabited by lobsters) did not exceed 1.6 µ/L. Thus, Miller et al. (2005) concluded that malathion should not be considered a causative agent of the lobster die-off.

Wilson et al. (2005) modeled a less conservative exposure scenario, which only considered New York pesticide application data within two zip codes of the coast, applied weekly loadings of pesticides and accounted for degradation. However, the approach used was still very conservative in assuming that all the pesticide applied on land reached Long Island Sound. Wilson et al. (2005) concluded that there is little evidence for bottom concentrations exceeding 0.001 µg/L for any of the pesticides considered. The results of both modeling exercises indicated that malathion concentrations, alone, in the water column of western Long Island Sound could not have been high enough to cause adult lobster mortality in 1999.

Summary

Five field studies evaluated the effects of malathion applied at rates ranging from 0.051 to 1 lb/A on aquatic ecosystems, particularly wetlands and watercress beds (Giles, 1970 [MRID 00058820]; Jensen et al., 1999; Tagatz et al., 1974; Kuhajda et al., 1996 [MRID 47587601]; Crane et al., 1995). These studies reported peak concentrations ranging from 0.34 to 8500 µg/L following malathion spraying (concentrations exceeding the EECs predicted by PRZM/EXAMS and PFAM in the screening-level assessment), but observed no persistent effects on the abundance and survival of fish or aquatic invertebrate communities. In particular, Kuhajda et al. (1996 [MRID 47587601]) measured peak malathion concentrations ranging from <LOD to 10.89 µg/L in 1993 and from 4.34 to 31.10 µg/L in 1994, but did not observe any significant effects on species abundance, number of taxa or diversity for fish or aquatic invertebrate communities over the three-year study. Although Kuhajda et al. (1996 [MRID 47587601]) observed a

significant reduction in brain AChE in rough shiners (*Notropis baileyi*), the biochemical change did not have a significant effect on the survival of the shiners.

The reduction of lobsters in Long Island Sound was evaluated through two independent modeling exercises (Miller et al., 2005; Wilson et al., 2005). In both cases, estimated exposure concentrations of malathion in the Sound were lower than the lowest toxicity value available for adult lobsters. Moreover, recent studies have failed to demonstrate a link between malathion exposure and the decline in lobster populations. Those populations have continued to decline in the absence of detectable concentrations of pesticides which bolsters the belief that warming temperatures is the main culprit (CDEEP, 2012; Collins, 2016; Middletown Press, 2016). Finally, all available lobster toxicity studies were rated as unacceptable by Cheminova because they did not follow standard testing guidelines and did not provide enough details to fully replicate the studies.

Mesocosm Studies

Outdoor Mesocosm Studies

Ebke (2002 [MRID 46525901]) conducted a GLP mesocosm study to investigate acute and chronic effects of malathion directly applied as a formulation (Malathion 440 g/L EW; Cheminova A/S, Lemvig, Denmark; 40.6% w/w purity) to aquatic communities in outdoor pond mesocosms (1,060 L each). The study involved a single and direct application of malathion to 16 stainless steel enclosures containing freshwater assemblages of pelagic and benthic organisms. There were six control enclosures. The study involved six treatment concentrations with three replicates each for the 0.6, 1.2, 2.5 and 5.0 µg a.i./L treatments and two replicates each for the 10 and 30 µg a.i./L treatments. Biological sampling for zooplankton, macrozoobenthos, emerging insects, phytoplankton, periphyton and macrophytes occurred on a weekly or bi-weekly basis for 99 days. Concentrations in treatment enclosures declined rapidly with half-life values ranging from 0.8 to 2.3 days. Sediment concentrations never exceeded the detection limit (DL = 0.3 µg/kg ww).

In the highest malathion treatment (30 µg a.i./L), there were no effects on periphyton, phytoplankton, productivity (determined via chlorophyll a levels), macrophytes, macroinvertebrates, emergent insects and functional endpoints (e.g., redox potential) (Ebke, 2002 [MRID 46525901]). There were transient effects in certain zooplankton species (*Daphniidae*, *Chydoridae*), but complete recovery occurred within 28 days. No species were eliminated from either of the 30 µg a.i./L enclosures. Similar results were observed in the 10 µg a.i./L treatment, except that only *Daphniidae* species were transiently affected in this treatment. No effects were observed in any of the remaining malathion treatments. The results confirm that cladoceran species are the most sensitive species to malathion exposure. However, in natural systems where malathion dissipation and degradation are expected to be rapid following application, recovery of cladocerans will also be rapid.

Relyea (2005) used outdoor mesocosms to examine the impact of malathion on the biodiversity of aquatic communities containing algae and 25 species of animals. Zooplankton and phytoplankton assemblages from six local ponds were added to polyethylene tanks containing 1000 L of well water, 300 g dry leaves and 25 g rabbit chow (initial nutrient source). Anuran larvae (*Rana sylvatica*, *Rana pipiens*, *Bufo americanus*, *Hyla versicolor* and *Pseudacris crucifer*), spotted salamander larvae (*Ambystoma maculatum*), snails (*Physa integra*, *Helisoma trivolvis* and *Stagnicola elodes*), damselfly larvae (*Lestes* sp.), dragonfly larvae (*Anax junius* and *Tramea* sp.), diving beetle larvae (*Dytiscus* sp. and *Acilius semisulcatus*) and hemipterans (*Notonecta undulata* and *Belastoma flumineum*) were added to mesocosms 22 to 24 days after the addition of zooplankton and phytoplankton communities. Either a negative control (250 mL of water) or 320 µg/L of commercial-grade malathion (50.6% purity; 162 µg a.i./L based on malathion purity) was applied to mesocosms and abundance and survival measurements were taken 12 days after the initial exposure. The effects of malathion treatment on (1) total species richness of the aquatic community, (2) species richness and biomass of four major functional groups in the community (i.e., predators (insects and salamanders), large herbivores (snails and tadpoles), zooplankton and periphyton algae) and (3) individual species abundance were examined.

Malathion exposures of 162 µg a.i./L reduced overall species richness by 30% compared to controls. There were significant decreases in predator richness and biomass and in zooplankton species richness, but no effects on large herbivore richness or biomass, zooplankton abundance, or periphyton biomass. This exposure level significantly reduced *Tramea* dragonfly survival and eliminated *Dytiscus* beetle and backswimmer (*Neonecta undulata*) populations. However, other insect predators (i.e., *Anax junius*, *Belostoma flumineum* and *Lestes* sp.) were unaffected. *Daphnia pulex* and *Daphnia ambigua* were effectively absent from malathion-treated mesocosms. However, only effects on *Daphnia pulex* were significant. Aside from an increase in *Eurytemora* abundance, no effects of malathion exposure on other zooplankton species (i.e., *Daphnia longiremis*, *Ceriodaphnia* sp., *Scapholebris* sp., *Eurycyclops agilis*, *Mesocyclops edax*, or *Leptochaptumorus* sp.) were observed. Exposure to 162 µg a.i./L malathion did not adversely affect the survival of snails or amphibian tadpoles. In fact, a 28 to 43% increase in leopard frog (*Rana pipiens*) survival and a 50 to 67% increase in wood frog (*Rana sylvatica*) survival in the malathion treatment group were observed compared to control. However, these effects were not statistically significant.

In summary, malathion exposures of 162 µg a.i./L eliminated or significantly reduced the abundance of a limited number of cladoceran and insect species, but had indirect positive effects on copepod (i.e., *Eurytemora*) and anuran tadpoles, which were likely due to malathion effects on predators. Relyea (2005) notes that this study observed the effects of malathion within a short time frame (~two weeks) and that many taxa have the potential to recover over longer periods of time if pesticide exposure is not a recurring event.

Relyea (2009 [MRID 48261129]) studied the effects of malathion on aquatic communities using outdoor mesocosms over a 57-day period. Water was supplied from nearby ponds and contained zooplankton, phytoplankton and periphyton. Algal and zooplankton communities were

allowed to develop in the mesocosm prior to addition of leopard frog (*Rana pipiens*) and gray tree frog (*Hyla versicolor*) tadpoles. Technical-grade malathion (99% purity) was added to the water at a nominal concentration of 10 µg/L. However, the actual concentration achieved was 5.8 µg/L. Survival of tadpoles in the control mesocosms was 96% and survival in the malathion mesocosms was also near 100% for both species. Mass at metamorphosis was comparable to control values. Average zooplankton abundance was significantly decreased compared to control levels for *Ceriodaphnia* sp., but not for *Leptodiaptomus minutus*, *Skistodiaptomus oregonensis* or *Daphnia pulex*. Likewise, the abundances of phytoplankton and periphyton were not significantly different from control. Thus, only the biomass of cladocerans was significantly reduced following exposure to 5.8 µg/L malathion. However, since *Ceriodaphnia* sp. abundance was averaged for the two sampling times (days 16 and 36), it is unknown when significant effects occurred and if the *Ceriodaphnia* sp. population was recovering following the initial exposure.

Hua and Relyea (2012) compared the effects of a malathion formulation (50% purity, Malathion Plus, Ortho Corporation, Marysville, OH) on two aquatic communities with geographically distinct amphibian assemblages from Pennsylvania and Oregon. Mesocosm studies were conducted with a factorial experimental design. Each mesocosm included (1) either wood frog (*Rana sylvatica*) or Cascades frog (*Rana cascadae*) larvae for the Pennsylvania and Oregon assemblages, respectively, (2) either a presence or absence of a zooplankton predator (larval north western salamanders (*Ambystoma gracile*) for the Pennsylvania assemblage or spotted salamanders (*Ambystoma laterale*) for the Oregon assemblage) and (3) one of three nominal malathion concentrations (0, 5, or 50 µg a.i./L). Originally, Malathion Plus (Ortho Corporation, Marysville, OH, 50% purity) was added to mesocosms at concentrations of 1 or 10 µg a.i./L. However, concentrations measured in samples collected four hours after dosing were an order of magnitude less than the nominal concentration. Thus, applied concentrations were increased to 5 and 50 µg a.i./L. Measured concentrations were 6 and 40 µg a.i./L in samples collected three hours after dosing. Abiotic response variables (temperature, pH, DO and light attenuation), zooplankton, phytoplankton and periphyton abundance were measured on day 22. Emergence of wood frogs and Cascades frogs was monitored from day 16 until day 40 and mass at metamorphosis was measured.

Copepod abundance increased in the 6 µg a.i./L treatment compared to controls, but did not differ from controls for the 40 µg a.i./L treatment. Cladoceran abundance was practically reduced to zero individuals in the 6 and 40 µg a.i./L treatment groups in all amphibian assemblages. Thus, the increase in copepod abundance at the 6 µg a.i./L treatment was likely due to reduced competition with cladocerans. However, at the 40 µg a.i./L treatment, the toxic effects of malathion on copepods appeared to offset the positive effects of reduced competition.

Phytoplankton abundance was greater in both malathion treatments compared to controls. This increase was likely due to overall reductions in zooplankton, which are the major consumers of phytoplankton. Malathion-related increases in phytoplankton also had effects on abiotic variables associated with increased photosynthesis, such as pH and DO. Conversely, periphyton biomass was significantly lower at the 40 µg a.i./L treatment compared to controls.

The reduction in periphyton biomass is likely an indirect effect of malathion treatment. Periphyton competes with phytoplankton for light and nutrients, thus the malathion-related increase in phytoplankton is likely associated with the reduction in periphyton.

Anuran and salamander survival was high across all treatments and ranged from 97.1% to 99.4%. Anuran mass at metamorphosis was increased in the malathion treatments relative to the control and Cascades frog metomorphs were larger than the wood frog metomorphs. The positive effect of malathion on anuran mass at metamorphosis may be due to a brief initial increase in periphyton abundance after insecticide application, which has been observed by Boone and Semlitsch (2002), Boone et al., (2005), Boone and Bridges-Britton (2006), Relyea and Diecks (2008) and Mackey and Boone (2009). Because wood frogs and cascade frogs have relatively short larval periods, they may be able to complete metamorphosis before declines in periphyton occur (Hua and Relyea, 2012). Salamander mass was significantly different between malathion treatments and amphibian assemblages. Salamander mass decreased significantly with increasing malathion concentrations and was likely due to the reduction in zooplankton prey.

Overall, Hua and Relyea (2012) found that malathion concentrations ranging from 6 to 40 µg a.i./L had direct lethal effects of cladoceran populations and indirect effects on abiotic variables, phytoplankton, periphyton and copepod abundance and amphibian mass at metamorphosis. However, these taxa were only sampled once and recovery could not be measured. Although mass at metamorphosis for both anurans and salamander was affected by malathion exposure, significant effects on amphibian survival were not observed. Amphibian survival is not affected likely because, even if prey base composition changes, overall abundance of the prey base is sufficient to not affect survival of the amphibian larvae.

Hua and Relyea (2014) conducted a mesocosm study to measure the direct and indirect effects of malathion on aquatic population and community-level variables. Technical-grade malathion (Chem Service, West Chester, PA) was applied at nominal concentrations of 10 or 40 µg/L to outdoor mesocosms containing algae, zooplankton, amphipods (*Crangonyx pseudocracilis*), isopods (*Asellus aquaticus*), snails (*Physa acuta*, *Helisoma trivolvis* and *Stagnacola elodes*) and spring-breeding tadpoles (wood frogs (*Rana sylvatica*), leopard frogs (*R. pipiens*), American toads (*Anaxyrus* [*Bufo*] *americanus*) and spring peepers (*Pseudacris crucifer*)). Tadpoles of two summer-breeding amphibians species (gray treefrogs (*Hyla versicolor*) and green frogs (*R. clamitans*)) were added approximately three weeks after insecticide application. All tanks were covered with 60% shade cloth to prevent organisms from entering or leaving the systems (Hua and Relyea, 2014). Water samples were taken four hours after dosing and after the addition of summer-breeding amphibian species to measure insecticide concentrations. Trace amounts of malathion (0.2 µg/L) were detected in the controls and measured concentrations of malathion in treatments were 2.8 (10 µg/L nominal concentration) and 12 µg/L (40 µg/L nominal concentration) after four hours. Malathion was not detected in water after addition of the summer-breeding amphibian species. The authors stated that because the first and second set of samples were processed two and five weeks after collection, respectively, storage degradation likely occurred (Hua and Relyea, 2014). Abiotic variables (temperature, pH, DO and

light attenuation), zooplankton, phytoplankton and periphyton abundance were measured two, four, nine and 18 weeks after malathion application. Snail abundance was assessed six and 18 weeks after dosing, detrivore (amphipods and isopods) abundance was measured 13 weeks after dosing and amphibian metamorphosis was recorded daily from four weeks after dosing. Survival to metamorphosis and mass at metamorphosis were measured. Mass of green frog tadpoles was measured at week 18 since they are an overwintering species and did not undergo metamorphosis over the course of the study.

Direct effects on cladoceran and copepod abundance were not observed at any time point following exposure to 10 µg/L malathion. However for the 40 µg/L treatment, significant reductions in zooplankton abundance relative to controls were observed at the two and four week sampling times for cladocerans and at the two week sampling time for copepods. Recovery of cladoceran abundance was observed by the nine week sampling time and copepod populations recovered by week four. The relatively rapid recoveries of cladoceran and copepod populations were likely due to the complete degradation of malathion by week three of the study. Malathion had no significant effects on amphipod abundance at the 10 µg/L concentration and no effects on isopod abundance at either concentration. However, the 40 µg/L malathion treatment severely reduced amphipod abundance. Detrivore abundance was only sampled once, therefore, recovery was not assessed. No significant effects of malathion on abiotic variables, phytoplankton, periphyton or snail abundance were observed.

Neither of the malathion treatments affected the survival to metamorphosis nor the mass at metamorphosis of spring-breeding amphibians. The 40 µg/L treatment reduced gray treefrog survival to less than 50%, but mass at metamorphosis was not affected by malathion. The mass of green frog tadpoles was not affected by either malathion treatment.

In summary, the 40 µg/L nominal malathion treatment (measured 12 µg/L) was associated with significant reductions in zooplankton and amphipod abundance, and gray treefrog survival (Hua and Relyea, 2014). However, recovery of zooplankton abundance was observed within nine weeks. Recovery was not measured for amphipods. The observed reduction in gray treefrog survival is likely due to indirect effects of malathion treatment since malathion had completely degraded at the time the summer-breeding tadpoles were added to the mesocosm (Hua and Relyea, 2014). Instead, the authors attribute gray treefrog mortality to possible formation of the malathion degradate, malaoxon. However, the authors do not report whether malaoxon was actually measured. In addition, formation of malaoxon in this mesocosm study is unlikely since, as described in Appendix B, no malaoxon was detected in hydrolysis and photolysis studies (Teeter, 1988 [MRID 40941201]; Miller, 1988 [MRID 40969301]; Carpenter, 1990 [MRID 41673001]; Sanders, 1988 [MRID 41039001]; Dykes et al., 1990 [MRID 41695501]; Spare and Cutchin, 1991 [MRID 42015201]); soil and water samples from two aquatic dissipation studies and two soil dissipation studies (Rice et al., 1990a,b [MRID 41748901, 41727701]; Jacobson and Lochhaas, 1992a,b [MRID 43042401, 43042402]; Judy et al., 1991a,b [MRID 42058401, 42058402]); and water-sediment systems treated with malathion (Knoch, 2001a [MRID 46769502]; Hiler and Mannella, 2012 [MRID 48906401]; Blumhorst, 1991a,b [MRID 42271601; 42216301]).

Halstead et al. (2014) conducted a freshwater mesocosm study to examine the effects of malathion on species- and ecosystem-level responses. The authors hypothesized that malathion would reduce zooplankton and arthropod abundance, leading to increases in non-arthropod herbivores and phytoplankton. Outdoor mesocosms covered with 60% shade cloth and containing zooplankton (Copepod nauplii, calanoidea spp., Cyclopoidea spp., *Ceriodaphnia* sp. and *Diaphanosoma* sp.), periphyton, phytoplankton, macrophytes (*Hydrilla verticillata* and *Utricularia macrorhiza*), amphibian larvae (*Osteopilus septentrionalis* and *Rana sphenocephala*), snails (*Melanoides tuberculate*, *Planorbella scalaris*, *P. trivolvis* and *Viviparus georgianus*) and macroarthropods (*Anax junius*, *Belostoma flumineum*, Corixidae sp., *Procambarus alleni* and *Ranatra* sp.) were exposed to 102 or 204 µg/L technical-grade malathion (98.4% purity, Chem Service, West Chester, PA; 100 and 200 µg a.i./L based on malathion purity) dissolved in acetone. Negative and solvent controls were also included. Weekly measurements of phytoplankton abundance, light availability and water temperature were taken and periphyton and zooplankton abundance, DO and pH were measured biweekly. Amphibian, snail and macroarthropod abundances were measured at the end of the 4-week experiment. The Bray-Curtis similarity index was used as the dissimilarity measure for the whole ecosystem.

No significant effects to amphibians, macroarthropods, snails, copepod nauplii, Calanoidea spp., Cyclopoidea spp., *Diaphanosoma* sp., periphyton or phytoplankton were observed in response to malathion exposure. *Ceriodaphnia* sp. abundance was significantly reduced compared to controls throughout the experiment (Halstead et al., 2014), but appeared to be increasing between weeks two and four. As such, recovery of *Ceriodaphnia* sp. could have been observed had the experiment been of longer duration. *Diaphanosoma* sp. abundance increased between weeks two and four of the experiment and was likely a result of reduced competition for nutrients with *Ceriodaphnia* sp. Bray-Curtis similarity was significantly different between control mesocosms and malathion-treated mesocosms. However, there was no evidence of a community-level dose response to the two malathion treatments.

Brogan and Relyea (2015) performed a factorial mesocosm to examine the mitigating effects of macrophytes on malathion toxicity to aquatic communities. Mesocosms contained one of four macrophyte treatments (0, 10, 50 or 100 *Elodea canadensis* shoots planted) and one of three malathion exposure scenarios was administered (no exposure, single pulse and repeated pulse). Microbial, algal and zooplankton communities were established in 1200 L mesocosms and on day 0 of the experiment, 20 gray treefrog (*Hyla versicolor*) tadpoles, 15 pond snails (*Physa acuta* and *Physa gyrina*), and seven ram snails (*Helisoma trivolis*) were added. Mesocosms were left undisturbed for 10 days prior to exposure. Technical-grade malathion (99% purity; Chem Service Inc., West Chester, PA) was applied at a target concentration of 18 µg/L to all treated mesocosms on day 19. However, since this application did not significantly reduce cladoceran populations, an additional application of 36 µg/L malathion was made on day 37 for the single pulse treatment and on days 37, 55 and 73 for the repeated-pulse treatment. Water samples were collected one hour after application and sent for analysis of malathion concentrations. However, results were unreliable and concentrations could not be verified.

The response variables sampled by Brogan and Relyea (2015) and sampling times are listed below:

- *Elodea* density on day 47 (qualitative measurement) and on day 320 (quantitative measurement)
- abiotic water quality variables (pH, dissolved oxygen (DO), light decay rate, temperature) on days 26, 47, 68 and 100
- cladoceran, copepod and rotifer densities on days 26, 47, 68 and 100
- phytoplankton abundance (measured as chlorophyll *a*) on days 26, 47, 68 and 100
- periphyton mass on days 26, 47, 68 and 100
- pond and ram horn snail abundance and mass on day 68
- gray tree frog survival and mass at metamorphosis checked daily after emergence of first metamorph on day 30 until the final gray treefrog emergence on day 94

Where included, *Elodea* density increased in all mesocosms over the course of the experiment. By the end of the experiment (day 320), there were no significant differences in biomass density between the 10 and 50 macrophyte treatments, but both groups contained 50% less biomass than the 100 macrophyte treatment. As expected based on the results of Brogan and Relyea (2013a,b; 2014), mesocosm pH values increased with increasing macrophyte treatments. *Elodea*-treated mesocosms had pH value at least 1.17 units higher than mesocosms without *Elodea*. In addition, inclusion of *Elodea* had no effect on temperature, but increased DO and reduced light decay rates.

Cladocerans were marginally affected by the initial 18 µg/L malathion treatment ($p = 0.061$), but there were no effects of *Elodea* or the *Elodea* x malathion interaction on cladoceran densities. After the 36 µg/L malathion treatment was applied, the single pulse exposure resulted in reduced cladoceran density by 76% in mesocosms without *Elodea*, but populations returned to control levels within 21 days. However, for the repeated pulse exposures, mesocosms without *Elodea* had cladoceran densities that were 97% lower than controls on days 47, 68 and 100. In contrast, no adverse effects of malathion treatment were observed on cladoceran densities for mesocosms that contained *Elodea*. Copepods and rotifers are much more resistant to malathion toxicity compared to cladocerans. Accordingly, no direct effects of malathion treatment on these taxa were observed by Brogan and Relyea (2015).

The reduction in cladocerans observed in mesocosms without *Elodea* did not result in phytoplankton blooms for the single pulse exposure, likely due to the rapid recovery of populations. However, phytoplankton blooms in the repeated pulse exposure were observed from day 68 to day 100. Phytoplankton blooms were not observed in any of the mesocosms containing *Elodea*, which indicates that macrophytes mitigated both the direct effect of malathion on cladoceran populations and the indirect effects on phytoplankton.

Ram horn snails may have been affected by malathion-induced phytoplankton blooms since reduced abundance of these organisms relative to controls were observed in the repeated pulse treatment mesocosms without *Elodea*. It is unlikely that this effect resulted from direct toxicity of malathion based on a 48-hour LC50 of 228,840 µg/L for the ramhorn snail (Tchounwou et al., 1991). Rather, ramhorn snails may have been affected by reduced production or quality of periphyton, resulting from phytoplankton blooms (Brogan and Relyea, 2015). Malathion exposures of 18 to 36 µg/L were not associated with any effects on gray treefrog survival or mass at metamorphosis, and survival was high across all mesocosms (86 ± 2%).

Groner and Relyea (2011) conducted a freshwater mesocosm study to examine the effects of malathion on common pond species. The authors hypothesized a trophic cascade of effects wherein zooplankton would be reduced, causing an increase in phytoplankton, which would in turn shade and reduce periphyton, thereby decreasing the growth and development of tadpoles. Additionally, they hypothesized that these effects would be greater in smaller weekly applications then they would in larger single applications. Aliquots of pond water were collected from local ponds, screened for predators, and added to 1200-L mesocosms. All tanks were covered with 60% shade cloth to prevent organisms from entering or leaving the systems. Leopard frog eggs (*R. pipiens*) were collected from one egg mass and hatched in laboratory conditions. Forty tadpoles were then added to each mesocosm and allowed to acclimate for six days before malathion was added into the experiment. Three exposure concentrations were selected and subsequently measured to be 3.1 µg a.i./L added once a week for four weeks and 34 and 384 µg a.i./L added once. However, the 34 µg/L concentration had to be estimated due to damage to the sample. Periphyton, phytoplankton, and zooplankton abundance was measured at seven and 21 days by measuring mass, chlorophyll *a*, and density, respectively. Zooplankton was identified to group of cladoceran or copepod. Amphibian endpoints were selected to be survival to metamorphosis, mass after metamorphosis, time to metamorphosis. After metamorphs reached Gosner stage 42 (Gosner, 1960), they were removed from experimental chambers. To simulate the potential for pond drying, 225-L of water were removed from each mesocosm every three days beginning on day 88. However, few tadpoles exposed to malathion remained in the experiment after the ponds were dried, so this method did not ultimately alter survival. Abiotic experimental conditions including DO, temperature, pH, and light extinction were measured on days seven and 21.

Malathion exposure was determined to significantly alter the structure of pond communities and tadpole survival. At seven days, cladoceran density in the two highest exposure concentrations was reduced by at least 93% compared to the control. At 21 days, cladoceran density was significantly reduced for all exposure concentrations when compared to the controls. At that time, copepod density was also significantly increased relative to the controls in the weekly treatment and the lowest single-application treatment, but not in the high single-application. Phytoplankton productivity was also significantly greater at this time point when compared to the controls. Additionally, periphyton mass was reduced in the low single-exposure treatment.

Tadpole survival was significantly decreased by all exposure treatments, by at least 8%. Single-exposure treatments did not significantly alter the time to metamorphosis but exposure to multiple treatments significantly increased the time to metamorphosis of tadpoles. Mass at

emergence was significantly decreased by multiple exposures but significantly increased in the single-exposure treatments.

In summary, Groner and Relyea (2011) found that malathion exposure can significantly alter the makeup of freshwater pond communities and that the observed differences can be different if exposure happens more than once, even at lower concentrations. They observed significant direct results for amphibians exposed to concentrations greater than 3.1 µg/L and differences in all measured factors of community structure 21 days after exposure to malathion. They hypothesized that the results indicating significant differences in amphibian growth were due to alterations in community structure, thereby changing food availability and competition with mesocosms.

Indoor Mesocosm Studies

Shrestha et al. (1987) conducted a laboratory and nursery pond study to assess the effects of malathion on zooplankton and fish populations in the context of fish aquaculture. Cladocerans and copepods prey on fish hatchlings, thus reducing fish harvests in aquaculture. However, other aquatic invertebrate taxa, such as rotifers, represent an important food source for early life stage fish (Shrestha et al. 1987). In the laboratory experiment, zooplankton and grass shrimp larvae were exposed to 0, 1000 and 1500 µg/L of a malathion emulsion (50% purity; 500 and 750 µg a.i./L based on malathion purity) for 48 hours before adding fish hatchlings to the test system. The zooplankton population was observed daily. Crustaceans and copepods died within a few hours of malathion treatment (500 and 750 µg a.i./L). However, neither treatment level affected fish fry or rotifer populations. The fish species used in the laboratory experiments were not specified and although the authors indicate that fish fry and rotifer mortality occurred in controls, survival rates for each taxon included in the experiment were not reported.

In the nursery pond experiments, 1000 and 1500 µg/L of malathion emulsion (50% purity; 500 and 750 µg a.i./L) were each applied to three ponds (Shrestha et al., 1987). The study did not report whether indoor or outdoor nursery ponds were tested. Copepod and cladoceran populations were significantly reduced following malathion application, but recovered to their initial population stage within eight to nine days. Thus, even at the high exposure concentrations tested, aquatic invertebrate populations rebounded rapidly. An increase in rotifer populations was observed following malathion application, likely as a result of reduced competition with copepods and cladocerans. Malathion applications in nursery ponds were made 48 hours before fish were added. Ponds were then stocked with either four to five day-old silver carp (*Hypophthalmichthys molitrix*) or two to three day-old Indian carp (mrigal carp (*Cirrhina mrigala*) and rohu (*Labeo rohita*) at a density of 300 per square meter. Fish survival for all species was significantly higher in nursery ponds where malathion was applied (56.6% survival for rohu in 750 µg a.i./L malathion treatment to 95.0% for silver carp in 750 µg a.i./L malathion treatment) compared to untreated ponds (26.0% survival for silver carp to 32.0% for rohu).

Sweilum (2006) investigated the effects of sub-lethal exposures of malathion on the growth rate, hematological properties, and production of juvenile Nile tilapia (*Oreochromis niloticus*). Glass

ponds (3-m^2) were filled with freshwater and exposed to 0, 500, 1000, or 2000 µg/L malathion for the 24-week duration of the experiment. Water was aerated, changed weekly in order to maintain pesticide concentrations, and filtered daily to remove fecal matter and detritus. Sixty fish were placed in each pond and fed six days per week. Abiotic conditions measured were temperature and DO (twice daily); pH (daily); and alkalinity, ammonia, nitrate, and phosphate (estimated monthly). Biweekly, plankton was collected, identified, and counted, and tilapia length and body weight was measured. Blood samples were collected from 15 fish from each pond and erythrocytes, hematocrit, hemoglobin, proteins, and serum glucose and lipids were measured. Proteins, glycogen, and lipids were measured in fish muscles. Malathion concentrations were determined in the liver, gills, and muscles of fish upon the termination of the study. Production was determined as a factor of mass per fish.

The number of phytoplankton and zooplankton organisms were significantly less in all exposed treatments when compared to the control. Tilapia growth rate was also significantly decreased by exposure to all levels of malathion used in this study. Additionally, the authors claim that malathion caused a decrease in survival and feeding rate of all exposed tilapia in this study but do not report statistics to support this result. Significant alterations in hematological and muscle composition were observed for all endpoints measured in this study at all concentrations except for muscle glycogen and blood glucose, for which significant results were only observed at concentrations above 1000 µg/L, and blood lipids, for which no significant differences were observed. All significant results observed were decreased relative to the control except for blood glucose and muscle glycogen, wherein exposure to malathion increased these parameters compared to the control. The authors also reported a trend of increased malathion residue in tilapia organs with increasing exposure concentration. Statistical analysis was not completed for the endpoint of production but the authors reported decreased production, net returns, and probability with increasing dose of the chemical.

In summary, Sweilum (2006) determined that exposure to malathion at a concentration of at least 500 µg/L can have significant effects on the growth and the makeup of the muscle and blood of Nile tilapia. The results for growth and survival of the fish may be in part due to the reduction in plankton caused by pesticide exposure. However, the author did not report the source or active ingredient of the chemical used, nor did he measure the actual experimental chemical concentration. Furthermore, many of the abiotic factors measured in this study were significantly different in exposure treatments when compared to the control. Additionally, the experimental design only utilized a single replicate for each exposure concentration.

Summary

Ten mesocosm studies are available that studied the effects of malathion on aquatic communities (Ebke, 2002 [MRID 46525901]; Relyea, 2005, 2009 [MRID 48261129]; Sweilum, 2006; Groner and Relyea, 2011; Hua and Relyea, 2012, 2014; Halstead et al., 2014; Brogan and Relyea, 2015; Shrestha et al., 1987). Studies attempted to quantify effects to fish, aquatic-phase amphibians, periphyton, phytoplankton, macrophytes, zooplankton and macroarthropods. Test concentrations ranged from 0.6 to 2000 µg/L. Effects on cladocerans, the most sensitive

taxon tested, were observed at concentrations ≥5.8 µg/L (Ebke, 2002 [MRID 46525901]; Relyea, 2005, 2009 [MRID 48261129]; Groner and Relyea, 2011; Hua and Relyea, 2012, 2014; Halstead et al., 2014; Shrestha et al., 1987). The effects observed on cladocerans are not unexpected, given that the acceptable 48-h LC50 for the water flea (*Daphnia magna*) is 0.70 µg a.i./L (Gries and Purghart, 2001a [MRID 47540303]). However, when measured, recovery of cladoceran populations occurred within eight days to seven weeks of when effects were originally observed (Ebke, 2002 [MRID 46525901]; Hua and Relyea, 2014; Brogan and Relyea, 2015; Shrestha et al., 1987). Other aquatic invertebrate taxa (e.g., copepods, rotifers, snails, amphipods, isopods, and insects) were considerably less sensitive to malathion. Reductions in copepod, amphipod and insect abundance were only observed in response to malathion exposures ≥40 µg/L (Relyea, 2005; Hua and Relyea, 2014; Shrestha et al., 1987). Copepod populations recovered within two weeks of the original effects being observed (Hua and Relyea, 2014; Shrestha et al., 1987). The study designs did not allow for measurement of recovery in amphipod and insect populations (Relyea, 2005; Hua and Relyea, 2014). Aquatic-phase amphibian survival was decreased for concentrations ≥ 3.1 µg/L (Groner and Relyea, 2011). Fish exposed to malathion at concentrations ≥500 µg/L had increased mortality and altered blood and muscle structure but these results are questionable due to poor experimental design and execution (Sweilum, 2006). Overall, snails, rotifers, isopods, periphyton, phytoplankton and macrophytes were resistant to malathion toxicity.

Effects of Macrophytes on Malathion Toxicity

Brogan and Relyea (2013a) measured the effects of the macrophyte *Elodea canadensis* on malathion toxicity to *Daphnia magna*. Daphnia were exposed to measured malathion concentrations of 0, 3.2, 4.7, 17.7 and 29.6 µg/L in the presence of different densities of *Elodea* (0, 344, 612, 899 and 1,102 g dry wt/m^3) for 48 hours. The 48-hour LC50 values for malathion decreased with increasing *Elodea* density, and an order of magnitude difference was observed between the 48-hour LC50 with no *Elodea* added (2.8 µg/L) and the LC50 at the highest *Elodea* density (25.2 µg/L). The effect of *Elodea* on the rate at which malathion toxicity decreases over time was also measured. Water samples were collected from each experimental container 2, 6, 10, and 48 hours after malathion had been applied, and new daphnia were exposed to these samples. Malathion toxicity at a given concentration decreased at a faster rate with increasing *Elodea* density. With no *Elodea*, the 4.7, 17.7, and 29.6 µg/L malathion concentrations caused greater than 50% mortality at all time points, but at the 1,102 g dry wt/m^3 *Elodea* density, daphnia mortality for all malathion concentrations and time points did not differ significantly from controls. Similar results were observed with three additional macrophyte species: *Myriophyllum spicatum*, *Ceratophyllum demersum*, and *Vallisneria americana*. The presence of all macrophytes species decreased malathion toxicity to daphnia and increased the rate at which toxicity decreases over time (Brogan and Relyea, 2013b).

Brogan and Relyea (2013a) observed an increase in pH from 8 with no added *Elodea* to 10 with 1,102 g dry wt/m^3 *Elodea*. Each unit increase in pH is associated with an order of magnitude decrease in the malathion half-life (Brogan and Relyea, 2013a). The authors, therefore, hypothesized that the effects of macrophyte density on malathion toxicity were due to their

effects on water pH. During photosynthesis, submerged plants remove carbon dioxide and increase dissolved oxygen in water, leading to an increase in pH (Brogan and Relyea, 2013b). Thus, the presence of macrophytes may reduce malathion toxicity by increasing the rate at which it is degraded.

Brogan and Relyea (2014) tested this hypothesis by comparing malathion toxicity to *Daphnia magna* under different pH conditions in the absence of macrophytes or in the presence of macrophytes with varying photosynthetic activity. Using a factorial experimental design, daphnia were exposed to five nominal concentrations of technical-grade malathion (0, 1, 5, 10, 50 µg/L; 99.1% purity) under four environmental conditions: (1) presence of *Elodea canadensis* in full sunlight, (2) presence of *Elodea* in complete shade, (3) addition of 1 M hydrochloric acid to maintain low pH conditions (~7.5) and (4) addition of 1 M sodium carbonate to maintain high pH conditions(~9.5). Malathion concentrations of 0, 1, 4, 36, and 36 µg/L were measured in water samples. The effects of malathion on daphnia neonates were observed over a 48-hour period.

Water pH levels measured one hour before malathion application, and 24 and 48 hours after malathion application were significantly higher in the no-plant/high pH and unshaded-plant conditions compared to the no-plant/low pH and shaded plant conditions. In addition, no significant effects of malathion treatment or the interaction between malathion treatment and plant-pH conditions on water pH were observed. The no-plant/low-pH and shaded plant conditions had pH values within 0.1 standard units of each other, and the no-plant/high pH and unshaded-plant conditions were within 0.32 units of each other.

Survival of daphnia neonates was significantly affected by plant-pH condition, malathion treatment and their interaction. For malathion concentrations of 4 and 6 µg/L, daphnia survival after 48 hours was 62-78% higher in the no-plant/high-pH and unshaded plant conditions compared to the no-plant/low-pH and shaded plant conditions. In addition, no significant differences in survival were observed between the no-plant/high-pH and unshaded plant conditions or between the no-plant/low-pH and shaded plant conditions. Although no statistically significant differences in daphnia survival were observed between any of the four conditions when daphnia were exposed to 36 µg/L malathion, higher survival was still observed in the no-plant/high-pH and unshaded plant conditions compared to the no-plant/low-pH and shaded plant conditions. These results strongly support the hypothesis that macrophytes mitigate malathion toxicity to daphnia through increases in pH since reductions in pH through the addition of sodium carbonate had the same mitigating effect as including photosynthetically active submerged plants in the test system.

Brogan and Relyea (2013a) had also proposed that sorption of malathion to the surface of macrophytes could be another mechanism for mitigation of malathion toxicity (Brogan and Relyea, 2013a). However, Brogan and Relyea (2014) demonstrated that “the mitigating effect of submerged plants on malathion toxicity could be predicted entirely based on the pH of the water and that sorption played no role in mitigation”. This finding is consistent with malathion’s low binding affinity to organic substrates (log Kow = 2.75) and the results of Gao et al. (2000), which showed weak sorption of malathion and other organophosphate pesticides to aquatic plants.

Based on average daily pH values from the USGS real-time surface water quality monitoring data, surface water pH values exceeding 8 are common, suggesting that pH-mediated insecticide mitigation may be a widespread phenomenon (Brogan and Relyea, 2014).

As described in the Mesocosm Studies section above, Brogan and Relyea (2015) performed a factorial mesocosm to examine the mitigating effects of macrophytes on malathion toxicity to aquatic communities. Mesocosms contained one of four macrophyte treatments (0, 10, 50 or 100 *Elodea canadensis* shoots planted) and one of three malathion exposure scenarios was administered (no exposure, single pulse and repeated pulse). Technical-grade malathion (99% purity; Chem Service Inc., West Chester, PA) was applied at a target concentration of 18 µg/L to all treated mesocosms on day 19. However, since this application did not significantly reduce cladoceran populations, an additional application of 36 µg/L malathion was made on day 37 for the single pulse treatment and on days 37, 55 and 73 for the repeated-pulse treatment. Effects on abiotic variables (pH, DO, temperature, light decay rate), macrophyte density, zooplankton (cladoceran, copepod and rotifer) density, phytoplankton abundance, periphyton mass, pond snail (*Physa acuta* and *Physa gyrina*) and ram snail (*Helisoma trivolis*) abundance and mass, and gray treefrog survival, time to metamorphosis and mass at metamorphosis were recorded.

As expected based on the results of Brogan and Relyea (2013a,b; 2014), mesocosm pH values increased with increasing macrophyte treatments. *Elodea*-treated mesocosms had pH value at least 1.17 units higher than mesocosms without *Elodea*. The single pulse exposure resulted in reduced cladoceran density in mesocosms without *Elodea*, but populations recovered within 21 days. The repeated pulse exposures in mesocosms without *Elodea* reduced cladoceran densities by 97% relative to controls, resulting in phytoplankton blooms and reduced abundance of ramhorn snails. However, no adverse effects of malathion treatment were observed on cladocerans or any other taxa for mesocosms that contained *Elodea*.

Malathion exposures of 18 to 36 µg/L were not associated with any effects on gray treefrog survival, time to metamorphosis and mass at metamorphosis. However, the 50 and 100 macrophyte treatments increased time to metamorphosis by approximately five days and reduced mass at metamorphosis by 25% relative to the 0 and 10 macrophyte treatments. *Elodea* treatment also had an effect on pond snail and ramhorn snail abundance, which was likely a result of observed reductions in periphyton with increasing macrophyte density. Thus, the direct effects of the single and repeated pulse exposures of malathion to cladoceran populations and indirect effects to phytoplankton abundance were successfully mitigated by all macrophyte treatments.

Summary

Brogan and Relyea (2013a,b; 2014; 2015) demonstrated that malathion toxicity to cladocerans is substantially reduced in the presence of macrophytes. This reduction in toxicity was confirmed to be due to the photosynthetic activity of macrophytes, which increases the pH of the test system, leading to increased degradation of malathion. Furthermore, the initial findings of the microcosm studies (Brogan and Relyea, 2013a,b; 2014) were supported by a recent outdoor

mesocosm study (Brogan and Relyea, 2015). Wildlife do not live in the inert environments used in standard toxicity tests, and factors such as pH, temperature, presence of organic matter and shading of water bodies, can influence the degree to which organisms are exposed to and affected by pesticides.

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Appendix H
Comparison of Acute Oral Toxicity of Organophosphates to Avian and Herptile Species

In the absence of toxicity data for terrestrial-phase amphibians and reptiles (collectively called herptiles), it is standard practice to use endpoints for avian species as a surrogate (EPA, 2008). This extrapolation has little, if any, empirical support. That is, the extrapolation is made out of necessity due to a paucity of herptile toxicity data, not because of scientific justification. Birds and herptiles belong to different taxonomic classes, and therefore have different metabolic rates, diets, respiratory and reproductive systems and ecology in general. As summarized by Fryday and Thompson (2009, 2012), some acute oral toxicity data for herptiles have been reported for organophosphate pesticides in sources that can be used to draw qualitative conclusions about the relative sensitivity of herptile receptors in comparison to birds[3]. Based on these data (summarized in Table H-1 and Table H-2), this appendix provides a qualitative discussion of the bias that may result from using avian surrogate toxicity data to represent potential effects to herptiles.

Overall, reported avian oral LD50s for organophosphate pesticides ranged from 0.0001 to 0.297 times that of the most sensitive reported terrestrial-phase amphibian and from 0.01 to 10.7 times that of the most sensitive reported reptile exposed to the same pesticide (Table H-1 and Table H-2, respectively). As birds were always more sensitive to organophosphates than terrestrial-phase amphibians, this suggests that their use as surrogate species for predicting effects to terrestrial-phase amphibians would likely overestimate risk. For reptiles, it should be noted that the most sensitive bird for a given pesticide (which would typically be adopted as the surrogate species in a risk assessment) was more sensitive than the most sensitive reptile for the corresponding pesticide in all cases except for phosphamidon (Table H-2). This finding also suggests that the use of surrogate avian receptor toxicity data are likely to result in an overestimate of risk to reptiles.

CONCLUSIONS

Based on the available data, it appears that terrestrial-phase amphibians may be considerably less sensitive with respect to acute oral toxicity to organophosphate pesticides than typical avian receptors. Similarly, the available data suggest that the sensitive bird species that are most likely to be used as surrogate species are more sensitive to organophosphate pesticides than reptiles. Therefore, use of avian toxicity data in ecological risk assessment is likely a conservative approach to assess risk to herptiles. This conclusion is limited by the uncertainty in the data (i.e., some herptile studies do not meet Cheminova's study evaluation criteria) and the small number of herptile species represented in the comparisons.

Further research on the acute oral toxicity of organophosphate pesticides to terrestrial-phase herptiles would clearly be valuable to help minimize uncertainty in future organophosphate pesticide risk assessments. To overcome these issues, Cheminova has conducted an acute oral toxicity study with malathion for a terrestrial-phase amphibian (the bullfrog, *Lithobates catesbeianus*) to address the existing data gap for the herptile risk assessment. The LD50 of

[3] e.g., Hudson et al. (1984 [MRID 00160000]); a source that is commonly used by EPA to support the registrations of pesticides in the US.

1672 mg a.i./kg bw for female bullfrogs from this study (single dose, 14-day observation period) by Fort (2015 [MRID 49693705]) falls within the range of LD50s presented in Table H-1 for amphibians. It is also lower than the LD50 reported by Hall and Clark (1982) for the reptile *Anolis carolinensis* exposed to malathion (Table H-2).

Table H-1 Comparison of amphibian and avian oral LD50s for organophosphate pesticides

Pesticide	*Avian Species LD50 (mg/kg bw)*	*Amphibian Species LD50 (mg/kg bw)*	*Range of Species Sensitivity Ratios*[a]	*Data Sources*
Chlorpyrifos	Pheasant (8.41 - 17.7)[i], Japanese quail (15.9 -17.8)[i], House sparrow (21)[i], Sandhill crane (25 - 50)[i], Rock dove (26.9)[i], Canada goose (40 - 80)[i], Chukar (60.7 -61.1)[i], California quail (68.3)[i], Mallard (75.6)[i], Mallard duckling (112)[i]	Bullfrog, *Rana catesbeiana* (>400)[i]	<0.02 - <0.28	[i] Hudson, et al., 1984 [MRID 00160000]
Demeton (Systox)	House finch (2.38)[i], Sharp-tailed grouse (4.76)[i], Mallard (7.19)[i], Pheasant (8.21)[i], Japanese quail (8.48)[i], Rock dove (8.48)[i], House sparrow (9.52)[i], California quail (10.6)[i], Chukar (15.1)[i]	Bullfrog, *Rana catesbeiana* (562)[i]	0.004 - 0.03	[i] Hudson, et al., 1984 [MRID 00160000]
Diazinon	Mallard (3.54)[i], Pheasant (4.33)[i]	Bullfrog, *Rana catesbeiana* (>2000)[i]	<0.0018 - <0.0022	[i] Hudson, et al., 1984 [MRID 00160000]
Dicrotophos (Bidrin)	California quail (1.89)[i], Rock dove (2)[i], Canada goose (2.28)[i], Sharp-tailed grouse (2.31)[i], House finch (2.83)[i], House sparrow (3)[i], Pheasant (3.21)[i], Mallard (4.24)[i], Japanese quail (4.32)[i], Chukar (9.63)[i]	Bullfrog, *Rana catesbeiana* (2000)[i]	0.001 - 0.005	[i] Hudson, et al., 1984 [MRID 00160000]
Dimethoate	Red-winged blackbird (6.6)[ii], Bobwhite quail (10.5)[iii], Ring-necked pheasant (14.1)[iii], Pheasant (20)[i], Blackbird (26)[iv], Mallard (41.7 - 63.5)[i], European starling (32)[ii], Domestic chicken (55)[v], Canary (147)[vi]	Indian/Asian Common toad, *Bufo melanostictus* (900-1900 at 24 h)[vii]	0.009 - 0.163	[i] Hudson et al., 1984 [MRID 00160000] [ii] Schafer, 1972 [MRID 00020560] [iii] Zok, 2001 [MRID 47769702] [iv] Worthing, 1979 [v] Redgrave et al., 1991 [MRID 42884401] [vi] Hubbard et al., 2013 [MRID 49096101] [vii] Pradhan and Dasgupta, 1992

Table H-1 Comparison of amphibian and avian oral LD50s for organophosphate pesticides

Pesticide	*Avian Species LD50 (mg/kg bw)*	*Amphibian Species LD50 (mg/kg bw)*	*Range of Species Sensitivity Ratios[a]*	*Data Sources*
Parathion	Fulvous tree duck (0.125 - 0.25)[i], Mallard duck (1.9 - 28.3)[ii], Pigeon (2.52)[i], House sparrow (3.36)[i], Sharp-tailed grouse (5.66)[i],Coturnix (5.86 - 5.95)[ii], Northern bobwhite (6 - 13)[ii], Ringed turtle-dove (12)[ii], Pheasant (12.4)[i], Gray partridge (16)[i], Chukar (24)[i]	Eurasian or Green toad, *Bufo viridis* (967)[iii]	0.0001 - 0.025	[i] Hudson et al., 1984 [MRID 00160000] [ii] See EPA Ecotox database for full references [iii] Edery and Schatzberg-Porath, 1960
Phorate (Thimat)	Mallard (0.616 - 2.55)[i], Pheasant (7.12)[i], Chukar (12.8)[i]	Bullfrog, *Rana catesbeiana* (85.2)[i]	0.007 - 0.150	[i] Hudson et al., 1984 [MRID 00160000]
Temephos (Abate)	California quail (18.9)[i], Pheasant (35.4)[i], House sparrow (35.4)[i], Rock dove (50.1)[i], Mallard (79.4)[i], Japanese quail (84.1)[i], Chukar (240)[i]	Bullfrog, *Rana catesbeiana* (>2000)[i]	<0.009 - <0.120	[i] Hudson et al., 1984 [MRID 00160000]
Tetraethyl pyrophosphate (TEPP)	Mallard (3.56)[i], Pheasant (4.22)[i], Chukar (10.1)[i],	Marsh frog, *Rana ridibunda* (34)[ii]; Bullfrog, *Rana catesbeiana* (112)[i]; Eurasian or Green toad, *Bufo viridis* (540)[ii]	0.105 – 0.297	[i] Hudson et al., 1984 [MRID 00160000] [ii] Edery and Schatzberg-Porath, 1960

[a] Species sensitivity ratios calculated by dividing the avian LD50s by the lowest available amphibian LD50. A ratio < 1 indicates that the avian receptor is more sensitive than the amphibian receptor

Table H-2 Comparison of reptile and avian oral LD50s for organophosphate pesticides

Pesticide	*Avian Species LD50 (mg/kg bw)*	*Reptile Species LD50 (mg/kg bw)*	*Range of Species Sensitivity Ratios[a]*	*Data Sources*
Azinphos-methyl	Northern bobwhite (32 – 33)[i], Ring-necked pheasant (74.9 – 283)[i], Chukar (84.2)[i], Mallard duck (136 – 540)[i], Dabbling duck (980)[i],	Green anole, *Anolis carolinensis* (98) [ii]	0.32 - 10	[i] See EPA Ecotox database for full references [ii] Hall and Clark, 1982
Malathion	Ring-necked pheasant (167)[i], Horned lark (403)[i], Domestic chicken (524.8)[i], Mallard duck (1485)[i]	Green anole, *Anolis carolinensis* (2324)[ii]	0.07 – 0.63	[i] See EPA Ecotox database for full references [ii] Hall and Clark, 1982
Methyl-parathion	Mallard duck (6.6 - 138)[i], Northern bobwhite (7.56 - 112.5)[i], Ring-necked pheasant (8.21)[i],	Green anole, *Anolis carolinensis* (82.7) [ii]	0.08 – 3.02	[i] See EPA Ecotox database for full references [ii] Hall and Clark, 1982

Table H-2 Comparison of reptile and avian oral LD50s for organophosphate pesticides				
Pesticide	***Avian Species LD50 (mg/kg bw)***	***Reptile Species LD50 (mg/kg bw)***	***Range of Species Sensitivity Ratios[a]***	***Data Sources***
	Red-winged blackbird (23.7)[i], Japanese quail (57)[i], Dabbling duck (250)[i]			
Parathion	Fulvous tree duck (0.125 - 0.25)[i], Mallard duck (1.9 - 28.3)[ii], Pigeon (2.52)[i], House sparrow (3.36)[i], Sharp-tailed grouse (5.66)[i], Coturnix (5.86 - 5.95)[ii], Northern bobwhite (6 - 13)[ii], Ringed turtle-dove (12)[ii], Pheasant (12.4)[i], Gray partridge (16)[i], Chukar (24)[i]	Green anole, *Anolis carolinensis* (8.9)[iii]; Caspian terrapin, *Mauremys caspica* (15)i[v]	0.01 – 2.7	[i] Hudson et al., 1984 [MRID 00160000] [ii] See EPA Ecotox database for full references [iii] Hall and Clark, 1982 [iv] Yawetz et al., 1983
Phosphamidon	White-winged dove (2.93)[i], Rock dove (3.66)[i], Mallard duck (3.81 - 26)[i], Ring-necked pheasant (4.24)[i], Chukar (11.8)[i], Sharp-tailed grouse (< 3)[i], Japanese quail (< 3.6)[i]	Oriental garden lizard, *Calotes versicolor* (1.1)[ii]	2.7 – 10.7	[i] See EPA Ecotox database for full references [ii] Meenakshi and Karpagaganapathi (1996)
Trichlorfon	Northern bobwhite (22.4 - < 106)[i], Ringed turtle-dove (32)[i], Mallard duck (36.8)[i], Red-winged blackbird (40)[i], European starling (47)[i], California quail (59.3)[i], Ring-necked pheasant (95.9)[i], Rock dove (123)[i], Domestic chicken (125)[i]	Gallot's lizard, *Gallotia gallotia* (>100)[ii, iii]	<0.224 - <1.25	[i] See EPA Ecotox database for full references [ii] Fossi et al. (1995) [iii] Sanchez-Hernandez and Walker (2000)

[a] Species sensitivity ratios calculated by dividing the avian LD50s by the lowest available reptile LD50. A ratio < 1 indicates that the avian receptor is more sensitive than the reptile receptor

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Appendix I
Calculation of avian dietary doses using Beavers et al. (1995 [MRID 43501501])

Birds

Beavers et al. (1995 [MRID 43501501]) conducted a one-generation reproduction dietary toxicity study with Fyfanon Technical (96.4% a.i.). The GLP study was conducted with 18-week-old northern bobwhite (*Colinus virginianus*). Treatment groups consisted of 16 pairs of birds approaching their first breeding season. Birds were housed in pens containing one male and one female bird each. The nominal treatment levels were 110, 350, or 1200 mg a.i./kg-diet ww (reported as ppm a.i.). A control group of equivalent size was maintained concurrently. Dietary concentrations were corrected for the purity of the test substance. Birds were exposed to the test diet for 21 weeks. Pre-test stability analyses showed that Day zero and Day seven feed concentration ranged from 95 to 97% and 83 to 96%, respectively, for nominal concentrations ranging from 50 to 1,200 mg a.i./kg. Diet samples collected during the test to confirm test substance concentrations for the 110, 350 and 1200 mg a.i./kg diets had means and standard deviations of 112 ± 2.66, 358 ± 25.8 and 1260 ± 43.1 mg a.i./kg.

Adult birds were observed daily during the test for signs of toxicity. Body weight was measured at test initiation and at test termination, as well as on weeks 2, 4, 6 and 8. Body weights were not measured during the egg laying due to possible adverse effects on production. Feed consumption was measured weekly for each pen. Eggs were collected daily. A suit of reproductive parameters were recorded by pen:

- Eggs laid
- Eggs cracked
- Eggs set
- Infertile or clear eggs
- Dead embryos (to Day 11)
- Viable embryos
- Dead embryos (to Day 21)
- Live three-week embryos
- Unhatched eggs
- Hatchlings
- Body weight of hatchlings
- 14-day old survivors
- Body weight of 14-day old survivors
- Egg shell thickness

The authors reported that no treatment related mortalities occurred in the control group or in the 110 and 350 mg a.i./kg nominal treatment groups. The overall NOEL and LOEL for all assessed effects were the nominal concentrations of 110 and 350 mg a.i./kg diet, respectively. See Beavers et al. (1995 [MRID 43501501]) for more details about the study.

Estimated test substance intake, or average daily dose, for the northern bobwhites in the NOEL and LOEL treatment groups were calculated using the formula in Equation BB-1.

$$DDD = \frac{DC\ x\ DFC}{BW}$$ **Equation BB-1**

where,

DDD is the daily dietary dose (mg a.i./kg bw/d)
DC is the measured dietary concentration tested (mg a.i./kg diet)
DFC is the daily feed consumption (g/bird/d)
BW is the mean body weight (g/bird)

Body weight and feed consumption values were averaged over the 21-week exposure period. The accuracy of the estimated mean daily dietary dosage may have been impacted by differences in individual feed consumption both within and between pens, and feed wastage, as well as variability in dietary concentration. The estimated daily dietary dose for the NOEL and LOEL treatment groups are 12.6 and 42.8 mg a.i./kg bw/d, respectively (Table I-1 and Table I-2).

Table I-1 Chronic avian malathion NOEL daily dose estimation[a]

Pen No.	Male Birds			Female Birds			Average Pen Body Weight (g)	Mean Food Intake (g/bird/day)	Mean Dose Per Pen (mg a.i./kg bw/d)
	Day 0 Body Weight (g)	Term Body Weight (g)	Mean Weight (g)[b]	Day 0 Body Weight (g)	Term Body Weight (g)	Mean Weight (g)[b]			
217	192	222	207	196	233	215	211	25	13.4
218	200	241	221	199	263	231	226	30	14.7
219	174	mortality	mortality	196	mortality	mortality	mortality	19	mortality
220	192	217	205	206	264	235	220	25	12.5
221	186	200	193	200	251	226	209	22	11.6
222	213	234	224	181	230	206	215	19	10.1
223	190	222	206	176	227	202	204	22	11.8
224	188	246	217	205	290	248	232	21	10.3
225	203	231	217	175	228	202	209	26	13.7
226	188	194	191	197	249	223	207	25	13.5
227	203	233	218	193	237	215	217	24	12.2
228	185	200	193	198	241	220	206	21	11.4
229	203	251	227	207	285	246	237	29	14.0
230	186	196	191	185	228	207	199	25	14.2
231	203	235	219	205	248	227	223	23	11.5
232	177	189	183	205	271	238	211	27	14.4
Overall Average Daily Dose (mg a.i./kg bw/d)									12.6

[a] Data from the nominal 110 mg a.i./kg diet treatment group in Beavers et al. (1995 [MRID 43501501]). The mean measured dietary concentration of 112 mg a.i./kg diet was used in these calculations.

[b] Day 0 and Term bodyweights were used in these calculations. Other body weight measurements collected in the first 8 weeks of exposure were not included to avoid bias results, as body weights were not measured during the egg laying period.

Table I-2 Chronic avian malathion LOEL daily dose estimation[a]

Pen No.	Male Birds			Female Birds			Average Pen Body Weight (g)	Mean Food Intake (g/bird/day)	Mean Dose Per Pen (mg a.i./kg bw/d)
	Day 0 Body Weight (g)	Term Body Weight (g)	Mean Weight (g)[b]	Day 0 Body Weight (g)	Term Body Weight (g)	Mean Weight (g)[b]			
233	190	229	209.5	200	263	231.5	221	27	44.1
234	203	mortality	mortality	211	mortality	mortality	mortality	19	mortality
235	197	229	213	209	215	212	213	27	45.6
236	189	197	193	186	223	204.5	199	18	33.2
237	195	208	201.5	183	223	203	202	23	40.4
238	211	268	239.5	176	176	176	208	26	44.39
239	195	197	196	196	223	209.5	203	29	51.8
240	176	218	197	181	210	195.5	196	17	30.8
241	176	192	184	212	249	230.5	207	31	53.9
242	193	255	224	191	229	210	217	24	38.9
243	199	214	206.5	184	234	209	208	28	47.5
244	177	183	180	195	252	223.5	202	21	36.9
245	203	223	213	189	196	192.5	203	27	48.3
246	187	234	210.5	195	249	222	216	23	38.4
247	186	204	195	196	210	203	199	25	45.7
248	198	231	214.5	202	273	237.5	226	26	41.9
Overall Average Daily Dose (mg a.i./kg bw/d)									42.8

[b] Data from the nominal 350 mg a.i./kg diet treatment group in Beavers et al. (1995 [MRID 43501501]). The mean measured dietary concentration of 358 mg a.i./kg diet was used in these calculations.

[c] Day 0 and Term bodyweights were used in these calculations. Other body weight measurements collected in the first 8 weeks of exposure were not included to avoid bias results, as body weights were not measured during the egg laying period.

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Appendix J
Acute to Chronic Ratio (ACR) Approach Applied to Calculate Chronic (Sublethal) Threshold Values for Herptiles

Although a supplemental chronic toxicity study was available for terrestrial herptiles exposed to malathion (Holem et al., 2008), this study used an unconventional exposure regime whereby Western fence lizards were administered three oral gavage doses of technical malathion with a 27-day observation period following each dose. Thus, this study was inappropriate for estimating a chronic effects metric (Breton et al., 2015 [MRID 49692301]).

An acute-to-chronic ratio (ACR) was derived using the best available data from other terrestrial vertebrates. A literature search was conducted to acquire data to support an ACR for herptiles, with consideration of other chemicals. However, no studies reporting acute and chronic effects for terrestrial-phase herptiles were found.

It is common practice to estimate a chronic endpoint from acute data when chronic data are lacking. The relationship between acute LD50s and chronic NOELs have been explored for terrestrial vertebrates, mainly birds and mammals (Kenaga, 1981; Layton et al., 1987; Elmegaard and Jagers op Akkerhuis, 2000; also discussed in Suter (2007)).

Layton et al. (1987) compiled paired endpoints for 41 chemicals from acute oral and 2-year toxicity tests with rats. The results are presented in Table J-1. The results suggest a range of over three orders of magnitude in LD50/NOEL within the same species, depending on the chemical, but in most cases the ACR estimates are less than 100, and the vast majority are less than 1000. Mammalian ACRs for organophosphates ranged from 5.2 for methylazinphos to 364.4 for Dowco 109.

Table J-1 ACRs for rats based on data provided in Layton et al. (1987)

Pesticide	*NOEL (mg/kg bw/d)*	*LD50 (mg/kg bw)*	*Rat NOEL/LD50 Min*	*ACR*
DDT	15	113	0.1327	7.5
Propylene glycol	3675	27000	0.1361	7.3
Thiourea	188	400	0.4700	2.1
Carbaryl	7	510	0.0137	72.9
Chlortetracycline HCl	750	3000	0.2500	4.0
Methoxychlor	120	5500	0.0218	45.8
DDD	90	3600	0.0250	40.0
Acrylamide	3	170	0.0176	56.7
Ovex	1.9	2000	0.0010	1052.6
Methyl methacrylate	8.4	8410	0.0010	1001.2
Methylazinphos[a]	2.5	13	0.1923	5.2
α-naphthylthiourea	7.5	100	0.0750	13.3
Pentachlorophenol	3	142	0.0211	47.3
1-phenylphenol	100	2700	0.0370	27.0
Biphenyl	50	3280	0.0152	65.6
Chlorpropamid	125	2000	0.0625	16.0
Chlorpropham	1500	6251	0.2400	4.2
Bis(2-ethylhexyl)phthalate	97.5	31000	0.0031	317.9
Methyl salicylate	50	887	0.0564	17.7
p-dioxane	14	5170	0.0027	369.3
Tergitol 08	290	5760	0.0503	19.9
Sodium 2,2-dichloropropionate	9.75	7744	0.0013	794.3
BHT	400	1700	0.2353	4.3
Sesone	15	11070	0.0014	738.0
Thiabendazole	50	3100	0.0161	62.0

Table J-1 ACRs for rats based on data provided in Layton et al. (1987)				
Pesticide	***NOEL (mg/kg bw/d)***	***LD50 (mg/kg bw)***	***Rat NOEL/LD50 Min***	***ACR***
Telodrin	0.38	8	0.0475	21.1
Ronnel[a]	5	1740	0.0029	348.0
Zytron[a]	0.75	270	0.0028	360.0
Crufomate[a]	2.25	750	0.0030	333.3
Bromacil	12.5	5200	0.0024	416.0
Dehydroacetic acid	75	1000	0.0750	13.3
Bis(4-chlorophenoxy)methane	22.5	5800	0.0039	257.8
Propanil	20	1384	0.0145	69.2
2,3,6,8-TCDD	0.000001	0.022	0.0000	22000.0
Tetrasul	0.5	3500	0.0001	7000.0
2,4,5-TES	15	805	0.0186	53.7
Trisodium nitrilotriacetate	22	2000	0.0110	90.9
Dowco 109[a]	2.25	820	0.0027	364.4
Benzalkonium chloride	94	400	0.2350	4.3
Butoxypolypropylene glycol	480	9100	0.0527	19.0
Pimaricin	25	2730	0.0092	109.2

[a] organophosphate chemicals

Using acceptable toxicity studies, ACRs for malathion were developed for three vertebrate species: northern bobwhite (*Colinus virginianus*), mallard (*Anas platyrhynchos*), and rat (*Rattus norvegicus*). The ACRs were developed using acute LD50s and chronic NOELs (in mg a.i./kg bw/d; Rodgers, 2002 [MRID 48153114]; Beavers et al., 1995 [MRID 43501501]; Hubbard and Beavers, 2012 [MRID 48963307]; Pedersen and Fletcher, 1993 [MRID 42782101]; Moore, 2003 [MRID 48153112]; Schroeder, 1990 [MRID 41583401]). For bobwhite, mallard and rat, the calculated ACRs are 27.4, >20.6 and 5.10, respectively (see Table J-2). These ACRs fall within the range of ACRs estimated by Layton et al. (1987) for mammals, which suggests only that these estimates are not wildly different from ACRs developed for other chemicals. In addition, the rat ACR for malathion is similar to that derived for methylazinphos.

To derive an effect metric for indirect effects to terrestrial-phase herptiles, ACRs were calculated using acute LD50s and chronic LOELs from the same studies listed above. For bobwhite, mallard and rat, the LOEL-based ACRs are 8.06, >10.2, and 3.28 (see Table J-2).

Table J-2 Derivation of terrestrial vertebrate ACRs for malathion with acceptable and supplemental toxicity data									
Common Name	***Scientific Name***	***LD50 (mg a.i./kg bw)***	***Chronic Study Type***	***Chronic Measure of Effects***	***Endpoint***	***Value (mg a.i./kg bw/d)***	***ACR (LD50/NOEL or LOEL)***	***Acute Reference (Cheminova Study Rating)***	***Chronic Reference (Cheminova Study Rating)***
Mallard	*Anas platyrhynchos*	>2250	Reproduction (20 weeks)	Growth (male parental body weight) and reproduction (egg fertility, viable embryos, eggshell thickness)	NOEL	109	**>20.6**	Hubbard and Beavers, 2012 [MRID 48963307] (Acceptable)	Pedersen and Fletcher, 1993 [MRID 42782101] (Supplemental)
					LOEL	221	**>10.2**		
Northern bobwhite	*Colinus virginianus*	345	Reproduction (21 weeks)	Reproduction (regressed ovaries, reduced egg hatch))	NOEL	12.6	**27.4**	Rodgers, 2002 [MRID 48153114] (Acceptable)	Beavers et al., 1995 [MRID 43501501] (Acceptable)
					LOEL	42.8	**8.06**		
Rat	*Rattus norvegicus*	**2010**	Reproduction (2-gen)	Growth (parental body weight)	NOEL	**394**	**5.10**	Moore, 2003 [MRID 48153112] (Acceptable)	Schroeder, 1990 [MRID 41583401] (Supplemental)
					LOEL	**612**	**3.28**		

The most conservative NOEL and LOEL-based ACRs of 27.4 and >10.2 were selected for herptiles. When the ACRs are applied to the author-reported LD50 of 1672 mg a.i./kg bw for female bullfrogs (*Lithobates catesbeianus*; Fort, 2015), the estimated chronic NOEL is 61.0 mg a.i./kg bw/d and the chronic LOEL is 164 mg a.i./kg bw/d. These value should be used to assess chronic risk to herptiles in the effects determinationt. Notably, the ACR-derived NOEL is more conservative than the survival and body weight NOEL of ≥96.5 mg a.i./kg bw obtained from Holem et al. (2008).

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Appendix K
Determination of Risk Designations for Aquatic Species

This appendix contains tables detailing how EPA designated risk categories (i.e., LOW, MED, HIGH, unknown or NA) for mortality, growth reproduction, behavior, sensory, and indirect effects to listed aquatic species. The information presented herein was extracted from the WoE tools provided on the provisional models webpage (https://www.epa.gov/endangered-species/provisional-models-endangered-species-pesticide-assessments#woe). Risk designations were ultimately used to determine the species and critical habitat calls (i.e., Effects Determination) for listed species (see Section 5.0 of this Document for further details).

The tables included in this appendix pertain to the species calls for listed aquatic animals and plants. Risk designations for (1) indirect effects due to listed aquatic animals with obligate relationships (i.e., potential effects to the species on which the obligate is dependent), and (2) chemical and abiotic stressors were not detailed herein due to: (a) the fact that the risk designations for these factors were not employed in the determination of the species call and critical habitat call in the “Summary Sheet” worksheets of both the Species_Aquatic_Animal_Template.xlsx and Species_Aquatic_Plant_Template.xlsx files in the WoE Tools, and (b) a paucity of time and resources to address the Agency’s Biological Evaluation. These risk designations warrant further scrutiny if the Agency intends on using them in future effects determinations.

Table K-1 Summary of mortality risk designation calculations for aquatic species								
Exposure	***Threshold***	***Fish and Aquatic-phase amphibians***	***Aquatic invertebrates***	***Aquatic Plants***	***Requirement for Risk Designation[a]***		***Cell ref.[b]***	***Description/ Comment***
					MED	***LOW***		
Drift and runoff: overall min peak EECs by bin (µg/L)	Mortality threshold (1/million) (µg/L)	3.97 (FW fish) 3.76 (EM fish) 15.7 (amphibians)	0.046	10,000 (non-vascular) 100,000 (vascular)	Cannot exceed	Cannot exceed	O100:O101; O349:O350	Counts scenarios where maximum and minimum EECs exceeded threshold
	Lowest LC50 (µg/L)	4.1 (FW fish) 33 (EM fish) 200 (amphibians)	0.06 (FW invertebrates) 0.4 (EM invertebrates) 76 (mollusks)	NA	Cannot exceed	Cannot exceed	Q100:Q101; Q349:Q350	
	HC5 (µg/L)	45.2 (FW fish) 42.8 (EM fish) 178 (amphibians)	0.39 (FW invertebrates) 1.7 (EM invertebrates) NA (mollusks)	NA	Cannot exceed	Cannot exceed	R100:R101; R349:R350	
	HC50 (µg/L)	934 (FW fish) 228 (EM fish) 1484 (amphibians)	144 (FW invertebrates) 39 (EM invertebrates) NA (mollusks)	NA	Cannot exceed	Cannot exceed	S100:S101; S349:S350	
Drift and runoff: overall max peak EECs by bin (µg/L)	Mortality threshold (1/million) (µg/L)	3.97 (FW fish) 3.76 (EM fish) 15.7 (amphibians)	0.046	10,000 (non-vascular) 100,000 (vascular)	ND	Cannot exceed	O94, O343	Determines whether maximum EEC exceeded threshold
	Lowest LC50 (µg/L)	4.1 (FW fish) 33 (EM fish) 200 (amphibians)	0.06 (FW invertebrates) 0.4 (EM invertebrates) 76 (mollusks)	NA	ND	Cannot exceed	Q94, Q343	
	HC5 (µg/L)	45.2 (FW fish) 42.8 (EM fish) 178 (amphibians)	0.39 (FW invertebrates) 1.7 (EM invertebrates) NA (mollusks)	NA	ND	Cannot exceed	R94, R343	
	HC50 (µg/L)	934 (FW fish) 228 (EM fish) 1484 (amphibians)	144 (FW invertebrates) 39 (EM	NA	ND	Cannot exceed	R94, R343	

Table K-1 Summary of mortality risk designation calculations for aquatic species								
Exposure	***Threshold***	***Fish and Aquatic-phase***	***Aquatic invertebrates***	***Aquatic Plants***	***Requirement for Risk Designation[a]***		***Cell ref.[b]***	***Description/ Comment***
			invertebrates) NA (mollusks)					

NA: not applicable
ND: not dependant (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceed by the exposure estimate)
[a] Shows requirement for the threshold-exposure comparison to achieve specified designation, if MED requirements are not met, the risk designation is HIGH.
[b] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Aquatic_Animal_Template.xlsx and Species_Aquatic_Plant_Template.xls files.

Table K-2 Summary of sublethal risk designation calculations for aquatic species

Exposure	*Threshold*	*Fish and Aquatic-phase amphibians*	*Aquatic invertebrates*	*Aquatic Plants*	*Requirement for Risk Designation[a]*		*Cell ref.[b]*	*Description/ Comment*
					MED	*LOW*		
Drift and runoff: overall min peak EECs by bin (µg/L)	Growth endpoint (µg/L)	8.6	0.1 (FW invertebrates) 0.29 (EM invertebrates and mollusks)	500 (non-vascular) 24,000 (vascular)	Cannot exceed	Cannot exceed	W100:W101; W349:W350	Counts scenarios where maximum and minimum EECs exceeded threshold
	Growth endpoint - LOAEC (µg/L)	10.9	0.25 (FW invertebrates) 0.58 (EM invertebrates and mollusks)	1200 (non-vascular) 47,000 (vascular)	Cannot exceed	Cannot exceed	X100:X101; X349:X350	
	Reproduction endpoint (µg/L)	220	0.06 (FW invertebrates) 0.58 (EM invertebrates and mollusks)	NA	Cannot exceed	Cannot exceed	Y100:Y101; Z349:Z350	
	Reproduction endpoint - LOAEC (µg/L)	690	0.1 (FW invertebrates) 1.2 (EM invertebrates and mollusks)	NA	Cannot exceed	Cannot exceed	Z100:Z101; Z349:Z350	
	Behavior endpoint (µg/L)	NA	0.048 (FW invertebrates) 0.5 (EM invertebrates and mollusks)	NA	Cannot exceed	Cannot exceed	AA100:AA101; AA349:AA350	
	Behavior endpoint - LOAEC (µg/L)	20	0.097 (FW invertebrates) 5.6 (EM invertebrates and mollusks)	NA	Cannot exceed	Cannot exceed	AB100:AB101; AB349:AB350	
	Sensory endpoint (µg/L)	NA	NA	NA	Cannot exceed	Cannot exceed	AC100:AC101; AC349:AC350	
	Sensory endpoint - LOAEC (µg/L)	NA	NA	NA	Cannot exceed	Cannot exceed	AD100:AD101; AD349:AD350	

Table K-2 Summary of sublethal risk designation calculations for aquatic species

Exposure	*Threshold*	*Fish and Aquatic-*	*Aquatic invertebrates*	*Aquatic Plants*	*Requirement for Risk Designation*[a]		*Cell ref.*[b]	*Description/ Comment*
Drift and runoff: overall max peak EECs by bin (µg/L)	Growth endpoint (µg/L)	8.6	0.1 (FW invertebrates) 0.29 (EM invertebrates and mollusks)	500 (non-vascular) 24,000 (vascular)	ND	Cannot exceed	W94, W343	Determines whether maximum EEC exceeded threshold
	Growth endpoint - LOAEC (µg/L)	10.9	0.25 (FW invertebrates) 0.58 (EM invertebrates and mollusks)	1200 (non-vascular) 47,000 (vascular)	ND	Cannot exceed	X94, X343	
	Reproduction endpoint (µg/L)	220	0.06 (FW invertebrates) 0.58 (EM invertebrates and mollusks)	NA	ND	Cannot exceed	Y94, Y343	
	Reproduction endpoint - LOAEC (µg/L)	690	0.1 (FW invertebrates) 1.2 (EM invertebrates and mollusks)	NA	ND	Cannot exceed	Z94, Z343	
	Behavior endpoint (µg/L)	NA	0.048 (FW invertebrates) 0.5 (EM invertebrates and mollusks)	NA	ND	Cannot exceed	AA94, AA343	
	Behavior endpoint - LOAEC (µg/L)	20	0.097 (FW invertebrates) 5.6 (EM invertebrates and mollusks)	NA	ND	Cannot exceed	AB94, AB343	
	Sensory endpoint (µg/L)	NA	NA	NA	ND	Cannot exceed	AC94, AC343	
	Sensory endpoint - LOAEC (µg/L)	NA	NA	NA	ND	Cannot exceed	AD94, AD343	

NA: not applicable

ND: not dependant (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceeded by the exposure estimate)

[a] Shows requirement for the threshold-exposure comparison to achieve specified designation, if MED requirements are not met, the risk designation is HIGH.

[b] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Aquatic_Animal_Template.xls and Species_Aquatic_Plant_Template.xls files.

Table K-3 Summary of risk designation calculations for aquatic species from indirect effects to prey items						
Exposure	***Prey item***	***Threshold***	***Value (µg/L)***	***Requirement for Risk Designation[a]*** ***LOW***	***Cell ref.[b]***	***Description/ Comment***
Drift and runoff: overall min and max peak EECs by bin (µg/L)	Aquatic Plants - non-vascular	Mortality threshold - Indirect	10,000	Cannot exceed	AG40:AG45, AG47:AG52	Calculates RQs for prey items consumed by the species in question.
		Sublethal threshold - Indirect	1200	Cannot exceed	AM40:AM45, AM47:AM52	
	Aquatic plants - vascular	Mortality threshold - Indirect	100,000	Cannot exceed	AH40:AH45, AH47:AH52	
		Sublethal threshold - Indirect	1200	Cannot exceed	AN40:AN45, AN47:AN52	
	Freshwater invertebrates	Mortality threshold - Indirect	0.22	Cannot exceed	AI40:AI45, AI47:AI52	
		Sublethal threshold - Indirect	0.097	Cannot exceed	AO40:AO45, AO47:AO52	
	Marine invertebrates	Mortality threshold - Indirect	0.22	Cannot exceed	AJ40:AJ45, AJ47:AJ52	
		Sublethal threshold - Indirect	0.097	Cannot exceed	AP40:AP45, AP47:AP52	
	Freshwater fish	Mortality threshold - Indirect	23.5	Cannot exceed	AK40:AK45, AK47:AK52	
		Sublethal threshold - Indirect	9	Cannot exceed	AQ40:AQ45, AQ47:AQ52	
	Marine Fish	Mortality threshold - Indirect	22.2	Cannot exceed	AL40:AL45, AL47:AL52	
		Sublethal threshold - Indirect	9	Cannot exceed	AR40:AR45, AR47:AR52	
Spray drift only: max EECs (µg/L)	Aquatic Plants - non-vascular	Mortality threshold - Indirect	10,000	Distance to threshold = 0 ft	M145:R145[c]	Calculates spray drift distance required for water concentrations to equal threshold concentrations.
		Sublethal threshold - Indirect	1200	Distance to threshold = 0 ft	M147:R147[c]	
	Aquatic plants - vascular	Mortality threshold - Indirect	100,000	Distance to threshold = 0 ft	M137:R137[c]	
		Sublethal threshold - Indirect	1200	Distance to threshold = 0 ft	M139:R139[c]	
	Freshwater invertebrates	Mortality threshold - Indirect	0.22	Distance to threshold = 0 ft	M60:R60[c]	
		Sublethal threshold - Indirect	0.097	Distance to threshold = 0 ft	M66:R66[c]	
	Marine invertebrates	Mortality threshold - Indirect	0.22	Distance to threshold = 0 ft	M77:R77[c]	

Table K-3 Summary of risk designation calculations for aquatic species from indirect effects to prey items						
Exposure	***Prey item***	***Threshold***	***Value (µg/L)***	***Requirement for Risk Designation[a]***	***Cell ref.[b]***	***Description/ Comment***
		Sublethal threshold - Indirect	0.097	Distance to threshold = 0 ft	M83:R83[c]	
	Freshwater fish	Mortality threshold - Indirect	23.5	Distance to threshold = 0 ft	M26:R26[c]	
		Sublethal threshold - Indirect	9	Distance to threshold = 0 ft	M32:R32[c]	
	Marine Fish	Mortality threshold - Indirect	22.2	Distance to threshold = 0 ft	M43:R43[c]	
		Sublethal threshold - Indirect	9	Distance to threshold = 0 ft	M49:R49[c]	
	Aquatic-phase amphibians	Mortality threshold - Indirect	92.6	Distance to threshold = 0 ft	M9:R9[c]	
		Sublethal threshold - Indirect	9	Distance to threshold = 0 ft	M15:R15[c]	

[a] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if LOW requirements are not met, the risk designation is HIGH.
[b] Unless otherwise specified all cell references refer to the 'WoE Matrix' worksheet in the Species_Aquatic_Animal_Template.xls.
[c] Cell references in 'Spray Drift all' worksheet of AquaWoE_v1.0.xls file.

Table K-4 Summary of risk designation calculations for aquatic species from indirect effects to habitat

Exposure	*Prey item*	*Threshold*	*Value (µg/L)*	*Requirement for Risk Designation[a]* *LOW*	*Cell ref.[b]*	*Description/ Comment*
Drift and runoff: overall max peak EECs (µg/L)	Aquatic Plants - vascular	Mortality threshold - Indirect	100,000	Cannot exceed	U107	Calculates RQs for habitat species.
		Sublethal threshold - Indirect	1200	Cannot exceed		
		Growth endpoint - LOAEC	47,000	Cannot exceed	U104	
		Reproduction endpoint - LOAEC	NA	Cannot exceed		
	Aquatic plants - non-vascular	Mortality threshold - Indirect	10,000	Cannot exceed	W107	
		Sublethal threshold - Indirect	1200	Cannot exceed		
		Growth endpoint - LOAEC	1200	Cannot exceed	W104	
		Reproduction endpoint - LOAEC	NA	Cannot exceed		
Spray drift only: max EECs (µg/L)	Aquatic Plants - vascular	Mortality threshold - Indirect	100,000	Distance to threshold = 0 ft	M137:R137[c]	Calculates spray drift distance required for water concentrations to equal threshold concentrations.
		Sublethal threshold - Indirect	1200	Distance to threshold = 0 ft	M139:R139[c]	
	Aquatic plants - non-vascular	Mortality threshold - Indirect	10,000	Distance to threshold = 0 ft	M145:R145[c]	
		Sublethal threshold - Indirect	1200	Distance to threshold = 0 ft	M147:R147[c]	

NA: not applicable

[a] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if LOW requirements are not met, the risk designation is HIGH.

[b] Unless otherwise specified all cell references refer to the 'WoE Matrix' worksheet in the Species_Aquatic_Animal_Template.xls.

[c] Cell references in 'Spray Drift all' worksheet of AquaWoE_v1.0.xls file

Appendix L
Determination of Risk Designations for Terrestrial Species

This appendix contains tables detailing how EPA designated risk categories (i.e., LOW, MED, HIGH, unknown or NA) for mortality, growth reproduction, behavior, sensory, and indirect effects to listed terrestrial species. The information presented herein was extracted from the WoE tools provided on the provisional models webpage (https://www.epa.gov/endangered-species/provisional-models-endangered-species-pesticide-assessments#woe). Risk designations were ultimately used to determine the species and critical habitat calls (i.e., effects determinations) for listed species (see Chapter 5 of this document for further details).

The first 12 tables of this appendix pertain to the species calls for listed terrestrial animals, the remaining four tables apply to listed terrestrial plants. Note that this appendix does not include reference to the quality of the studies or accuracy of the endpoints presented. Some of these studies are discussed in Section 4 of this response document.

Risk designations for (1) indirect effects due to listed terrestrial animals with obligate relationships (i.e., potential effects to the species on which the obligate is dependent), and (2) chemical and abiotic stressors were not detailed herein due to: (a) the fact that the risk designations for these factors were not employed in the determination of the species calls and critical habitat calls in the “Summary Sheet” worksheet of both the Species_Animal_Template.xlsx and Species_Plant_Template.xlsx files in the WoE Tools, and (b) a paucity of time and resources to address the Agency’s Biological Evaluation. These risk designations warrant further scrutiny if the Agency intends on using them in future biological evaluations and effects determinations.

Table L-1 Summary of mortality risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*					*MED*	*LOW*	
1 lb a.i./A once (“minimum single application rate”)	Upper bound, maximum of dietary items	mg/kg diet	Mortality threshold (1/million) (mg/kg bw)	18.4 mg/kg bw based on E35348	20.6 mg/kg bw (no reference provided in TED tool)	20.6 mg/kg bw (no reference provided in TED tool)	ND	Cannot exceed	AO13
1 lb a.i./A once (“minimum single application rate”)	Upper bound, maximum of dietary items	mg/kg diet	Lowest LD50 (mg/kg bw)	209 mg/kg bw based on E35348	136 mg/kg bw (M48966605)	2324 mg/kg bw (E36970)	Cannot exceed	Cannot exceed	If(T8=”NA”, AQ13, AR13)
1 lb a.i./A once (“minimum single application rate”)	Upper bound, maximum of dietary items	mg/kg diet	HC50 (mg/kg bw)	NA	331 mg/kg bw (no reference provided in TED tool)	NA	Cannot exceed	Cannot exceed	
4 lb a.i./A once (“upper bound single application rate”)	Upper bound, maximum of dietary items	mg/kg diet	Mortality threshold (1/million) (mg/kg bw)	18.4 mg/kg bw based on E35348	20.6 mg/kg bw (no reference provided in TED tool)	20.6 mg/kg bw (no reference provided in TED tool)	ND	Cannot exceed	AO11
4 lb a.i./A once (“upper bound single application rate”)	Upper bound, maximum of dietary items	mg/kg diet	Lowest LD50 (mg/kg bw)	209 mg/kg bw based on E35348	136 mg/kg bw (M48966605)	2324 mg/kg bw (E36970)	Cannot exceed	Cannot exceed	If(T8=”NA”, AQ11, AR11)
4 lb a.i./A once (“upper bound single application rate”)	Upper bound, maximum of dietary items	mg/kg diet	HC50 (mg/kg bw)	NA	331 mg/kg bw (no reference provided in TED tool)	NA	Cannot exceed	Cannot exceed	
1 lb a.i./A once (“minimum single application rate”)	Mean, minimum of dietary items	mg/kg diet	Mortality threshold (1/million) (mg/kg bw)	18.4 mg/kg bw based on E35348	20.6 mg/kg bw (no reference provided in TED tool)	20.6 mg/kg bw (no reference provided in TED tool)	ND	Cannot exceed	AT14
1 lb a.i./A once (“minimum single application rate”)	Mean, minimum of dietary items	mg/kg diet	Lowest LD50 (mg/kg bw)	209 mg/kg bw based on E35348	136 mg/kg bw (M48966605)	2324 mg/kg bw (E36970)	Cannot exceed	Cannot exceed	If(T8=”NA”, AV14, AW14)

Table L-1 Summary of mortality risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*					*MED*	*LOW*	
1 lb a.i./A once ("minimum single application rate")	Mean, minimum of dietary items	mg/kg diet	HC50 (mg/kg bw)	NA	331 mg/kg bw (no reference provided in TED tool)	NA	Cannot exceed	Cannot exceed	
1 lb a.i./A once ("minimum single application rate")	Maximum of alternate exposure routes (drinking water, dermal and inhalation)	mg/kg bw	Mortality threshold (1/million) (mg/kg bw)	18.4 mg/kg bw based on E35348	20.6 mg/kg bw (no reference provided in TED tool)	20.6 mg/kg bw (no reference provided in TED tool)	ND	Cannot exceed	AL14
4 lb a.i./A once ("upper bound single application rate")	Maximum of alternate exposure routes (drinking water, dermal and inhalation)	mg/kg bw	Mortality threshold (1/million) (mg/kg bw)	18.4 mg/kg bw based on E35348	20.6 mg/kg bw (no reference provided in TED tool)	20.6 mg/kg bw (no reference provided in TED tool)	ND	Cannot exceed	AL12
1 lb a.i./A once ("minimum single application rate")	NA	lb a.i./A	Application rate-based mortality threshold (lb a.i./A)	NA	NA	NA	ND	Cannot exceed	BV20
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Application rate-based mortality threshold (lb a.i./A)	NA	NA	NA	ND	Cannot exceed	BW20
6 lb a.i./A once ("single maximum application rate")	NA	lb a.i./A	Application rate-based mortality threshold (lb a.i./A)	NA	NA	NA	ND	Cannot exceed	BV20 on 'Alt' worksheet
6 lb a.i./A once ("single maximum application rate")	Upper bound	mg/kg diet	Lowest LD50 (mg/kg bw)	209 mg/kg bw based on E35348	136 mg/kg bw (M48966605)	2324 mg/kg bw (E36970)	Cannot exceed	ND	If(T8="NA", AQ13, AR13) on 'Alt' worksheet
6 lb a.i./A once ("single maximum application rate")	Upper bound	mg/kg diet	HC50 (mg/kg bw)	NA	331 mg/kg bw (no reference provided in TED tool)	NA	Cannot exceed	ND	

Table L-1 Summary of mortality risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*					*MED*	*LOW*	
4 lb a.i./A twice with a 7 d retreatment interval (“multiple application scenario”)	Upper bound	mg/kg diet	Lowest LD50 (mg/kg bw)	209 mg/kg bw based on E35348	136 mg/kg bw (M48966605)	2324 mg/kg bw (E36970)	Cannot exceed	ND	If(T8=”NA”, AQ11, AR11) on ‘Alt’ worksheet
4 lb a.i./A twice with a 7 d retreatment interval (“multiple application scenario”)	Upper bound	mg/kg diet	HC50 (mg/kg bw)	NA	331 mg/kg bw (no reference provided in TED tool)	NA	Cannot exceed	ND	
6 lb a.i./A once (“single maximum application rate”)	Mean	mg/kg diet	Lowest LD50 (mg/kg bw)	209 mg/kg bw based on E35348	136 mg/kg bw (M48966605)	2324 mg/kg bw (E36970)	Cannot exceed	ND	If(T8=”NA”, AV14, AW14) on ‘Alt’ worksheet
6 lb a.i./A once (“single maximum application rate”)	Mean	mg/kg diet	HC50 (mg/kg bw)	NA	331 mg/kg bw (no reference provided in TED tool)	NA	Cannot exceed	ND	

ND = Not dependent (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceed by the exposure estimate)

[a] The risk designation for mortality is determined in cell DI10 of the ‘WoE Matrix’ worksheet in the Species_Animal_Template.xlsx file. This table traces the precedents for this cell.

[b] Presents inputs thresholds without species specific body mass scaling

[c] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH. Formulae resulting in a risk designation of “unknown” or “NA” are not explicitly presented herein.

[d] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Animal_Template.xlsx file.

Table L-2 Summary of mortality risk designation calculations for terrestrial invertebrates[a]

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation[b]*		*Cell ref.[c]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg bw	1/million mortality (mg/kg bw)	0.184 mg/kg bw	E94337 based on Thai honey bee, contact, LD50 = 1.6 mg/kg-bw; slope=5; TGAI	ND	Cannot Exceed	BB20
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg bw	1/million mortality (mg/kg bw)	0.184 mg/kg bw	E94337 based on Thai honey bee, contact, LD50 = 1.6 mg/kg-bw; slope=5; TGAI	ND	Cannot Exceed	BC20
1 lb a.i./A once ("minimum single application rate")	NA	mg/kg soil	1/million mortality (mg/kg soil)	0.66 mg/kg soil	E52962 based Earthworm; 50% EC	ND	Cannot Exceed	BD20
4 lb a.i./A once ("upper bound single application rate")	NA	mg/kg soil	1/million mortality (mg/kg soil)	0.66 mg/kg soil	E52962 based Earthworm; 50% EC	ND	Cannot Exceed	BE20
1 lb a.i./A once ("minimum single application rate")	NA	lb a.i./A	Direct mortality (1/million)	0.0012 lb a.i./A	E89288 based on hemlock sawfly. TGAI, slope 5.6, contact	ND	Cannot Exceed	BF20
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Direct mortality (1/million)	0.0012 lb a.i./A	E89288 based on hemlock sawfly. TGAI, slope 5.6, contact	ND	Cannot Exceed	BG20
6 lb a.i./A once ("maximum single application rate")	Upper bound	mg/kg bw	1/million mortality (mg/kg bw)	0.184 mg/kg bw	E94337 based on Thai honey bee, contact, LD50 = 1.6 mg/kg-bw; slope=5; TGAI	ND	Cannot Exceed	BB20 on Alt worksheet

Table L-2 Summary of mortality risk designation calculations for terrestrial invertebrates[a]								
Exposure			***Threshold***	***Value***	***Reference***	***Requirement for Risk Designation[b]***		***Cell ref.[c]***
Use Pattern	***Dietary Residue Assumption (e.g., mean or upper bound)***	***Units***				***MED***	***LOW***	
4 lb a.i./A twice with a 7 d retreatment interval ("multiple application scenario")	Upper bound	mg/kg bw	1/million mortality (mg/kg bw)	0.184 mg/kg bw	E94337 based on Thai honey bee, contact, LD50 = 1.6 mg/kg-bw; slope=5; TGAI	ND	Cannot Exceed	BC20 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	NA	mg/kg soil	1/million mortality (mg/kg soil)	0.66 mg/kg soil	E52962 based Earthworm; 50% EC	ND	Cannot Exceed	BD20 on Alt worksheet
4 lb a.i./A twice with a 7 d retreatment interval ("multiple application scenario")	NA	mg/kg soil	1/million mortality (mg/kg soil)	0.66 mg/kg soil	E52962 based Earthworm; 50% EC	ND	Cannot Exceed	BE20 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	NA	lb a.i./A	Direct mortality (1/million)	0.0012 lb a.i./A	E89288 based on hemlock sawfly. TGAI, slope 5.6, contact	ND	Cannot Exceed	BF20 on Alt worksheet
1 lb a.i./A once ("minimum single application rate")	Upper bound, dietary item with minimum days exceeding	mg/kg diet	Direct mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AR20
1 lb a.i./A once ("minimum single application rate")	Upper bound, dietary item with maximum days exceeding	mg/kg diet	Direct mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AS20
4 lb a.i./A once ("upper bound single application rate")	Upper bound, dietary item with minimum days exceeding	mg/kg diet	Direct mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AT20
4 lb a.i./A once ("upper bound single application rate")	Upper bound, dietary item with maximum days exceeding	mg/kg diet	Direct mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AU20

Table L-2 Summary of mortality risk designation calculations for terrestrial invertebrates[a]								
Exposure			***Threshold***	***Value***	***Reference***	***Requirement for Risk Designation[b]***		***Cell ref.[c]***
Use Pattern	***Dietary Residue Assumption (e.g., mean or upper bound)***	***Units***				***MED***	***LOW***	
1 lb a.i./A once ("minimum single application rate")	Upper bound, dietary item with minimum days exceeding	mg/kg diet	Indirect mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AR21
1 lb a.i./A once ("minimum single application rate")	Upper bound, dietary item with maximum days exceeding	mg/kg diet	Indirect mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AS21
4 lb a.i./A once ("upper bound single application rate")	Upper bound, dietary item with minimum days exceeding	mg/kg diet	Indirect mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AT21
4 lb a.i./A once ("upper bound single application rate")	Upper bound, dietary item with maximum days exceeding	mg/kg diet	Indirect mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AU21
1 lb a.i./A once ("minimum single application rate")	Upper bound, dietary item with minimum days exceeding	mg/kg diet	Direct and indirect (lowest LC50)	1.3 mg/kg diet	From Bee Rex calculator; honeybee, LC50=0.38 ug ai/bee; MRID 05001991, 05004151	ND	Cannot Exceed	AR22
1 lb a.i./A once ("minimum single application rate")	Upper bound, dietary item with maximum days exceeding	mg/kg diet	Direct and indirect (lowest LC50)	1.3 mg/kg diet	From Bee Rex calculator; honeybee, LC50=0.38 ug ai/bee; MRID 05001991, 05004151	ND	Cannot Exceed	AS22

Table L-2 Summary of mortality risk designation calculations for terrestrial invertebrates[a]

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation[b]*		*Cell ref.[c]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
4 lb a.i./A once ("upper bound single application rate")	Upper bound, dietary item with minimum days exceeding	mg/kg diet	Direct and indirect (lowest LC50)	1.3 mg/kg diet	From Bee Rex calculator; honeybee, LC50=0.38 ug ai/bee; MRID 05001991, 05004151	ND	Cannot Exceed	AT22
4 lb a.i./A once ("upper bound single application rate")	Upper bound, dietary item with maximum days exceeding	mg/kg diet	Direct and indirect (lowest LC50)	1.3 mg/kg diet	From Bee Rex calculator; honeybee, LC50=0.38 ug ai/bee; MRID 05001991, 05004151	ND	Cannot Exceed	AU22
6 lb a.i./A once ("maximum single application rate")	Upper bound, dietary item with minimum days exceeding	mg/kg diet	Direct mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AR20 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, dietary item with maximum days exceeding	mg/kg diet	Direct mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AS20 on Alt worksheet
4 lb a.i./A twice with a 7 d retreatment interval ("multiple application scenario")	Upper bound, dietary item with minimum days exceeding	mg/kg diet	Direct mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AT20 on Alt worksheet
4 lb a.i./A twice with a 7 d retreatment interval ("multiple application scenario")	Upper bound, dietary item with maximum days exceeding	mg/kg diet	Direct mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AU20 on Alt worksheet

Table L-2 Summary of mortality risk designation calculations for terrestrial invertebrates[a]

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation[b]*		*Cell ref.[c]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
6 lb a.i./A once ("maximum single application rate")	Upper bound, dietary item with minimum days exceeding	mg/kg diet	Indirect mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AR21 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, dietary item with maximum days exceeding	mg/kg diet	Indirect mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AS21 on Alt worksheet
4 lb a.i./A twice with a 7 d retreatment interval ("multiple application scenario")	Upper bound, dietary item with minimum days exceeding	mg/kg diet	Indirect mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AT21 on Alt worksheet
4 lb a.i./A twice with a 7 d retreatment interval ("multiple application scenario")	Upper bound, dietary item with maximum days exceeding	mg/kg diet	Indirect mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	ND	Cannot Exceed	AU21on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, dietary item with minimum days exceeding	mg/kg diet	Direct mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	Cannot Exceed	Cannot Exceed	AR22 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, dietary item with maximum days exceeding	mg/kg diet	Direct mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	Cannot Exceed	Cannot Exceed	AS22 on Alt worksheet
4 lb a.i./A twice with a 7 d retreatment interval ("multiple application scenario")	Upper bound, dietary item with minimum days exceeding	mg/kg diet	Direct mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	Cannot Exceed	Cannot Exceed	AT22 on Alt worksheet

Table L-2 Summary of mortality risk designation calculations for terrestrial invertebrates[a]

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation[b]*		*Cell ref.[c]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
4 lb a.i./A twice with a 7 d retreatment interval ("multiple application scenario")	Upper bound, dietary item with maximum days exceeding	mg/kg diet	Direct mortality	1.3 mg/kg diet	Referenced only as "surrogate" in Ted tool	Cannot Exceed	Cannot Exceed	AU22 on Alt worksheet
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg bw	Direct lowest LC50	1.64 mg/kg bw	E94337	Cannot Exceed	ND	BB22
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg bw	Direct lowest LC50	1.64 mg/kg bw	E94337	Cannot Exceed	ND	BC22
1 lb a.i./A once ("minimum single application rate")	NA	mg/kg soil	Direct lowest LC50	7.54 mg/kg soil	E52962	Cannot Exceed	ND	If AT17 = "yes" then BD22[d]
4 lb a.i./A once ("upper bound single application rate")	NA	mg/kg soil	Direct lowest LC50	7.54 mg/kg soil	E52962	Cannot Exceed	ND	If AT17 = "yes" then BE22[d]
1 lb a.i./A once ("minimum single application rate")	NA	lb a.i./A	Direct mortality (1/million)	0.0012 lb a.i./A	E89288 based on hemlock sawfly. TGAI, slope 5.6, contact	Cannot Exceed	ND	If AT17 = "yes" then BF22[d]

ND = Not dependent (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceed by the exposure estimate)

[a] [a]The risk designation for mortality is determined in cell DI10 of the 'WoE Matrix' worksheet in the Species_Animal_Template.xlsx file. This table traces the precedents for this cell.

[b] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH. Formulae resulting in a risk designation of "unknown" or "NA" are not explicitly presented herein.

[c] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Animal_Template.xlsx file.

[d] A check for whether the listed terrestrial invertebrate is a soil-dweller (in which case AT17 should contain "yes")

Table L-3 Summary of growth risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (Mean or Upper Bound)*	*Units*					*MED*	*LOW*	
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg /kg diet	Growth NOEL (mg/kg diet)	1700 mg/kg diet from MRID 41583401	304 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	304 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	ND	Cannot exceed	Q19:AA19
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg /kg diet	Growth LOEL (mg/kg diet)	5000 mg/kg diet (no reference provided in Ted tool)	551 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	551 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	ND	Cannot exceed	Q20:AA20
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound	mg /kg diet	Growth NOEL (mg/kg diet)	1700 mg/kg diet from MRID 41583401	304 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	304 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	ND	Cannot exceed	Q19:AA19 in Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound	mg /kg diet	Growth LOEL (mg/kg diet)	5000 mg/kg diet (no reference provided in Ted tool)	551 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	551 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	Cannot exceed	Cannot exceed	Q20:AA20 in Alt worksheet

Table L-3 Summary of growth risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (Mean or Upper Bound)*	*Units*					*MED*	*LOW*	
1 lb a.i./A once ("minimum single application rate"	NA	lb a.i./A	Application-rate based threshold (lb a.i./A)	Growth (NA for malathion)	Reproduction (NA for malathion)	0.53 lb a.i./A (based on reported changes in length, biomass from E89113)	ND	Cannot exceed	BV21
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Application-rate based threshold (lb a.i./A)	Growth (NA for malathion)	Reproduction (NA for malathion)	0.53 lb a.i./A (based on reported changes in length, biomass from E89113)	ND	Cannot exceed	BW21
1 lb a.i./A once ("minimum single application rate"	Upper bound	mg /kg diet	Growth NOEL (mg/kg diet)	1700 mg/kg diet from MRID 41583401	304 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	304 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	ND	Cannot exceed	Q45:AA45
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg /kg diet	Growth LOEL (mg/kg diet)	5000 mg/kg diet (no reference provided in Ted tool)	551 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	551 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	ND	Cannot exceed	Q46:AA46
6 lb a.i./A once ("maximum single application rate"	Upper bound	mg /kg diet	Growth NOEL (mg/kg diet)	1700 mg/kg diet from MRID 41583401	304 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	304 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	ND	Cannot exceed	Q45:AA45 in Alt worksheet

Table L-3 Summary of growth risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (Mean or Upper Bound)*	*Units*					*MED*	*LOW*	
6 lb a.i./A once ("maximum single application rate"	Upper bound	mg /kg diet	Growth LOEL (mg/kg diet)	5000 mg/kg diet (no reference provided in Ted tool)	551 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	551 mg/kg diet from M48963301 (NOEC, acute dietary, ring-necked pheasant, bd wt gain d0-5, LOEC 551)	Cannot exceed	Cannot exceed	Q46:AA46 in Alt worksheet
6 lb a.i./A once ("maximum single application rate"	NA	lb a.i./A	Application-rate based threshold (lb a.i./A)	Growth (NA for malathion)	Reproduction (NA for malathion)	0.53 lb a.i./A (based on reported changes in length, biomass from E89113)	ND	Cannot exceed	BV21 in Alt worksheet
All modelled for aquatic exposure	Minimum EEC	µg/L	Growth NOEC (µg/L)	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Lowest of aquatic dietary items	ND	Cannot exceed	AF19
All modelled for aquatic exposure	Maximum EEC	µg/L	Growth NOEC (µg/L)	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Lowest of aquatic dietary items	ND	Cannot exceed	AG19
All modelled for aquatic exposure	Minimum EEC	µg/L	Growth LOEC (µg/L)	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Cannot exceed	Cannot exceed	AF20
All modelled for aquatic exposure	Maximum EEC	µg/L	Growth LOEC (µg/L)	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Cannot exceed	Cannot exceed	AG20

ND = Not dependent (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceed by the exposure estimate)

[a] The risk designation for growth is determined in cell DI19 of the 'WoE Matrix' worksheet in the Species_Animal_Template.xlsx file. This table traces the precedents for this cell.

[b] Presents inputs thresholds without species specific body mass scaling

[c] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH. Formulae resulting in a risk designation of "unknown" or "NA" are not explicitly presented herein.

[d] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Animal_Template.xlsx file.

Table L-4 Summary of growth risk designation calculations for terrestrial invertebrates[a]								
Exposure			***Threshold***	***Value***	***Reference***	***Requirement for Risk Designation[b]***		***Cell ref.[c]***
Use Pattern	***Dietary Residue Assumption (e.g., mean or upper bound)***	***Units***				***MED***	***LOW***	
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg bw	Direct growth NOEC	NA	No value provided	ND	Cannot Exceed	BB24
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg bw	Direct growth LOEC	NA	No value provided	ND	Cannot Exceed	BB25
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg bw	Direct growth NOEC	NA	No value provided	ND	Cannot Exceed	BC24
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg bw	Direct growth LOEC	NA	No value provided	ND	Cannot Exceed	BC25
1 lb a.i./A once ("minimum single application rate")	NA	mg/kg soil	Direct growth NOEC	NA	No value provided	ND	Cannot Exceed	BD24
1 lb a.i./A once ("minimum single application rate")	NA	mg/kg soil	Direct growth LOEC	45600 mg/kg soil	No reference provided in TED tool	ND	Cannot Exceed	BD25
4 lb a.i./A once ("upper bound single application rate")	NA	mg/kg soil	Direct growth NOEC	NA	No value provided	ND	Cannot Exceed	BE24
4 lb a.i./A once ("upper bound single application rate")	NA	mg/kg soil	Direct growth LOEC	45600 mg/kg soil	No reference provided in TED tool	ND	Cannot Exceed	BE25
1 lb a.i./A once ("minimum single application rate")	NA	lb a.i./A	Growth LOEL	0.456 lb a.i./A	E158669 (based on emergence of parasitoid wasp, LOAEC value)	ND	Cannot Exceed	BF24

Table L-4 Summary of growth risk designation calculations for terrestrial invertebrates[a]								
Exposure			***Threshold***	***Value***	***Reference***	***Requirement for Risk Designation[b]***		***Cell ref.[c]***
Use Pattern	***Dietary Residue Assumption (e.g., mean or upper bound)***	***Units***				***MED***	***LOW***	
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Growth LOEL	0.456 lb a.i./A	E158669 (based on emergence of parasitoid wasp, LOAEC value)	Cannot Exceed	Cannot Exceed	BG24
6 lb a.i./A once ("maximum single application rate"	Upper bound	mg/kg bw	Direct growth NOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	BB24 on Alt worksheet
6 lb a.i./A once ("maximum single application rate"	Upper bound	mg/kg bw	Direct growth LOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	BB25 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound	mg/kg bw	Direct growth NOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	BC24 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound	mg/kg bw	Direct growth LOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	BC25 on Alt worksheet
6 lb a.i./A once ("maximum single application rate"	NA	mg/kg soil	Direct growth NOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	BD24 on Alt worksheet
6 lb a.i./A once ("maximum single application rate"	NA	mg/kg soil	Direct growth LOEC	45600 mg/kg soil	No reference provided in TED tool	Cannot Exceed	Cannot Exceed	BD25 on Alt worksheet

Table L-4 Summary of growth risk designation calculations for terrestrial invertebrates[a]

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation[b]*		*Cell ref.[c]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
4 lb a.i./A, twice with a seven day retreatment interval (“multiple application scenario”)	NA	mg/kg soil	Direct growth NOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	BE24 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval (“multiple application scenario”)	NA	mg/kg soil	Direct growth LOEC	45600 mg/kg soil	No reference provided in TED tool	Cannot Exceed	Cannot Exceed	BE25 on Alt worksheet
6 lb a.i./A once (“maximum single application rate"	NA	lb a.i./A	Growth LOEL	0.456 lb a.i./A	E158669 (based on emergence of parasitoid wasp, LOAEC value)	Cannot Exceed	Cannot Exceed	BF24 on Alt worksheet
1 lb a.i./A once (“minimum single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct growth NOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AR24
1 lb a.i./A once (“minimum single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct growth LOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AR25
1 lb a.i./A once (“minimum single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct growth NOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AS24

Table L-4 Summary of growth risk designation calculations for terrestrial invertebrates[a]

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation[b]*		*Cell ref.[c]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
1 lb a.i./A once ("minimum single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct growth LOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AS25
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct growth NOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AT24
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct growth LOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AT25
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct growth NOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AU24
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct growth LOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AU25
6 lb a.i./A once ("maximum single application rate"	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct growth NOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AR24 on Alt worksheet
6 lb a.i./A once ("maximum single application rate"	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct growth LOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AR25 on Alt worksheet

Table L-4 Summary of growth risk designation calculations for terrestrial invertebrates[a]

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation[b]*		*Cell ref.[c]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
6 lb a.i./A once ("maximum single application rate"	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct growth NOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AS24 on Alt worksheet
6 lb a.i./A once ("maximum single application rate"	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct growth LOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AS25 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct growth NOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AT24 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct growth LOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AT25 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct growth NOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AU24 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct growth LOEC	NA	No value provided	Cannot Exceed	Cannot Exceed	AU25 on Alt worksheet

ND = Not dependent (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceed by the exposure estimate)

[a] The risk designation for growth is determined in cell DI19 of the 'WoE Matrix' worksheet in the Species_Animal_Template.xlsx file. This table traces the precedents for this cell.

[b] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH. Formulae resulting in a risk designation of "unknown" or "NA" are not explicitly presented herein.
[c] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Animal_Template.xlsx file.

Table L-5 Summary of reproductive risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (Mean or Upper Bound)*	*Units*					*MED*	*LOW*	
4 lb a.i./A once	Upper bound, all dietary items	mg/kg diet	Reproduction NOEL (mg/kg diet)	825 mg/kg diet based on MRID 00152569, 40812001 (Developmental rabbit study, increase in mean # of resorption sites/dose; dose-based study converted to mg/kg-diet)	110 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350 (used as sublethal indirect endpoint))	110 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350)	ND	Cannot exceed	Q21:AA21
4 lb a.i./A once	Upper bound, all dietary items	mg/kg diet	Reproduction LOEL (mg/kg diet)	1650 mg/kg diet based on MRID 00152569, 40812001 (Developmental rabbit study, increase in mean # of resorption sites/dose; dose-based study converted to mg/kg-diet)	350 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350)	350 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350)	ND	Cannot exceed	Q22:AA22

Table L-5 Summary of reproductive risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (Mean or Upper Bound)*	*Units*					*MED*	*LOW*	
4 lb a.i./A twice, 7 day retreatment interval	Upper bound, all dietary items	mg/kg diet	Reproduction NOEL (mg/kg diet)	825 mg/kg diet based on MRID 00152569, 40812001 (Developmental rabbit study, increase in mean # of resorption sites/dose; dose-based study converted to mg/kg-diet)	110 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350 (used as sublethal indirect endpoint))	110 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350)	ND	Cannot exceed	Q21:AA21 in Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval	Upper bound, all dietary items	mg/kg diet	Reproduction LOEL (mg/kg diet)	1650 mg/kg diet based on MRID 00152569, 40812001 (Developmental rabbit study, increase in mean # of resorption sites/dose; dose-based study converted to mg/kg-diet)	350 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350)	350 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350)	Cannot exceed	Cannot exceed	Q22:AA22 in Alt worksheet
1 lb a.i./A once ("minimum single application rate"	NA	lb a.i./A	Application-rate based threshold (lb a.i./A)	Reproduction (NA for malathion)	Behavior (NA for malathion)	Reproduction (NA for malathion)	ND	Cannot exceed	BV22
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Application-rate based threshold (lb a.i./A)	Reproduction (NA for malathion)	Behavior (NA for malathion)	Reproduction (NA for malathion)	ND	Cannot exceed	BW22

Table L-5 Summary of reproductive risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (Mean or Upper Bound)*	*Units*					*MED*	*LOW*	
1 lb a.i./A once ("minimum single application rate")	Upper bound, all dietary items	mg/kg diet	Reproduction NOEL (mg/kg diet)	825 mg/kg diet based on MRID 00152569, 40812001 (Developmental rabbit study, increase in mean # of resorption sites/dose; dose-based study converted to mg/kg-diet)	110 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350 (used as sublethal indirect endpoint))	110 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350)	ND	Cannot exceed	Q47:AA47
1 lb a.i./A once ("minimum single application rate")	Upper bound, all dietary items	mg/kg diet	Reproduction LOEL (mg/kg diet)	1650 mg/kg diet based on MRID 00152569, 40812001 (Developmental rabbit study, increase in mean # of resorption sites/dose; dose-based study converted to mg/kg-diet)	350 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350)	350 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350)	ND	Cannot exceed	Q48:AA48

Table L-5 Summary of reproductive risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (Mean or Upper Bound)*	*Units*					*MED*	*LOW*	
6 lb a.i./A once ("maximum single application rate")	Upper bound, all dietary items	mg/kg diet	Reproduction NOEL (mg/kg diet)	825 mg/kg diet based on MRID 00152569, 40812001 (Developmental rabbit study, increase in mean # of resorption sites/dose; dose-based study converted to mg/kg-diet)	110 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350 (used as sublethal indirect endpoint))	110 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350)	ND	Cannot exceed	Q47:AA47 in Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, all dietary items	mg/kg diet	Reproduction LOEL (mg/kg diet)	1650 mg/kg diet based on MRID 00152569, 40812001 (Developmental rabbit study, increase in mean # of resorption sites/dose; dose-based study converted to mg/kg-diet)	350 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350)	350 mg/kg diet based on M43510501 (based on reproduction, NOEC, LOAEC is 350)	Cannot exceed	Cannot exceed	Q48:AA48 in Alt worksheet
6 lb a.i./A once ("maximum single application rate")	NA	lb a.i./A	Application-rate based threshold (lb a.i./A)	Reproduction (NA for malathion)	Behavior (NA for malathion)	Reproduction (NA for malathion)	ND	Cannot exceed	BV22 in Alt worksheet
All modelled for aquatic exposure	Minimum EEC	µg/L	Reproduction NOEC (µg/L)	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Lowest of aquatic dietary items	ND	Cannot exceed	AF21

Table L-5 Summary of reproductive risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (Mean or Upper Bound)*	*Units*					*MED*	*LOW*	
All modelled for aquatic exposure	Maximum EEC	µg/L	Reproduction NOEC (µg/L)	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Lowest of aquatic dietary items	ND	Cannot exceed	AG21
All modelled for aquatic exposure	Minimum EEC	µg/L	Reproduction LOEC (µg/L)	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Lowest of aquatic dietary items	ND	Cannot exceed	AF22
All modelled for aquatic exposure	Maximum EEC	µg/L	Reproduction LOEC (µg/L)	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Lowest of aquatic dietary items	ND	Cannot exceed	AG22

ND = Not dependent (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceed by the exposure estimate)

[a] The risk designation for reproduction is determined in cell DI26 of the 'WoE Matrix' worksheet in the Species_Animal_Template.xlsx file. This table traces the precedents for this cell.

[b] Presents inputs thresholds without species specific body mass scaling

[c] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH. Formulae resulting in a risk designation of "unknown" or "NA" are not explicitly presented herein.

[d] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Animal_Template.xlsx file.

Table L-6 Summary of reproduction risk designation calculations for terrestrial invertebrates[a]

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation[b]*		*Cell ref.[c]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg bw	Direct reproduction NOEL	NA	No value provided	ND	Cannot exceed	BB26
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg bw	Direct reproduction LOEL	NA	No value provided	ND	Cannot exceed	BB27
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg bw	Direct reproduction NOEL	NA	No value provided	ND	Cannot exceed	BC26
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg bw	Direct reproduction LOEL	NA	No value provided	ND	Cannot exceed	BC27
1 lb a.i./A once ("minimum single application rate")	NA	mg/kg soil	Direct reproduction NOEL	NA	No value provided	ND	Cannot exceed	BD26
1 lb a.i./A once ("minimum single application rate")	NA	mg/kg soil	Direct reproduction LOEL	1100 mg/kg soil	Reportedly based on progeny counts/numbers	ND	Cannot exceed	BD27
4 lb a.i./A once ("upper bound single application rate")	NA	mg/kg soil	Direct reproduction NOEL	NA	No value provided	ND	Cannot exceed	BE26
4 lb a.i./A once ("upper bound single application rate")	NA	mg/kg soil	Direct reproduction LOEL	1100 mg/kg soil	Reportedly based on progeny counts/numbers	ND	Cannot exceed	BE27

Table L-6 Summary of reproduction risk designation calculations for terrestrial invertebrates[a]

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation[b]*		*Cell ref.[c]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
1 lb a.i./A once ("minimum single application rate")	NA	lb a.i./A	Direct reproduction LOEL	0.456 lb a.i./A	E158669, parasitoid wasp, LOAEC value (no NOAEC reported)	ND	Cannot exceed	BF25
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Direct reproduction LOEL	0.456 lb a.i./A	E158669, parasitoid wasp, LOAEC value (no NOAEC reported)	ND	Cannot exceed	BG25
6 lb a.i./A once ("maximum single application rate")	Upper bound	mg/kg bw	Direct reproduction NOEL	NA	No value provided	ND	Cannot exceed	BB26 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound	mg/kg bw	Direct reproduction LOEL	NA	No value provided	Cannot exceed	Cannot exceed	BB27 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound	mg/kg bw	Direct reproduction NOEL	NA	No value provided	ND	Cannot exceed	BC26 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound	mg/kg bw	Direct reproduction LOEL	NA	No value provided	Cannot exceed	Cannot exceed	BC27 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	NA	mg/kg soil	Direct reproduction NOEL	NA	No value provided	ND	Cannot exceed	BD26 on Alt worksheet

Table L-6 Summary of reproduction risk designation calculations for terrestrial invertebrates[a]

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation[b]*		*Cell ref.[c]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
6 lb a.i./A once ("maximum single application rate")	NA	mg/kg soil	Direct reproduction LOEL	1100 mg/kg soil	Reportedly based on progeny counts/numbers	Cannot exceed	Cannot exceed	BD27 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	NA	mg/kg soil	Direct reproduction NOEL	NA	No value provided	ND	Cannot exceed	BE26 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	NA	mg/kg soil	Direct reproduction LOEL	1100 mg/kg soil	Reportedly based on progeny counts/numbers	Cannot exceed	Cannot exceed	BE27 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	NA	lb a.i./A	Direct reproduction LOEL	0.456 lb a.i./A	E158669, parasitoid wasp, LOAEC value (no NOAEC reported)	ND	Cannot exceed	BF25 on Alt worksheet
1 lb a.i./A once ("minimum single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct reproduction NOEL	NA	No value provided	ND	Cannot exceed	AR26
1 lb a.i./A once ("minimum single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct reproduction LOEL	NA	No value provided	Cannot exceed	Cannot exceed	AR27

Table L-6 Summary of reproduction risk designation calculations for terrestrial invertebrates[a]

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation[b]*		*Cell ref.[c]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
1 lb a.i./A once ("minimum single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct reproduction NOEL	NA	No value provided	ND	Cannot exceed	AS26
1 lb a.i./A once ("minimum single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct reproduction LOEL	NA	No value provided	Cannot exceed	Cannot exceed	AS27
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct reproduction NOEL	NA	No value provided	ND	Cannot exceed	AT26
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct reproduction LOEL	NA	No value provided	Cannot exceed	Cannot exceed	AT27
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct reproduction NOEL	NA	No value provided	ND	Cannot exceed	AU26
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct reproduction LOEL	NA	No value provided	Cannot exceed	ND	AU27
6 lb a.i./A once ("maximum single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct reproduction NOEL	NA	No value provided	Cannot exceed	ND	AR26 on Alt worksheet

Table L-6 Summary of reproduction risk designation calculations for terrestrial invertebrates[a]

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation[b]*		*Cell ref.[c]*
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
6 lb a.i./A once ("maximum single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct reproduction LOEL	NA	No value provided	Cannot exceed	ND	AR27 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct reproduction NOEL	NA	No value provided	Cannot exceed	ND	AS26 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct reproduction LOEL	NA	No value provided	Cannot exceed	ND	AS27 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct reproduction NOEL	NA	No value provided	Cannot exceed	ND	AT26 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct reproduction LOEL	NA	No value provided	Cannot exceed	ND	AT27 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct reproduction NOEL	NA	No value provided	Cannot exceed	ND	AU26 on Alt worksheet

Table L-6 Summary of reproduction risk designation calculations for terrestrial invertebrates[a]

Exposure			Threshold	Value	Reference	Requirement for Risk Designation[b]		Cell ref.[c]
Use Pattern	Dietary Residue Assumption (e.g., mean or upper bound)	Units				MED	LOW	
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct reproduction LOEL	NA	No value provided	Cannot exceed	ND	AU27 on Alt worksheet

ND = Not dependent (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceed by the exposure estimate)
[a] The risk designation for reproduction is determined in cell DI26 of the 'WoE Matrix' worksheet in the Species_Animal_Template.xlsx file. This table traces the precedents for this cell.
[b] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH. Formulae resulting in a risk designation of "unknown" or "NA" are not explicitly presented herein.
[c] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Animal_Template.xlsx file.

Table L-7 Summary of behavioral risk designation calculations for terrestrial vertebrates[a]

Exposure			Threshold	Mammal[b]	Birds[b]	Terrestrial Herptiles[b]	Requirement for Risk Designation[c]		Cell ref.[d]
Use Pattern	Dietary Residue Assumption (Mean or Upper Bound)	Units					MED	LOW	
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg diet	Behavior NOEL (mg/kg diet)	NA (no value provided in Ted tool)	NA (no value provided in Ted tool)	NA (no value provided in Ted tool)	ND	Cannot exceed	Q23:AA23
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg diet	Behavior LOEL (mg/kg diet)	640 mg/kg diet (E153607, based on alterations in general activity in rat)	400 mg/kg diet (E89120, LOEC, no NOEC, sleeping time in chickens)	400 mg/kg diet (E89120, LOEC, no NOEC, sleeping time in chickens)	ND	Cannot exceed	Q24:AA24

Table L-7 Summary of behavioral risk designation calculations for terrestrial vertebrates[a]

Exposure			Threshold	Mammal[b]	Birds[b]	Terrestrial Herptiles[b]	Requirement for Risk Designation[c]		Cell ref.[d]
Use Pattern	Dietary Residue Assumption (Mean or Upper Bound)	Units					MED	LOW	
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound	mg/kg diet	Behavior NOEL (mg/kg diet)	NA (no value provided in Ted tool)	NA (no value provided in Ted tool)	NA (no value provided in Ted tool)	ND	Cannot exceed	Q23:AA23 in Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound	mg/kg diet	Behavior LOEL (mg/kg diet)	640 mg/kg diet (E153607, based on alterations in general activity in rat)	400 mg/kg diet (E89120, LOEC, no NOEC, sleeping time in chickens)	400 mg/kg diet (E89120, LOEC, no NOEC, sleeping time in chickens)	Cannot exceed	Cannot exceed	Q24:AA24 in Alt worksheet
1 lb a.i./A once ("minimum single application rate")	NA	lb a.i./A	Application-rate based threshold (lb a.i./A)	Behavior (NA for malathion)	Sensory (NA for malathion)	Behavior (NA for malathion)	ND	Cannot exceed	BV23
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Application-rate based threshold (lb a.i./A)	Behavior (NA for malathion)	Sensory (NA for malathion)	Behavior (NA for malathion)	ND	Cannot exceed	BW24

Table L-7 Summary of behavioral risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammal[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (Mean or Upper Bound)*	*Units*					*MED*	*LOW*	
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg diet	Behavior NOEL (mg/kg diet)	NA (no value provided in Ted tool)	NA (no value provided in Ted tool)	NA (no value provided in Ted tool)	ND	Cannot exceed	Q49:AA49
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg diet	Behavior LOEL (mg/kg diet)	640 mg/kg diet (E153607, based on alterations in general activity in rat)	400 mg/kg diet (E89120, LOEC, no NOEC, sleeping time in chickens)	400 mg/kg diet (E89120, LOEC, no NOEC, sleeping time in chickens)	ND	Cannot exceed	Q50:AA50
6 lb a.i./A once ("maximum single application rate"	Upper bound	mg/kg diet	Behavior NOEL (mg/kg diet)	NA (no value provided in Ted tool)	NA (no value provided in Ted tool)	NA (no value provided in Ted tool)	ND	Cannot exceed	Q49:AA49 in Alt worksheet
6 lb a.i./A once ("maximum single application rate"	Upper bound	mg/kg diet	Behavior LOEL (mg/kg diet)	640 mg/kg diet (E153607, based on alterations in general activity in rat)	400 mg/kg diet (E89120, LOEC, no NOEC, sleeping time in chickens)	400 mg/kg diet (E89120, LOEC, no NOEC, sleeping time in chickens)	Cannot exceed	Cannot exceed	Q50:AA50 in Alt worksheet
6 lb a.i./A once ("maximum single application rate"	NA	lb a.i./A	Application-rate based threshold (lb a.i./A)	Behavior (NA for malathion)	Sensory (NA for malathion)	Behavior (NA for malathion)	ND	Cannot exceed	BV23 in Alt worksheet
All uses	Minimum EEC	µg/L	Behavioral NOEL	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Lowest of aquatic dietary items	ND	Cannot exceed	AF23

Table L-7 Summary of behavioral risk designation calculations for terrestrial vertebrates[a]

Exposure			Threshold	Mammal[b]	Birds[b]	Terrestrial Herptiles[b]	Requirement for Risk Designation[c]		Cell ref.[d]
Use Pattern	Dietary Residue Assumption (Mean or Upper Bound)	Units					MED	LOW	
All uses	Maximum EEC	µg/L	Behavioral NOEL	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Lowest of aquatic dietary items	ND	Cannot exceed	AG23
All uses	Minimum EEC	µg/L	Behavioral LOEL	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Cannot exceed	Cannot exceed	AF24
All uses	Maximum EEC	µg/L	Behavioral LOEL	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Lowest of aquatic dietary items	Cannot exceed	Cannot exceed	AG24
4 lb a.i./A once ("upper bound single application rate")	Upper bound, highest dietary concentration among feed items	mg/kg diet	Direct Sublethal threshold (mg/kg diet)	20 mg/kg diet (M43942901, AChE inhibition, LOEC, no NOEC)	110 mg/kg diet (M43510501, based on reproduction, NOEC, LOAEC is 350)	110 mg/kg diet (M43510501, based on reproduction, NOEC, LOAEC is 350)	Cannot exceed	Cannot exceed	Q18:AA18

ND = Not dependent (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceed by the exposure estimate)

[a] The risk designation for behavioral effects is determined in cell DI33 of the 'WoE Matrix' worksheet in the Species_Animal_Template.xlsx file. This table traces the precedents for this cell.

[b] Presents inputs thresholds without species specific body mass scaling

[c] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH. Formulae resulting in a risk designation of "unknown" or "NA" are not explicitly presented herein.

[d] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Animal_Template.xlsx file.

Table L-8 Summary of behavioral risk designation calculations for terrestrial invertebrates

Exposure			Threshold	Value	Reference	Requirement for Risk Designation[b]		Cell ref.[c]
Use Pattern	Dietary Residue Assumption (e.g., mean or upper bound)	Units				MED	LOW	

Table L-8 Summary of behavioral risk designation calculations for terrestrial invertebrates

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation*[b]		*Cell ref.*[c]
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg bw	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	BB28
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg bw	Direct behavior LOEL	NA	Value not provided	ND	Cannot exceed	BB29
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg bw	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	BC28
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg bw	Direct behavior LOEL	NA	Value not provided	ND	Cannot exceed	BC29
1 lb a.i./A once ("minimum single application rate")	NA	mg/kg soil	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	BD28
1 lb a.i./A once ("minimum single application rate")	NA	mg/kg soil	Direct behavior LOEL	NA	Value not provided	ND	Cannot exceed	BD29
4 lb a.i./A once ("upper bound single application rate")	NA	mg/kg soil	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	BE28
4 lb a.i./A once ("upper bound single application rate")	NA	mg/kg soil	Direct behavior LOEL	NA	Value not provided	ND	Cannot exceed	BE29
1 lb a.i./A once ("minimum single application rate")	NA	lb a.i./A	Direct behavior LOEL	NA	Value not provided	ND	Cannot exceed	BF26
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Direct behavior LOEL	NA	Value not provided	ND	Cannot exceed	BG26

Table L-8 Summary of behavioral risk designation calculations for terrestrial invertebrates								
Exposure			***Threshold***	***Value***	***Reference***	***Requirement for Risk Designation[b]***		***Cell ref.[c]***
Use Pattern	***Dietary Residue Assumption (e.g., mean or upper bound)***	***Units***				***MED***	***LOW***	
6 lb a.i./A once ("maximum single application rate"	Upper bound	mg/kg bw	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	BB28 on Alt worksheet
6 lb a.i./A once ("maximum single application rate"	Upper bound	mg/kg bw	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	BB29 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound	mg/kg bw	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	BC28 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound	mg/kg bw	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	BC29 on Alt worksheet
6 lb a.i./A once ("maximum single application rate"	NA	mg/kg soil	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	BD28 on Alt worksheet
6 lb a.i./A once ("maximum single application rate"	NA	mg/kg soil	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	BD29 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	NA	mg/kg soil	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	BE28 on Alt worksheet

Table L-8 Summary of behavioral risk designation calculations for terrestrial invertebrates

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation*[b]		*Cell ref.*[c]
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
4 lb a.i./A, twice with a seven day retreatment interval (“multiple application scenario”)	NA	mg/kg soil	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	BE29 on Alt worksheet
6 lb a.i./A once (“maximum single application rate"	NA	lb a.i./A	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	BF26 on Alt worksheet
1 lb a.i./A once (“minimum single application rate”)	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	AR28
1 lb a.i./A once (“minimum single application rate”)	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	AR29
1 lb a.i./A once (“minimum single application rate”)	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	AS28
1 lb a.i./A once (“minimum single application rate”)	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	AS29
4 lb a.i./A once (“upper bound single application rate”)	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	AT28

Table L-8 Summary of behavioral risk designation calculations for terrestrial invertebrates

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation*[b]		*Cell ref.*[c]
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	AT29
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	AU28
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	AU29
6 lb a.i./A once ("maximum single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	AR28 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	AR29 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	AS28 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	AS29 on Alt worksheet

Table L-8 Summary of behavioral risk designation calculations for terrestrial invertebrates

Exposure			*Threshold*	*Value*	*Reference*	*Requirement for Risk Designation*[b]		*Cell ref.*[c]
Use Pattern	*Dietary Residue Assumption (e.g., mean or upper bound)*	*Units*				*MED*	*LOW*	
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	AT28 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	AT29 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct behavior NOEL	NA	Value not provided	ND	Cannot exceed	AU28 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct behavior LOEL	NA	Value not provided	Cannot exceed	Cannot exceed	AU29 on Alt worksheet

ND = Not dependent (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceed by the exposure estimate)

[a] The risk designation for behavioral effects is determined in cell DI33 of the 'WoE Matrix' worksheet in the Species_Animal_Template.xlsx file. This table traces the precedents for this cell.

[b] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH. Formulae resulting in a risk designation of "unknown" or "NA" are not explicitly presented herein.

[c] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Animal_Template.xlsx file.

Table L-9 Summary of sensory risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	***Dietary Residue Assumption (Mean or Upper Bound)***	***Units***					***MED***	***LOW***	
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg diet	Sensory NOEL (mg/kg diet)	Sensory (NA for malathion)	Sensory (NA for malathion)	Sensory (NA for malathion)	ND	Cannot exceed	Q25:AA25
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg diet	Sensory LOEL (mg/kg diet)	Sensory (NA for malathion)	Sensory (NA for malathion)	Sensory (NA for malathion)	ND	Cannot exceed	Q26:AA26
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound	mg/kg diet	Sensory NOEL (mg/kg diet)	Sensory (NA for malathion)	Sensory (NA for malathion)	Sensory (NA for malathion)	ND	Cannot exceed	Q25:AA25 in Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound	mg/kg diet	Sensory LOEL (mg/kg diet)	Sensory (NA for malathion)	Sensory (NA for malathion)	Sensory (NA for malathion)	Cannot exceed	Cannot exceed	Q26:AA26 in Alt worksheet

Table L-9 Summary of sensory risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	***Dietary Residue Assumption (Mean or Upper Bound)***	***Units***					***MED***	***LOW***	
1 lb a.i./A once ("minimum single application rate")	NA	lb a.i./A	Application-rate based threshold (lb a.i./A)	Sensory (NA for malathion)	Mortality (NA for malathion)	Sensory (NA for malathion)	ND	Cannot exceed	BV24
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Application-rate based threshold (lb a.i./A)	Sensory (NA for malathion)	Mortality (NA for malathion)	Sensory (NA for malathion)	ND	Cannot exceed	BW24
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg diet	Sensory NOEL (mg/kg diet)	Sensory (NA for malathion)	Sensory (NA for malathion)	Sensory (NA for malathion)	ND	Cannot exceed	Q51:AA51
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg diet	Sensory LOEL (mg/kg diet)	Sensory (NA for malathion)	Sensory (NA for malathion)	Sensory (NA for malathion)	ND	Cannot exceed	Q52:AA52
6 lb a.i./A once ("maximum single application rate")	Upper bound	mg/kg diet	Sensory NOEL (mg/kg diet)	Sensory (NA for malathion)	Sensory (NA for malathion)	Sensory (NA for malathion)	ND	Cannot exceed	Q51:AA51 in Alt worksheet

Table L-9 Summary of sensory risk designation calculations for terrestrial vertebrates[a]

Exposure			*Threshold*	*Mammals[b]*	*Birds[b]*	*Terrestrial Herptiles[b]*	*Requirement for Risk Designation[c]*		*Cell ref.[d]*
Use Pattern	*Dietary Residue Assumption (Mean or Upper Bound)*	*Units*					*MED*	*LOW*	
6 lb a.i./A once ("maximum single application rate")	Upper bound	mg/kg diet	Sensory LOEL (mg/kg diet)	Sensory (NA for malathion)	Sensory (NA for malathion)	Sensory (NA for malathion)	Cannot exceed	Cannot exceed	Q52:AA52 in Alt worksheet
6 lb a.i./A once ("maximum single application rate")	NA	lb a.i./A	Application-rate based threshold (lb a.i./A)	Sensory (NA for malathion)	Mortality (NA for malathion)	Sensory (NA for malathion)	ND	Cannot exceed	BV24 in Alt worksheet
All uses	Minimum EEC	µg/L	Sensory NOEL	Lowest of aquatic dietary items (NA for malathion)	Lowest of aquatic dietary items (NA for malathion)	Lowest of aquatic dietary items (NA for malathion)	ND	Cannot exceed	AF25
All uses	Maximum EEC	µg/L	Sensory NOEL	Lowest of aquatic dietary items (NA for malathion)	Lowest of aquatic dietary items (NA for malathion)	Lowest of aquatic dietary items (NA for malathion)	ND	Cannot exceed	AG25
All uses	Minimum EEC	µg/L	Sensory NOEL	Lowest of aquatic dietary items (NA for malathion)	Lowest of aquatic dietary items (NA for malathion)	Lowest of aquatic dietary items (NA for malathion)	Cannot exceed	Cannot exceed	AF26
All uses	Maximum EEC	µg/L	Sensory NOEL	Lowest of aquatic dietary items (NA for malathion)	Lowest of aquatic dietary items (NA for malathion)	Lowest of aquatic dietary items (NA for malathion)	Cannot exceed	Cannot exceed	AG26

ND = Not dependent (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceed by the exposure estimate)

[a] The risk designation for sensory effects is determined in cell DI41 of the 'WoE Matrix' worksheet in the Species_Animal_Template.xlsx file. This table traces the precedents for this cell.

[b] Presents inputs thresholds without species specific body mass scaling

[c] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH. Formulae resulting in a risk designation of "unknown" or "NA" are not explicitly presented herein.

[d] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Animal_Template.xlsx file.

Table L -10 Summary of sensory risk designation calculations for terrestrial invertebrates[a]

Exposure			Threshold	Value	Reference	Requirement for Risk Designation[b]		Cell ref.[c]
Use Pattern	Dietary Residue Assumption (e.g., mean or upper bound)	Units				MED	LOW	
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg bw	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	BB30
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg bw	Direct sensory LOEL	NA	No value provided	ND	Cannot exceed	BB31
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg bw	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	BC30
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg bw	Direct sensory LOEL	NA	No value provided	ND	Cannot exceed	BC31
1 lb a.i./A once ("minimum single application rate")	NA	mg/kg soil	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	BD30
1 lb a.i./A once ("minimum single application rate")	NA	mg/kg soil	Direct sensory LOEL	NA	No value provided	ND	Cannot exceed	BD31
4 lb a.i./A once ("upper bound single application rate")	NA	mg/kg soil	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	BE30
4 lb a.i./A once ("upper bound single application rate")	NA	mg/kg soil	Direct sensory LOEL	NA	No value provided	ND	Cannot exceed	BE31
1 lb a.i./A once ("minimum single application rate")	NA	lb a.i./A	Direct sensory LOEL	NA	No value provided	ND	Cannot exceed	BF27
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Direct sensory LOEL	NA	No value provided	ND	Cannot exceed	BG27

Table L -10 Summary of sensory risk designation calculations for terrestrial invertebrates[a]

Exposure			Threshold	Value	Reference	Requirement for Risk Designation[b]		Cell ref.[c]
Use Pattern	Dietary Residue Assumption (e.g., mean or upper bound)	Units				MED	LOW	
6 lb a.i./A once ("maximum single application rate"	Upper bound	mg/kg bw	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	BB30 on Alt worksheet
6 lb a.i./A once ("maximum single application rate"	Upper bound	mg/kg bw	Direct sensory LOEL	NA	No value provided	ND	Cannot exceed	BB31 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound	mg/kg bw	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	BC30 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	Upper bound	mg/kg bw	Direct sensory LOEL	NA	No value provided	ND	Cannot exceed	BC31 on Alt worksheet
6 lb a.i./A once ("maximum single application rate"	NA	mg/kg soil	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	BD30 on Alt worksheet
6 lb a.i./A once ("maximum single application rate"	NA	mg/kg soil	Direct sensory LOEL	NA	No value provided	ND	Cannot exceed	BD31 on Alt worksheet
4 lb a.i./A, twice with a seven day retreatment interval ("multiple application scenario")	NA	mg/kg soil	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	BE30 on Alt worksheet

Table L -10 Summary of sensory risk designation calculations for terrestrial invertebrates[a]

Exposure			Threshold	Value	Reference	Requirement for Risk Designation[b]		Cell ref.[c]
Use Pattern	Dietary Residue Assumption (e.g., mean or upper bound)	Units				MED	LOW	
4 lb a.i./A, twice with a seven day retreatment interval (“multiple application scenario”)	NA	mg/kg soil	Direct sensory LOEL	NA	No value provided	ND	Cannot exceed	BE31 on Alt worksheet
6 lb a.i./A once (“maximum single application rate"	NA	lb a.i./A	Direct sensory LOEL	NA	No value provided	Cannot exceed	Cannot exceed	BF27 on Alt worksheet
1 lb a.i./A once (“minimum single application rate”)	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	AR30
1 lb a.i./A once (“minimum single application rate”)	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct sensory LOEL	NA	No value provided	Cannot exceed	Cannot exceed	AR31
1 lb a.i./A once (“minimum single application rate”)	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	AS30
1 lb a.i./A once (“minimum single application rate”)	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct sensory LOEL	NA	No value provided	Cannot exceed	Cannot exceed	AS31
4 lb a.i./A once (“upper bound single application rate”)	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	AT30

Table L -10 Summary of sensory risk designation calculations for terrestrial invertebrates[a]

Exposure			Threshold	Value	Reference	Requirement for Risk Designation[b]		Cell ref.[c]
Use Pattern	Dietary Residue Assumption (e.g., mean or upper bound)	Units				MED	LOW	
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct sensory LOEL	NA	No value provided	Cannot exceed	Cannot exceed	AT31
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	AU30
4 lb a.i./A once ("upper bound single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct sensory LOEL	NA	No value provided	Cannot exceed	Cannot exceed	AU31
6 lb a.i./A once ("maximum single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	AR30 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct sensory LOEL	NA	No value provided	Cannot exceed	Cannot exceed	AR31 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	AS30 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct sensory LOEL	NA	No value provided	Cannot exceed	Cannot exceed	AS31 on Alt worksheet

Table L -10 Summary of sensory risk designation calculations for terrestrial invertebrates[a]

Exposure			Threshold	Value	Reference	Requirement for Risk Designation[b]		Cell ref.[c]
Use Pattern	Dietary Residue Assumption (e.g., mean or upper bound)	Units				MED	LOW	
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	AT30 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, minimum number of days exceeding	mg/kg diet	Direct sensory LOEL	NA	No value provided	Cannot exceed	Cannot exceed	AT31 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct sensory NOEL	NA	No value provided	ND	Cannot exceed	AU30 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound, of dietary items, maximum number of days exceeding	mg/kg diet	Direct sensory LOEL	NA	No value provided	Cannot exceed	Cannot exceed	AU31 on Alt worksheet

ND = Not dependent (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceed by the exposure estimate)

[a] The risk designation for sensory effects is determined in cell DI41 of the 'WoE Matrix' worksheet in the Species_Animal_Template.xlsx file. This table traces the precedents for this cell.

[b] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH. Formulae resulting in a risk designation of "unknown" or "NA" are not explicitly presented herein.

[c] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Animal_Template.xlsx file.

Table L -11 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to prey/feed items[a]

Exposure			Feed Item/Prey											Cell ref.[b]
Use Pattern	*Residue (e.g., Mean or Upper Bound)*	*Units*	*Threshold*	*Aq. Inverts*	*Fish and Aquatic Amphib.*	*Aquatic Plants (non-vascular)*	*Aquatic Plants (vascular)*	*Terr. Monocots*	*Terr. Dicots*	*Terr. Inverts*	*Terr. Herptiles*	*Birds*	*Mammals*	
All modelled aquatic scenarios	Overall max EEC from species-specific HUCs	µg/L	Indirect Mortality (µg/L)	0.22 µg/L	20 µg/L	10,000 µg/L	100,000 µg/L	NA	NA	NA	NA	NA	NA	AE197
4 lb a.i./A once ("upper bound single application rate")	Upper bound	mg/kg diet (bw)	Indirect Mortality (mg/kg bw)	NA	NA	NA	NA	NA	NA	0.909 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	108 mg/kg bw based on young rats, 1-d E35348	AE197
4 lb a.i./A once ("upper bound single application rate")	Upper bound; Conc. in diet of all potential feed items of prey taxon	mg/kg diet	Indirect Mortality (mg/kg diet)	NA	NA	NA	NA	NA	NA	1.3 mg/kg diet (cited as "surrogates")	1210 mg/kg diet (no reference provided in Ted tool)	1210 mg/kg diet (no reference provided in Ted tool)	1080 mg/kg diet (Converted mortality threshold (dose-based) to dietary-based using WHO 2009 conversion for young rat)	AE197
4 lb a.i./A once ("upper bound single application rate")	NA	4 lb a.i./A	Indirect Mortality (lb a.i./A)	NA	NA	NA	NA	NA	NA	0.00875 lb a.i./A (E89288; mortality; LD50)	NA	NA	NA	AE197
4 lb a.i./A once ("upper bound single application rate")	Function of pore water concentration estimate and Koc	(mg/kg soil)	Indirect Mortality (mg/kg soil)	NA	NA	NA	NA	NA	NA	3.89 mg/kg soil (E52962)	NA	NA	NA	AE197
All modelled aquatic scenarios	Overall max EEC from species-specific HUCs	µg/L	Indirect Sublethal (µg/L)	0.097 µg/L	9 µg/L	1200 µg/L	1200 µg/L	NA	NA	NA	NA	NA	NA	AE197
4 lb a.i./A once ("upper bound single application rate")	Upper bound	(mg/kg diet (bw))	Indirect Sublethal (mg/kg bw)	NA	NA	NA	NA	NA	NA	0.909 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	108 mg/kg bw based on young rats, 1-d E35348	AE197

Table L -11 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to prey/feed items[a]

Exposure			Feed Item/Prey											Cell ref.[b]
Use Pattern	Residue (e.g., Mean or Upper Bound)	Units	Threshold	Aq. Inverts	Fish and Aquatic Amphib.	Aquatic Plants (non-vascular)	Aquatic Plants (vascular)	Terr. Monocots	Terr. Dicots	Terr. Inverts	Terr. Herptiles	Birds	Mammals	
4 lb a.i./A once ("upper bound single application rate")	Upper bound	Conc. in diet of all potential feed items of prey taxon (mg/kg diet)	Indirect Sublethal (mg/kg diet)	NA	NA	NA	NA	NA	NA	1.3 mg/kg diet (cited as "surrogates")	1210 mg/kg diet (no reference provided in Ted tool)	1210 mg/kg diet (no reference provided in Ted tool)	1080 mg/kg diet (Converted mortality threshold (dose-based) to dietary-based using WHO 2009 conversion for young rat)	AE197
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Indirect Sublethal (lb a.i./A)	NA	NA	NA	NA	NA	NA	0.00875 lb a.i./A (E89288; mortality; LD50)	NA	NA	NA	AE197
4 lb a.i./A once ("upper bound single application rate")	Upper bound	Function of pore water concentration estimate and Koc (mg/kg soil)	Indirect Sublethal (mg/kg soil)	NA	NA	NA	NA	NA	NA	7.54 mg/kg soil (mortality; E52962)	NA	NA	NA	AE197
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Indirect pre-emergent (lb a.i./A)	NA	NA	NA	NA	4.64 lb a.i./A overall minimum of lowest pre-emergent LOECs and EC25s from monocots and dicots	4.64 lb a.i./A overall minimum of lowest pre-emergent LOECs and EC25s from monocots and dicots	NA	NA	NA	NA	AE197
4 lb a.i./A once ("upper bound single application rate")	NA	lb a.i./A	Indirect post-emergent (lb a.i./A)	NA	NA	NA	NA	0.5 lb a.i./A overall minimum of lowest post-emergent LOECs and EC25s from monocots and dicots	0.5 lb a.i./A overall minimum of lowest post-emergent LOECs and EC25s from monocots and dicots	NA	NA	NA	NA	AE197

Table L -11 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to prey/feed items[a]

Exposure			Feed Item/Prey											Cell ref.[b]
Use Pattern	*Residue (e.g., Mean or Upper Bound)*	*Units*	*Threshold*	*Aq. Inverts*	*Fish and Aquatic Amphib.*	*Aquatic Plants (non-vascular)*	*Aquatic Plants (vascular)*	*Terr. Monocots*	*Terr. Dicots*	*Terr. Inverts*	*Terr. Herptiles*	*Birds*	*Mammals*	
All modelled aquatic scenarios	Overall max EEC from species-specific HUCs	µg/L	Indirect Mortality (µg/L)	0.22 µg/L	20 µg/L	10,000 µg/L	NA	NA	NA	NA	NA	NA	NA	AE210
1 lb a.i./A once ("minimum single application rate")	Upper bound	mg/kg diet (bw)	Indirect Mortality (mg/kg bw)	NA	NA	NA	NA	NA	NA	0.909 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	108 mg/kg bw based on young rats, 1-d E35348	AE210
1 lb a.i./A once ("minimum single application rate")	Upper bound; Conc. in diet of all potential feed items of prey taxon	mg/kg diet	Indirect Mortality (mg/kg diet)	NA	NA	NA	NA	NA	NA	1.3 mg/kg diet (cited as "surrogates")	1210 mg/kg diet (no reference provided in Ted tool)	1210 mg/kg diet (no reference provided in Ted tool)	1080 mg/kg diet (Converted mortality threshold (dose-based) to dietary-based using WHO 2009 conversion for young rat)	AE210
1 lb a.i./A once ("minimum single application rate")	NA	4 lb a.i./A	Indirect Mortality (lb a.i./A)	NA	NA	NA	NA	NA	NA	0.00875 lb a.i./A (E89288; mortality; LD50)	NA	NA	NA	AE210
1 lb a.i./A once ("minimum single application rate")	Function of pore water concentration estimate and Koc	(mg/kg soil)	Indirect Mortality (mg/kg soil)	NA	NA	NA	NA	NA	NA	3.89 mg/kg soil (E52962)	NA	NA	NA	AE210
All modelled aquatic scenarios	Overall max EEC from species-specific HUCs	µg/L	Indirect Sublethal (µg/L)	0.097 µg/L	9 µg/L	1200 µg/L	1200 µg/L	NA	NA	NA	NA	NA	NA	AE210
1 lb a.i./A once ("minimum single application rate")	Upper bound	(mg/kg diet (bw))	Indirect Sublethal (mg/kg bw)	NA	NA	NA	NA	NA	NA	0.909 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	108 mg/kg bw based on young rats, 1-d E35348	AE210

Table L -11 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to prey/feed items[a]

Exposure			Feed Item/Prey											Cell ref.[b]
Use Pattern	Residue (e.g., Mean or Upper Bound)	Units	Threshold	Aq. Inverts	Fish and Aquatic Amphib.	Aquatic Plants (non-vascular)	Aquatic Plants (vascular)	Terr. Monocots	Terr. Dicots	Terr. Inverts	Terr. Herptiles	Birds	Mammals	
1 lb a.i./A once ("minimum single application rate")	Upper bound	Conc. in diet of all potential feed items of prey taxon (mg/kg diet)	Indirect Sublethal (mg/kg diet)	NA	NA	NA	NA	NA	NA	1.3 mg/kg diet (cited as "surrogates")	1210 mg/kg diet (no reference provided in Ted tool)	1210 mg/kg diet (no reference provided in Ted tool)	1080 mg/kg diet (Converted mortality threshold (dose-based) to dietary-based using WHO 2009 conversion for young rat)	AE210
1 lb a.i./A once ("minimum single application rate")	NA	lb a.i./A	Indirect Sublethal (lb a.i./A)	NA	NA	NA	NA	NA	NA	0.00875 lb a.i./A (E89288; mortality; LD50)	NA	NA	NA	AE210
1 lb a.i./A once ("minimum single application rate")	Upper bound	Function of pore water concentration estimate and Koc (mg/kg soil)	Indirect Sublethal (mg/kg soil)	NA	NA	NA	NA	NA	NA	7.54 mg/kg soil (mortality; E52962)	NA	NA	NA	AE210
1 lb a.i./A once ("minimum single application rate")	NA	lb a.i./A	Indirect pre-emergent (lb a.i./A)	NA	NA	NA	NA	4.64 lb a.i./A overall minimum of lowest pre-emergent LOECs and EC25s from monocots and dicots	4.64 lb a.i./A overall minimum of lowest pre-emergent LOECs and EC25s from monocots and dicots	NA	NA	NA	NA	AE210
1 lb a.i./A once ("minimum single application rate")	NA	lb a.i./A	Indirect post-emergent (lb a.i./A)	NA	NA	NA	NA	0.5 lb a.i./A overall minimum of lowest post-emergent LOECs and EC25s from monocots and dicots	0.5 lb a.i./A overall minimum of lowest post-emergent LOECs and EC25s from monocots and dicots	NA	NA	NA	NA	AE210

Table L -11 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to prey/feed items[a]

Exposure			Feed Item/Prey											Cell ref.[b]
Use Pattern	Residue (e.g., Mean or Upper Bound)	Units	Threshold	Aq. Inverts	Fish and Aquatic Amphib.	Aquatic Plants (non-vascular)	Aquatic Plants (vascular)	Terr. Monocots	Terr. Dicots	Terr. Inverts	Terr. Herptiles	Birds	Mammals	
All modelled aquatic scenarios	Overall max EEC from species-specific HUCs	µg/L	Indirect Mortality (µg/L)	0.22 µg/L	20 µg/L	10,000 µg/L	NA	NA	NA	NA	NA	NA	NA	AE197 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound	mg/kg diet (bw)	Indirect Mortality (mg/kg bw)	NA	NA	NA	NA	NA	NA	0.909 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	108 mg/kg bw based on young rats, 1-d E35348	AE197 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound; Conc. in diet of all potential feed items of prey taxon	mg/kg diet	Indirect Mortality (mg/kg diet)	NA	NA	NA	NA	NA	NA	1.3 mg/kg diet (cited as "surrogates")	1210 mg/kg diet (no reference provided in Ted tool)	1210 mg/kg diet (no reference provided in Ted tool)	1080 mg/kg diet (Converted mortality threshold (dose-based) to dietary-based using WHO 2009 conversion for young rat)	AE197 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	NA	4 lb a.i./A	Indirect Mortality (lb a.i./A)	NA	NA	NA	NA	NA	NA	0.00875 lb a.i./A (E89288; mortality; LD50)	NA	NA	NA	AE197 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Function of pore water concentration estimate and Koc	(mg/kg soil)	Indirect Mortality (mg/kg soil)	NA	NA	NA	NA	NA	NA	3.89 mg/kg soil (E52962)	NA	NA	NA	AE197 on Alt worksheet
All modelled aquatic scenarios	Overall max EEC from species-specific HUCs	µg/L	Indirect Sublethal (µg/L)	0.097 µg/L	9 µg/L	1200 µg/L	1200 µg/L	NA	NA	NA	NA	NA	NA	AE197 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound	(mg/kg diet (bw))	Indirect Sublethal (mg/kg bw)	NA	NA	NA	NA	NA	NA	0.909 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	108 mg/kg bw based on young rats, 1-d E35348	AE197 on Alt worksheet

Table L -11 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to prey/feed items[a]

Exposure			Feed Item/Prey											Cell ref.[b]
Use Pattern	Residue (e.g., Mean or Upper Bound)	Units	Threshold	Aq. Inverts	Fish and Aquatic Amphib.	Aquatic Plants (non-vascular)	Aquatic Plants (vascular)	Terr. Monocots	Terr. Dicots	Terr. Inverts	Terr. Herptiles	Birds	Mammals	
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound	Conc. in diet of all potential feed items of prey taxon (mg/kg diet)	Indirect Sublethal (mg/kg diet)	NA	NA	NA	NA	NA	NA	1.3 mg/kg diet (cited as "surrogates")	1210 mg/kg diet (no reference provided in Ted tool)	1210 mg/kg diet (no reference provided in Ted tool)	1080 mg/kg diet (Converted mortality threshold (dose-based) to dietary-based using WHO 2009 conversion for young rat)	AE197 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	NA	lb a.i./A	Indirect Sublethal (lb a.i./A)	NA	NA	NA	NA	NA	NA	0.00875 lb a.i./A (E89288; mortality; LD50)	NA	NA	NA	AE197 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	Upper bound	Function of pore water concentration estimate and Koc (mg/kg soil)	Indirect Sublethal (mg/kg soil)	NA	NA	NA	NA	NA	NA	7.54 mg/kg soil (mortality; E52962)	NA	NA	NA	AE197 on Alt worksheet
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	NA	lb a.i./A	Indirect pre-emergent (lb a.i./A)	NA	NA	NA	NA	4.64 lb a.i./A overall minimum of lowest pre-emergent LOECs and EC25s from monocots and dicots	4.64 lb a.i./A overall minimum of lowest pre-emergent LOECs and EC25s from monocots and dicots	NA	NA	NA	NA	AE197 on Alt worksheet

Table L -11 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to prey/feed items[a]

Exposure			Feed Item/Prey											Cell ref.[b]
Use Pattern	*Residue (e.g., Mean or Upper Bound)*	*Units*	*Threshold*	*Aq. Inverts*	*Fish and Aquatic Amphib.*	*Aquatic Plants (non-vascular)*	*Aquatic Plants (vascular)*	*Terr. Monocots*	*Terr. Dicots*	*Terr. Inverts*	*Terr. Herptiles*	*Birds*	*Mammals*	
4 lb a.i./A twice, 7 day retreatment interval ("multiple application scenario")	NA	lb a.i./A	Indirect post-emergent (lb a.i./A)	NA	NA	NA	NA	0.5 lb a.i./A overall minimum of lowest post-emergent LOECs and EC25s from monocots and dicots	0.5 lb a.i./A overall minimum of lowest post-emergent LOECs and EC25s from monocots and dicots	NA	NA	NA	NA	AE197 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Overall max EEC from species-specific HUCs	µg/L	Indirect Mortality (µg/L)	0.22 µg/L	20 µg/L	10,000 µg/L	NA	NA	NA	NA	NA	NA	NA	AE210 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound	mg/kg diet (bw)	Indirect Mortality (mg/kg bw)	NA	NA	NA	NA	NA	NA	0.909 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	108 mg/kg bw based on young rats, 1-d E35348	AE210 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound; Conc. in diet of all potential feed items of prey taxon	mg/kg diet	Indirect Mortality (mg/kg diet)	NA	NA	NA	NA	NA	NA	1.3 mg/kg diet (cited as "surrogates")	1210 mg/kg diet (no reference provided in Ted tool)	1210 mg/kg diet (no reference provided in Ted tool)	1080 mg/kg diet (Converted mortality threshold (dose-based) to dietary-based using WHO 2009 conversion for young rat)	AE210 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	NA	4 lb a.i./A	Indirect Mortality (lb a.i./A)	NA	NA	NA	NA	NA	NA	0.00875 lb a.i./A (E89288; mortality; LD50)	NA	NA	NA	AE210 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Function of pore water concentration estimate and Koc	(mg/kg soil)	Indirect Mortality (mg/kg soil)	NA	NA	NA	NA	NA	NA	3.89 mg/kg soil (E52962)	NA	NA	NA	AE210 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound	µg/L	Indirect Sublethal (µg/L)	0.097 µg/L	9 µg/L	1200 µg/L	1200 µg/L	NA	NA	NA	NA	NA	NA	AE210 on Alt worksheet

Table L -11 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to prey/feed items[a]

Exposure			Feed Item/Prey											Cell ref.[b]
Use Pattern	Residue (e.g., Mean or Upper Bound)	Units	Threshold	Aq. Inverts	Fish and Aquatic Amphib.	Aquatic Plants (non-vascular)	Aquatic Plants (vascular)	Terr. Monocots	Terr. Dicots	Terr. Inverts	Terr. Herptiles	Birds	Mammals	
6 lb a.i./A once ("maximum single application rate")	Upper bound	(mg/kg diet (bw))	Indirect Sublethal (mg/kg bw)	NA	NA	NA	NA	NA	NA	0.909 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	69 mg/kg bw (no reference provided in Ted tool)	108 mg/kg bw based on young rats, 1-d E35348	AE210 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound	Conc. in diet of all potential feed items of prey taxon (mg/kg diet)	Indirect Sublethal (mg/kg diet)	NA	NA	NA	NA	NA	NA	1.3 mg/kg diet (cited as "surrogates")	1210 mg/kg diet (no reference provided in Ted tool)	1210 mg/kg diet (no reference provided in Ted tool)	1080 mg/kg diet (Converted mortality threshold (dose-based) to dietary-based using WHO 2009 conversion for young rat)	AE210 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	NA	lb a.i./A	Indirect Sublethal (lb a.i./A)	NA	NA	NA	NA	NA	NA	0.00875 lb a.i./A (E89288; mortality; LD50)	NA	NA	NA	AE210 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	Upper bound	Function of pore water concentration estimate and Koc (mg/kg soil)	Indirect Sublethal (mg/kg soil)	NA	NA	NA	NA	NA	NA	7.54 mg/kg soil (mortality; E52962)	NA	NA	NA	AE210 on Alt worksheet
6 lb a.i./A once ("maximum single application rate")	NA	lb a.i./A	Indirect pre-emergent (lb a.i./A)	NA	NA	NA	NA	4.64 lb a.i./A overall minimum of lowest pre-emergent LOECs and EC25s from monocots and dicots	4.64 lb a.i./A overall minimum of lowest pre-emergent LOECs and EC25s from monocots and dicots	NA	NA	NA	NA	AE210 on Alt worksheet

Table L -11 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to prey/feed items[a]

Exposure			Feed Item/Prey											
Use Pattern	*Residue (e.g., Mean or Upper Bound)*	*Units*	*Threshold*	*Aq. Inverts*	*Fish and Aquatic Amphib.*	*Aquatic Plants (non-vascular)*	*Aquatic Plants (vascular)*	*Terr. Monocots*	*Terr. Dicots*	*Terr. Inverts*	*Terr. Herptiles*	*Birds*	*Mammals*	*Cell ref.[b]*
6 lb a.i./A once ("maximum single application rate")	NA	lb a.i./A	Indirect post-emergent (lb a.i./A)	NA	NA	NA	NA	0.5 lb a.i./A overall minimum of lowest post-emergent LOECs and EC25s from monocots and dicots	0.5 lb a.i./A overall minimum of lowest post-emergent LOECs and EC25s from monocots and dicots	NA	NA	NA	NA	AE210 on Alt worksheet

[a] The risk designation for indirect effects due to direct effects to prey/feed items is determined in cell DI54 of the 'WoE Matrix' worksheet in the Species_Animal_Template.xlsx file. This table traces the precedents for this cell. Notably, there is no MED risk designation for indirect effects to prey in the WoE tools. Thus any exceedances in the rows of this table indicate a HIGH risk designation.

[b] Unless otherwise specified all cell references refer to the WoE Matrix worksheet in the Species_Animal_Template.xlsx file.

Table L -12 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to habitat[a]

Use Pattern	*Exposure*	*Threshold*	*Value (lb a.i./A)*	*Reference*	*Requirement for Risk Designation if Aquatic Association if no Aquatic Association[b]*		*Requirement for Risk Designation if Aquatic Association[b]*		*Cell ref.[c]*
					MED	*LOW*	*MED*	*LOW*	
6 lb a.i./A once ("maximum single application rate")	Runoff to dry areas estimate = 0.06 lb a.i./A	Monocot pre-emergent LOEC for growth	4.64	MRID49076001; highest conc tested (censored valule, >4.64 lb a.i./A)	ND	Cannot Exceed	ND	Cannot Exceed	K301
6 lb a.i./A once ("maximum single application rate")	Runoff to dry areas estimate = 0.06 lb a.i./A	Dicot pre-emergent LOEC for growth	4.64	MRID49076001; >4.64	ND	Cannot Exceed	ND	Cannot Exceed	K302
6 lb a.i./A once ("maximum single application rate")	Runoff to dry areas estimate = 0.06 lb a.i./A	Monocot pre-emergent EC25 growth	4.64	MRID49076001; highest conc tested	ND	Cannot Exceed	ND	Cannot Exceed	O301
6 lb a.i./A once ("maximum single application rate")	Runoff to dry areas estimate = 0.06 lb a.i./A	Dicot pre-emergent EC25 growth	4.64	MRID49076001; >4.64	ND	Cannot Exceed	ND	Cannot Exceed	O302
4 lb a.i./A twice with a 7 d retreatment interval	Runoff to dry areas estimate = 0.04 lb a.i./A	Monocot pre-emergent LOEC for growth	4.64	MRID49076001; highest conc tested (censored valule, >4.64 lb a.i./A)	ND	Cannot Exceed	ND	Cannot Exceed	L301
4 lb a.i./A twice with a 7 d retreatment interval	Runoff to dry areas estimate = 0.04 lb a.i./A	Dicot pre-emergent LOEC for growth	4.64	MRID49076001; >4.64	ND	Cannot Exceed	ND	Cannot Exceed	L302
4 lb a.i./A twice with a 7 d retreatment interval	Runoff to dry areas estimate = 0.04 lb a.i./A	Monocot pre-emergent EC25 growth	4.64	MRID49076001; highest conc tested	ND	Cannot Exceed	ND	Cannot Exceed	P301

Table L -12 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to habitat[a]

Use Pattern	*Exposure*	*Threshold*	*Value (lb a.i./A)*	*Reference*	*Requirement for Risk Designation if Aquatic Association if no Aquatic Association[b]*		*Requirement for Risk Designation if Aquatic Association[b]*		*Cell ref.[c]*
					MED	*LOW*	*MED*	*LOW*	
4 lb a.i./A twice with a 7 d retreatment interval	Runoff to dry areas estimate = 0.04 lb a.i./A	Dicot pre-emergent EC25 growth	4.64	MRID49076001; >4.64	ND	Cannot Exceed	ND	Cannot Exceed	P302
6 lb a.i./A once ("maximum single application rate")	Runoff to semi-aquatic areas = 0.6 lb a.i./A	Monocot pre-emergent LOEC for growth	4.64	MRID49076001; highest conc tested (censored valule, >4.64 lb a.i./A)	ND	ND	ND	Cannot Exceed	K306
6 lb a.i./A once ("maximum single application rate")	Runoff to semi-aquatic areas = 0.6 lb a.i./A	Dicot pre-emergent LOEC for growth	4.64	MRID49076001; >4.64	ND	ND	ND	Cannot Exceed	K307
6 lb a.i./A once ("maximum single application rate")	Runoff to semi-aquatic areas = 0.6 lb a.i./A	Monocot pre-emergent EC25 growth	4.64	MRID49076001; highest conc tested	ND	ND	ND	Cannot Exceed	O306
6 lb a.i./A once ("maximum single application rate")	Runoff to semi-aquatic areas = 0.6 lb a.i./A	Dicot pre-emergent EC25 growth	4.64	MRID49076001; >4.64	ND	ND	ND	Cannot Exceed	O307
4 lb a.i./A twice with a 7 d retreatment interval	Runoff to semi-aquatic areas = 0.4 lb a.i./A	Monocot pre-emergent LOEC for growth	4.64	MRID49076001; highest conc tested (censored valule, >4.64 lb a.i./A)	ND	ND	ND	Cannot Exceed	L306

Table L -12 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to habitat[a]

Use Pattern	*Exposure*	*Threshold*	*Value (lb a.i./A)*	*Reference*	*Requirement for Risk Designation if Aquatic Association if no Aquatic Association[b]*		*Requirement for Risk Designation if Aquatic Association[b]*		*Cell ref.[c]*
					MED	*LOW*	*MED*	*LOW*	
4 lb a.i./A twice with a 7 d retreatment interval	Runoff to semi-aquatic areas = 0.4 lb a.i./A	Dicot pre-emergent LOEC for growth	4.64	MRID49076001; >4.64	ND	ND	ND	Cannot Exceed	L307
4 lb a.i./A twice with a 7 d retreatment interval	Runoff to semi-aquatic areas = 0.4 lb a.i./A	Monocot pre-emergent EC25 growth	4.64	MRID49076001; highest conc tested	ND	ND	ND	Cannot Exceed	P306
4 lb a.i./A twice with a 7 d retreatment interval	Runoff to semi-aquatic areas = 0.4 lb a.i./A	Dicot pre-emergent EC25 growth	4.64	MRID49076001; >4.64	ND	ND	ND	Cannot Exceed	P307
6 lb a.i./A once ("maximum single application rate")	Application rate	Lowest of pre-emergent LOECs and EC25s for growth of monocots and dicots	4.64	MRID49076001; highest conc tested (censored valule, >4.64 lb a.i./A)	Cannot Exceed	Cannot Exceed	Cannot Exceed	Cannot Exceed	R311
6 lb a.i./A once ("maximum single application rate")	Application rate	Lowest of post-emergent LOECs and EC25s for growth of monocots and dicots	0.5	No reference provided in Ted tool	Cannot Exceed	Cannot Exceed	Cannot Exceed	Cannot Exceed	R312

Table L -12 Summary of indirect risk designation calculations for terrestrial animals due to potential effects to habitat[a]

Use Pattern	*Exposure*	*Threshold*	*Value (lb a.i./A)*	*Reference*	*Requirement for Risk Designation if Aquatic Association if no Aquatic Association[b]*		*Requirement for Risk Designation if Aquatic Association[b]*		*Cell ref.[c]*
					MED	*LOW*	*MED*	*LOW*	
4 lb a.i./A twice with a 7 d retreatment interval	Application rate	Lowest of pre-emergent LOECs and EC25s for growth monocots and dicots	4.64	MRID49076001; highest conc tested (censored valule, >4.64 lb a.i./A)	Cannot Exceed	Cannot Exceed	Cannot Exceed	Cannot Exceed	S311
4 lb a.i./A twice with a 7 d retreatment interval	Application rate	Lowest of post-emergent LOECs and EC25s for growth of monocots and dicots	0.5	No reference provided in Ted tool	Cannot Exceed	Cannot Exceed	Cannot Exceed	Cannot Exceed	S312

ND = Not dependent (i.e., the specified risk designation (column header) is not dependent on whether the threshold is exceed by the exposure estimate)

[a] The risk designation for indirect effects due to potential effects to habitat is determined in cell DI61 of the 'WoE Matrix' worksheet in the Species_Animal_Template.xlsx file. This table traces the precedents for this cell.

[b] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH. Formulae resulting in a risk designation of "unknown" or "NA" are not explicitly presented herein.

[c] Unless otherwise specified all cell references refer to the Alt worksheet in the Species_Animal_Template.xlsx file.

Table L-13 Summary of mortality risk designation calculations for terrestrial plants[a]

Exposure	*Threshold*	*Monocot Value (lb a.i./A)*	*Dicot Value (lb a.i./A)*	*Reference*	*Requirement for Risk Designation[b]*	
					MED	*LOW*
1 lb a.i./A once ("minimum single application rate")	Direct mortality (NOEL)	2.94	2.94	Reportedly, a NOAEC value was not available so the LOAEC is used. E162475 is cited.	One or more threshold can be exceeded	Cannot exceed
1 lb a.i./A once ("minimum single application rate")	Indirect mortality (LOEL)	2.94	2.94			Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Direct mortality (NOEL)	2.94	2.94			Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Indirect mortality (LOEL)	2.94	2.94			Cannot exceed
6 lb a.i./A once ("maximum single application rate")	Direct mortality (NOEL)	2.94	2.94		Cannot exceed	Cannot exceed
6 lb a.i./A once ("maximum single application rate")	Indirect mortality (LOEL)	2.94	2.94		Cannot exceed	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Direct mortality (NOEL)	2.94	2.94		Cannot exceed	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Indirect mortality (LOEL)	2.94	2.94		Cannot exceed	Cannot exceed

[a] The risk designation for mortality of terrestrial plants is determined in cell DI10 on the 'WoE Matrix' worksheet in the Species_Plant_Template.xlsx file. This table traces the precedents for this cell.

[b] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH.

Table L -14 Summary of growth risk designation calculations for terrestrial plants[a]

Exposure	*Threshold*	*Monocot Value (lb a.i./A)*	*Reference*	*Dicot Value (lb a.i./A)*	*Reference*	*Requirement for Risk Designation[b]*	
						MED	*LOW*
1 lb a.i./A once ("minimum single application rate")	Direct pre-emergence NOEL for growth	4.64	Reportedly, noeffects were observed at the highest concentration tested for pre- and post-emergence exposure to monocots; however, the maximum allowable application rate for malathion was not tested (i.e., 5.1 lbs/A).	4.64	Reportedly, no effects were observed at the highest concentration tested for pre-emergence exposure to dicots.	One or more threshold can be exceeded	Cannot exceed
1 lb a.i./A once ("minimum single application rate")	Direct pre-emergence LOEL for growth	4.64	MRID49076001; highest conc tested	4.64	MRID49076001; >4.64		Cannot exceed
1 lb a.i./A once ("minimum single application rate")	Direct post-emergence NOEL for growth	4.7	MRID49076002; highest conc tested	0.25	E068422; based on 12% reduction in soybean weight		Cannot exceed
1 lb a.i./A once ("minimum single application rate")	Direct post-emergence LOEL for growth	4.7	MRID49076002; highest conc tested	0.5	No reference provided		Cannot exceed

Table L -14 Summary of growth risk designation calculations for terrestrial plants[a]

Exposure	*Threshold*	*Monocot Value (lb a.i./A)*	*Reference*	*Dicot Value (lb a.i./A)*	*Reference*	*Requirement for Risk Designation[b]*	
						MED	*LOW*
4 lb a.i./A once ("upper bound single application rate")	Direct pre-emergence NOEL for growth	4.64	"No effects were observed at the highest concentration tested for pre- and post-emergence exposure to monocots; however, the maximum allowable application rate for malathion was not tested (i.e., 5.1 lbs/A)."	4.64	Reportedly, no effects were observed at the highest concentration tested for pre-emergence exposure to dicots.		Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Direct pre-emergence LOEL for growth	4.64	MRID49076001; highest conc tested	4.64	MRID49076001; >4.64		Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Direct post-emergence NOEL for growth	4.7	MRID49076002; highest conc tested	0.25	E068422; based on 12% reduction in soybean weight		Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Direct post-emergence LOEL for growth	4.7	MRID49076002; highest conc tested	0.5	No reference provided		Cannot exceed

Table L -14 Summary of growth risk designation calculations for terrestrial plants[a]

Exposure	*Threshold*	*Monocot Value (lb a.i./A)*	*Reference*	*Dicot Value (lb a.i./A)*	*Reference*	*Requirement for Risk Designation[b]*	
						MED	*LOW*
6 lb a.i./A once ("maximum single application rate")	Direct pre-emergence NOEL for growth	4.64	"No effects were observed at the highest concentration tested for pre- and post-emergence exposure to monocots; however, the maximum allowable application rate for malathion was not tested (i.e., 5.1 lbs/A)."	4.64	Reportedly, no effects were observed at the highest concentration tested for pre-emergence exposure to dicots.	Cannot exceed	Cannot exceed
6 lb a.i./A once ("maximum single application rate")	Direct pre-emergence LOEL for growth	4.64	MRID49076001; highest conc tested	4.64	MRID49076001; >4.64	Cannot exceed	Cannot exceed
6 lb a.i./A once ("maximum single application rate")	Direct post-emergence NOEL for growth	4.7	MRID49076002; highest conc tested	0.25	E068422; based on 12% reduction in soybean weight	Cannot exceed	Cannot exceed
6 lb a.i./A once ("maximum single application rate")	Direct post-emergence LOEL for growth	4.7	MRID49076002; highest conc tested	0.5	No reference provided	Cannot exceed	Cannot exceed

Table L -14 Summary of growth risk designation calculations for terrestrial plants[a]

Exposure	*Threshold*	*Monocot Value (lb a.i./A)*	*Reference*	*Dicot Value (lb a.i./A)*	*Reference*	*Requirement for Risk Designation[b]*	
						MED	*LOW*
4 lb a.i./A twice with a 7 d retreatment interval	Direct pre-emergence NOEL for growth	4.64	"No effects were observed at the highest concentration tested for pre- and post-emergence exposure to monocots; however, the maximum allowable application rate for malathion was not tested (i.e., 5.1 lbs/A)."	4.64	Reportedly, no effects were observed at the highest concentration tested for pre-emergence exposure to dicots.	Cannot exceed	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Direct pre-emergence LOEL for growth	4.64	MRID49076001; highest conc tested	4.64	MRID49076001; >4.64	Cannot exceed	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Direct post-emergence NOEL for growth	4.7	MRID49076002; highest conc tested	0.25	E068422; based on 12% reduction in soybean weight	Cannot exceed	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Direct post-emergence LOEL for growth	4.7	MRID49076002; highest conc tested	0.5	No reference provided	Cannot exceed	Cannot exceed

[a] The risk designation for growth of terrestrial plants is determined in cell DI15 on the 'WoE Matrix' worksheet in the Species_Plant_Template.xlsx file. This table traces the precedents for this cell.

[b] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH.

Table L-15 Summary of reproduction risk designation calculations for terrestrial plants[a]

Exposure	*Threshold*	*Monocot Value (lb a.i./A)*	*Dicot Value (lb a.i./A)*	*Reference*	*Requirement for Risk Designation[b]*	
					MED	*LOW*
1 lb a.i./A once ("minimum single application rate")	Direct reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).	One or more threshold can be exceeded	Cannot exceed
1 lb a.i./A once ("minimum single application rate")	Indirect reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).		Cannot exceed
1 lb a.i./A once ("minimum single application rate")	Direct reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).		Cannot exceed
1 lb a.i./A once ("minimum single application rate")	Indirect reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).		Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Direct reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).		Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Indirect reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).		Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Direct reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).		Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Indirect reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).		Cannot exceed
6 lb a.i./A once ("maximum single application rate")	Direct reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).	Cannot exceed	Cannot exceed
6 lb a.i./A once ("maximum single application rate")	Indirect reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).	Cannot exceed	Cannot exceed

Table L-15 Summary of reproduction risk designation calculations for terrestrial plants[a]

Exposure	*Threshold*	*Monocot Value (lb a.i./A)*	*Dicot Value (lb a.i./A)*	*Reference*	*Requirement for Risk Designation[b]*	
					MED	*LOW*
6 lb a.i./A once ("maximum single application rate")	Direct reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).	Cannot exceed	Cannot exceed
6 lb a.i./A once ("maximum single application rate")	Indirect reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).	Cannot exceed	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Direct reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).	Cannot exceed	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Indirect reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).	Cannot exceed	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Direct reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).	Cannot exceed	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Indirect reproduction	5.1	5.1	The NOAEC value represents the maximum allowable application rate for malathion (5.1 lbs a.i./A).	Cannot exceed	Cannot exceed

[a] The risk designation for reproduction of terrestrial plants is determined in cell DI21 on the 'WoE Matrix' worksheet in the Species_Plant_Template.xlsx file. This table traces the precedents for this cell.

[b] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if MED requirements are not met, the risk designation is HIGH.

Table L-16 Summary of indirect habitat risk designation calculations for terrestrial plants

Exposure	*Threshold*	*Monocot Value (lb a.i./A)*	*Reference*	*Dicot Value (lb a.i./A)*	*Reference*	*Requirement for LOW Risk Designation[a]*
1 lb a.i./A once ("minimum single application rate")	Pre-emergent Growth LOEL	4.64	MRID49076001; highest conc tested	4.64	MRID49076001; >4.64	Cannot exceed
1 lb a.i./A once ("minimum single application rate")	Pre-emergent Growth EC25	4.64	MRID49076001; highest conc tested	4.64	MRID49076001; >4.64	Cannot exceed
1 lb a.i./A once ("minimum single application rate")	Post-emergent Growth LOEL	4.7	MRID49076002; highest conc tested	0.5	No reference provided in TED tool	Cannot exceed
1 lb a.i./A once ("minimum single application rate")	Post-emergent Growth EC25	4.7	MRID49076002; highest conc tested	4.72	MRID49076001; >4.72	Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Pre-emergent Growth LOEL	4.64	MRID49076001; highest conc tested	4.64	MRID49076001; >4.64	Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Pre-emergent Growth EC25	4.64	MRID49076001; highest conc tested	4.64	MRID49076001; >4.64	Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Post-emergent Growth LOEL	4.7	MRID49076002; highest conc tested	0.5	No reference provided in TED tool	Cannot exceed
4 lb a.i./A once ("upper bound single application rate")	Post-emergent Growth EC25	4.7	MRID49076002; highest conc tested	4.72	MRID49076001; >4.72	Cannot exceed
6 lb a.i./A once ("maximum single application rate")	Pre-emergent Growth LOEL	4.64	MRID49076001; highest conc tested	4.64	MRID49076001; >4.64	Cannot exceed
6 lb a.i./A once ("maximum single application rate")	Pre-emergent Growth EC25	4.64	MRID49076001; highest conc tested	4.64	MRID49076001; >4.64	Cannot exceed
6 lb a.i./A once ("maximum single application rate")	Post-emergent Growth LOEL	4.7	MRID49076002; highest conc tested	0.5	No reference provided in TED tool	Cannot exceed

Table L-16 Summary of indirect habitat risk designation calculations for terrestrial plants

Exposure	*Threshold*	*Monocot Value (lb a.i./A)*	*Reference*	*Dicot Value (lb a.i./A)*	*Reference*	*Requirement for LOW Risk Designation[a]*
6 lb a.i./A once ("maximum single application rate")	Post-emergent Growth EC25	4.7	MRID49076002; highest conc tested	4.72	MRID49076001; >4.72	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Pre-emergent Growth LOEL	4.64	MRID49076001; highest conc tested	4.64	MRID49076001; >4.64	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Pre-emergent Growth EC25	4.64	MRID49076001; highest conc tested	4.64	MRID49076001; >4.64	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Post-emergent Growth LOEL	4.7	MRID49076002; highest conc tested	0.5	No reference provided in TED tool	Cannot exceed
4 lb a.i./A twice with a 7 d retreatment interval	Post-emergent Growth EC25	4.7	MRID49076002; highest conc tested	4.72	MRID49076001; >4.72	Cannot exceed

[a] The risk designation indirect effects to listed terrestrial plants due to potential direct effects to habitat is determined in cell DI39 on the 'WoE Matrix' worksheet in the Species_Plant_Template.xlsx file. This table traces the precedents for this cell.
[b] Shows requirement for the threshold-exposure comparison in order to achieve specified designation, if LOW requirements are not met, the risk designation is MED.

Appendix M
Malathion Mosquito Adulticide Use

The malathion mosquito adulticide use pattern has been treated as an additional use pattern in a wide-area generic group in the main body of this report. EPA has previously conducted ecological risk assessments for mosquito adulticide uses of malathion (U.S. EPA 2007, 2009). In addition, the most recent biological evaluations (BE) (EPA, 2016a) examined the potential risks to listed species and critical habitat from adulticide use. However, the EPA assessments and malathion BE do not properly characterize the geographic extent of the use pattern, fate and behavior of malathion, potential environmental concentrations, or the potential for effects and ultimately risk. Therefore, risk has been significantly overstated for mosquito adulticide uses by the EPA assessments.

This appendix describes the approach taken to characterize potential exposure of listed species and the critical habitat on which they depend to the mosquito adulticide uses of malathion.

Adulticide Use

Application of the malathion adulticide is performed by certified applicators, using very specialized equipment. Mosquito adulticide applications are typically made by dedicated Mosquito Abatement or Control Districts (e.g. Benton County, WA - Mosquito Control District) and other state or local departments of public health. Mosquito Control Districts are local government entities established to protect the public from disease and nuisance associated with mosquitoes within specific boundaries. Applications are based on monitoring of mosquito populations, complaint calls, or may arise from an emergency situation (e.g., hurricane), or public health threat (e.g. Zika virus/malaria).

Spatial Extent of Use[4]

To characterize the use and geographic scope of malathion adulticide in the contiguous United States, data were summarized from four sources. The first was a survey conducted by Angela Beehler of the Benton County WA Mosquito Control District, for the American Mosquito Control Association (AMCA). The second was a survey of sales and distributors conducted by Cheminova Inc./FMC. The third was from publically available usage information from Florida, and the fourth was from publically available usage information from California. Each of these four datasets are described below.

The usage information from the AMCA is based on a series of surveys that covered the United States (Angela Beehler, personal communication 2015)(Figure M-1). There are 1060 U.S. members of the AMCA, and they were asked if they use malathion, and if so, how many applications they make per year (Table M-1). This list of potential users includes organized mosquito control districts that did not answer the email survey, but that would be capable of making mosquito adulticide applications. Figure M-1 shows the spatial extent of the members

[4] Portions of this section (Spatial Extent of Use) was originally prepared by Compliance Services International (CSI) with modification by Intrinsik Environmental Sciences Inc.

who responded to the survey (provided by Angela Beehler, Benton, WA, MCD pers. comm. e-mail: June 1, 2016).

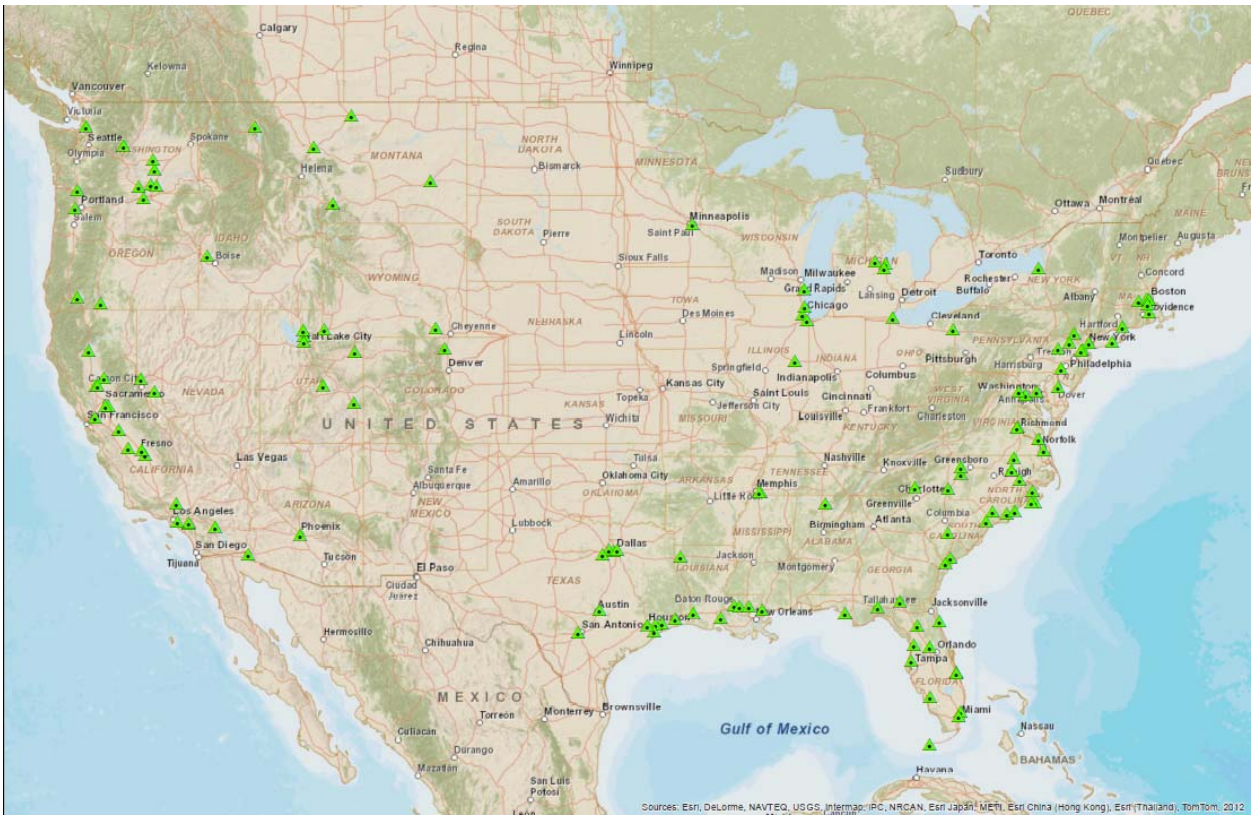


Figure M-1 Spatial extent of AMCA members surveyed in the conterminous US

Only a small number of the responding AMCA members reported using malathion in their mosquito control programs. These members are spatially represented in Figure M-2 (provided by Angela Beehler, Benton, WA, MCD pers. comm. e-mail: June 1, 2016). AMCA members who reported use of malathion also responded to the survey question “On average, how often do you use these products in the areas noted”. For malathion, applications ranged from 1 to a maximum of 10 per year (Table M-1). In some cases, members reported that malathion was used in resistance management programs and thus it may be rotated with other products (e.g. pyrethroids) on occasion.

Table M-1 AMCA Survey Responses to Number of Malathion Applications per Year						
	***Number of Reported Malathion Applications Per Y**ear*					
	1-2	***3-4***	***5-6***	***7-8***	***9-10***	***Total***
Number of Districts	6	6	2	2	10	26
					Range	1 – 10
					Mean	5.2

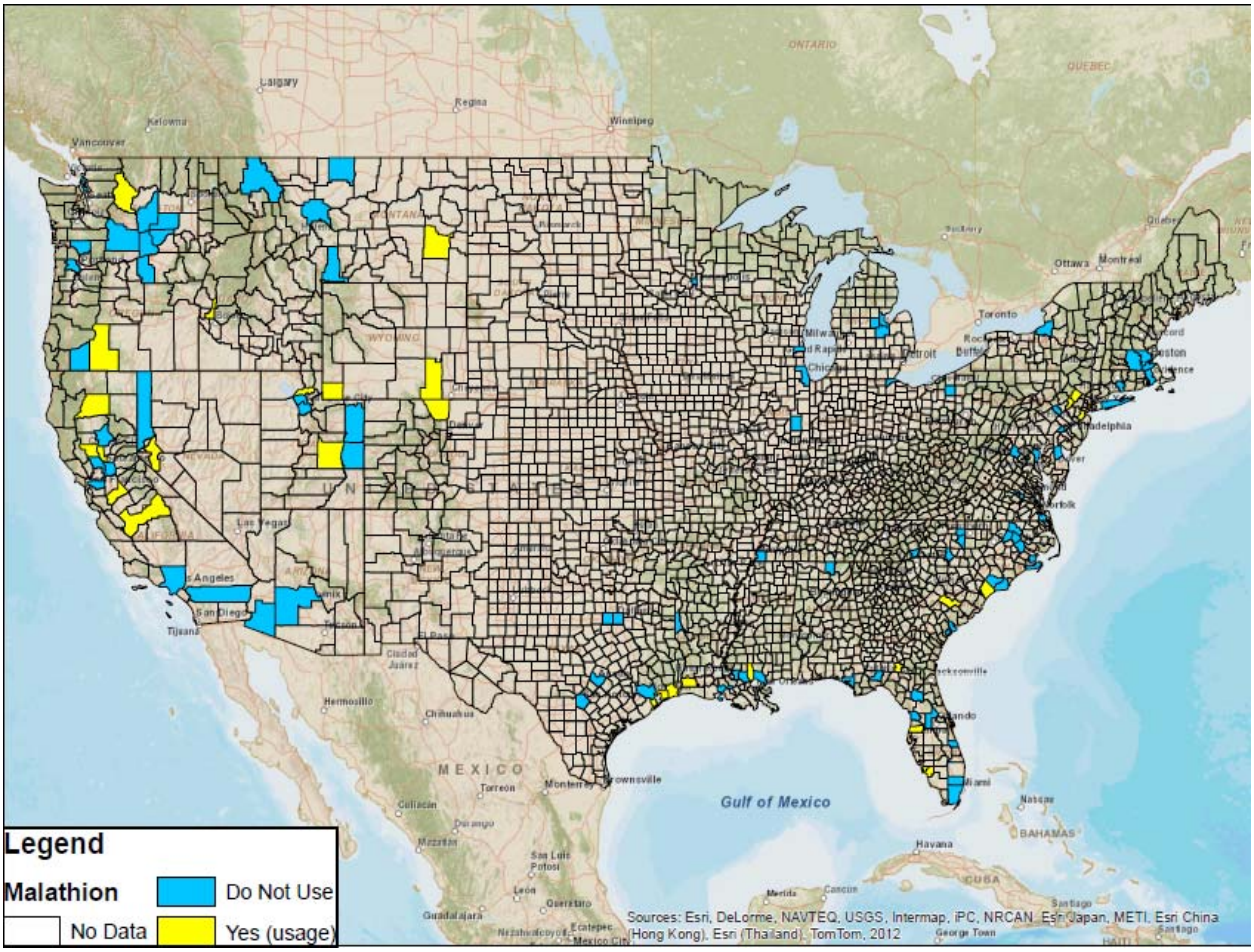


Figure M-2 AMCA survey responses specific to malathion use in the conterminous US

To supplement the AMCA association list, entities that reported use in the California and Florida pesticide databases were added. Then, based on information from Cheminova, a list of entities that purchased malathion in the last three years was obtained. Finally, regional and state vector control associations were asked to provide their member lists and malathion users. It is believed that these various data sources identified as using malathion mosquito adulticide provide the best available information on the coverage of the entities that have recently applied malathion as a ULV mosquito adulticide in the contiguous 48 states. The spatial resolution of the data is at

the county level which is a coarse resolution and can be considered conservative. These data have been provided to the EPA.

To obtain malathion adulticide sales data, Cheminova/FMC tabulated all sales locations of its mosquito adulticide product, for Cheminova/FMC and its major distributor, for the years 2011 to 2014. These sales locations were either assigned to states and counties directly, or assigned to cities or ULV applicators. The complete dataset will be provided to EPA under separate cover as detailed sales information is claimed as confidential business information. For the purposes of public dissemination, the results are provided herein with resolution at the county level (Table M-2). Key points derived from these data are: (a) Scope of use -- although mosquitoes are widely distributed throughout the contiguous 48 states, actual applications for adult mosquito control have been made in only 159 counties in less than half of the states; (b) Volume of use – malathion usage has dropped considerably since 2004. The relatively restricted geographic extent of use is a direct result of the fact that most ultra-low volume applications to control adult mosquitoes are made by Mosquito Abatement Districts or other local, state, or federal entities. Malathion adulticide use has decreased over the last few decades due to increasing preference for pyrethroids. However, because of the limited adulticide toolbox and because of growing resistance to the pyrethroids, OP use including malathion is expected to remain an important tool for resistance management.

Table M-2 Cheminova sales data[a] by county for 2011 to 2014 (Cheminova A/S)

Usage by State/County	*2011 (Oct - Dec)*	*2012*	*2013*	*2014 (YTD)*
TEXAS				
Austin County				x
Brazoria County			x	x
Broward County		x		
Calhoun County		x		
Chambers County	x	x	x	x
Galveston County		x	x	x
Harris County	x	x	x	x
Jefferson County	x	x	x	x
Orange County	x	x	x	x
LOUISIANA				
Calcasieu Parish			x	x
Jefferson Davis Parish		x	x	
FLORIDA				
Dade County	x	x	x	x
Indian River County		x		
Manatee County				x
Orange County		x	x	x
Pasco County			x	x
MISSISSIPPI				
Rankin County			x	x
Washington County		x	x	x
SOUTH CAROLINA				
Charleston County		x	x	x
Georgetown County			x	
Horry County		x	x	x
UTAH				
Salt Lake County		x	x	x
NEBRASKA				

Table M-2 Cheminova sales data[a] by county for 2011 to 2014 (Cheminova A/S)				
Usage by State/County	***2011 (Oct - Dec)***	***2012***	***2013***	***2014 (YTD)***
Wayne County				x
CALIFORNIA				
Stanislaus County				x
COLORADO				
Mesa County			x	
Moffat County			x	
Rio Blanco County				x
WYOMING				
Laramie County		x		x
NEW MEXICO				
San Juan County			x	
GEORGIA				
Lee County			x	

[a] Malathion adulticide is also sold from other minor suppliers and are not captured in Cheminova's sales data.

In addition to these broad county level tabulations of potential mosquito adulticide use sites, data were also obtained for the last 10 years from two states, California and Florida. The purpose of this exercise was to attempt to gauge the use rates, numbers of applications, and application intervals of ULV mosquito adulticide applications for these states, if possible.

Florida data were obtained from the website: http://www.freshfromflorida.com/Divisions-Offices/Agricultural-Environmental-Services/Consumer-Resources/Mosquito-Control/Reports

The dataset covers the years 2003 to 2014. Florida mosquito control data are summarized by active ingredient and county. Gallons of product, pounds of active ingredient, and acres treated are generally available for each county-year combination. Application methods are broken out by aerial and ground. Unfortunately, this Florida database does not provide the timing of applications, the number of applications, or the interval between applications. To estimate the intensity of usage in a particular county, the total county acreages were obtained, and then the total number of acres treated by air or ground were divided by the county acreage. This measure provides a crude estimate of the intensity of air or ground applications in each county.

The California data cover the years 2004 to 2013, and were obtained from the website: http://calpip.cdpr.ca.gov/main.cfm

Only data for non-agricultural applications were summarized. There is a category called Public Health Applications that relates most closely to the ULV mosquito adulticide uses. Data were obtained by county for each year. Timing of applications was included in the database. However, unfortunately, there were several pieces of missing data, including whether the application was made by air or ground and the application rate per acre. As had been done for the Florida data, county acreages were obtained. To estimate the number of acres that could be treated, the total pounds of malathion applied was divided by the maximum ground application rate. This estimated number of acres treated was then divided by the total acreage in the county to arrive at an index of use intensity for that county.

The four datasets were combined to generate a list of counties where ULV malathion mosquito adulticide applications have been made. To ensure that the potential for drift was taken into account, all counties adjacent to the counties where malathion ULV applications were reported to have been made were also included. The results of this tabulation are shown in Figure M-3. Based on the best available data available, ULV malathion applications have been made in 159 counties, and there are 514 adjacent counties for a total of 573 counties with potential for exposure.

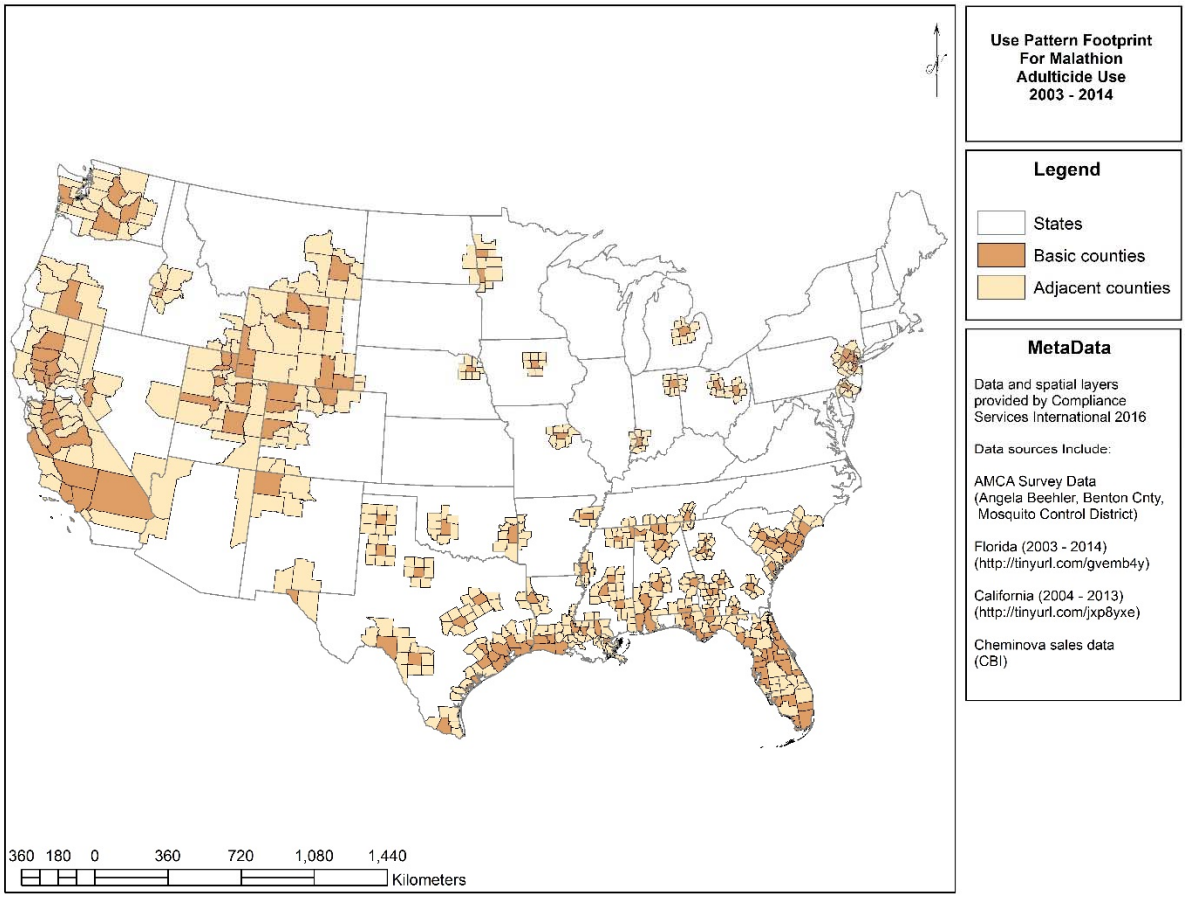


Figure M-3 Best available commercial use pattern footprint for malathion adulticide use (based on data covering 2003 - 2014)

In the Agencies BE for malathion (EPA, 2016a) (Chapter 4 and Appendix 4-5) the Agency suggests, but does not clearly state, that malathion adulticide use has the potential to occur across the entire spatial extent of the US including Alaska, Hawaii, and the US territories. This is the base assumption used in the BE (EPA, 2016a) with respect to the spatial extent of malathion adulticide use. This assumption is likely based on the fact that the malathion adulticide label does not place restrictions on where or when the adulticide may be used. The label is deliberately designed that way to allow for the public health managers maximum flexibility to respond to public health threats (e.g., Zika virus, malaria etc). On federal public lands, the decision to use adulticides would be made based on local policies for land

management. We are aware of an effort by FWS to finalize a national policy for use of pesticides (including mosquito adulticides and larvicides) on federal lands. State lands have varying policies in place. Thus, there are restrictions on pesticide use on public lands and readily available commercial data that EPA has not attempted to collect and apply to characterize the use footprint for adulticides.

Based on the best available commercial data, the analysis presented in this section, as summarized in Figure M-3, clearly indicate the assumption of use across the entire spatial extent of the United States is incorrect. The spatial area identified is considerably smaller than the area considered in the malathion BE (EPA, 2016a). Therefore, the number of species (and critical habitat) that may potentially be exposed to malathion is considerably fewer than ***all*** listed species and their critical habitat as stated in the malathion BE (EPA, 2016a). This will be explored further in the following sections. The *Endangered Species Act* (1973) is silent on accounting for factors that in future may expand the action area, specifically related to the federal action being evaluated (in this case re-registration of a pesticide under FIFRA). Cumulative effects analyses may account for potential other activities *unrelated to the federal action* (e.g. State, municipal, private activities) (FWS/NMFS, 1998). Thus, consideration of the potential for the spatial expansion of adulticide use beyond what the best scientific and commercial data indicates during the evaluation of the federal action under the ESA is unwarranted. EPA's own organophosphate cumulative analysis indicated that cumulative risk from adulticides was anticipated to be a minor issue albeit for human health (EPA, 2002)

To protect public health from vectored diseases, adulticide applications need to be part of the toolbox available to public health agencies and these agencies need the flexibility to use the adulticides when and where needed in order to protect public health. While needed flexibility may lead EPA to conclude that the entire U.S. is a potential use area for an adulticide, this conclusion is not a realistic characterization of actual adulticide use in the U.S. In fact, adulticides are used in relatively limited geographic locations in the US. The spatial data on malathion adulticide use represents the best available scientific and commercial data. Similar data are likely available for other currently-registered adulticides, so the current use footprint can be defined. During the 15 year period between registration reviews, actual use of any single adulticide may expand or contract based on federal and local policies, resistance management needs, and to address public health threats for which the mosquito acts as a vector (e.g., West Nile virus, dengue, encephalitis, Chikungunya, Zika virus). However, it is highly unlikely that the use area for adulticides will expand dramatically over the next 15 years.

There is also a temporal component to the use of adulticides that has not been captured by EPA in the BE. While there may be active mosquito populations throughout the year in a few extreme southern parts of the US, other parts of the country may only have active mosquito seasons for 1 or 2 months each year. The temporal use also includes resistance management efforts of local mosquito control districts. For example, many districts rely on pyrethroids as their primary tool to control adult and larval mosquitoes. To offset growing resistance to pyrethroids, these districts may rotate in an OP once or twice per spray season, or alternate the use of pyrethroids and OPs from year to year.

Finally, adulticide use on public lands may also be restricted based on state and local land use policies. For example, managers of state or federal-owned lands make decisions on a case-by-case basis whether the allow mosquito control (either adulticide or larvicide), what products may be used and under what conditions. There is also a federal policy in development on the use of pesticides on federal lands that, when finalized this year, will impact how and when mosquitocides may be used. EPA has not collected this information and without it, EPA cannot accurately characterize how any mosquitocide is being used on public lands. In addition, on public lands, managers must submit a biological assessment prepared pursuant to ESA Section 7(a)(2) to evaluate the potential effects of mosquito control activities to listed species under the jurisdiction of the Services (e.g., USACE, 2014)

The spatial data on malathion adulticide use described herein represent the best available scientific and commercial data currently available, spatially characterizing the application of malathion adulticide throughout the U.S. This is equivalent to the best scientific and commercially available data to characterize crop footprints (e.g. Crop Data Layer (CDL), AgCensus data etc) for individual crops. The review period for pesticides is every 15 years under the *Food Quality Protection Act* (FQPA). Thus, spatial changes in use patterns such as adulticide application will be re-evaluated every 15 years and changes will be captured at that point. **No other use pattern being evaluated by the Agency is treated in the same way as the adulticides**. Changes in commodity production (e.g. corn for ethanol) are accounted for every 15 years using the best available data (e.g., CDL) and other data (e.g. AgCensus) to capture changes to crop use patterns over time (e.g., use of the last 5 – 10 years of the CDL to account for changes in the crop footprint).

The data in this section therefore, represent the best available characterization of the malathion adulticide use pattern for the purposes of this review under the ESA and will be applied in the assessment.

Adulticide Spray Drift

Malathion adulticide applications are intended to drift to maximize the amount of time that adult mosquitoes will contact the droplets in flight and subsequently be killed. Weidhaus et al. (1970) calculated that a minimum lethal dose of malathion to kill a single mosquito is contained in a 25 µm droplet. Haile et al. (1982) estimated a relationship between mosquito mortality and droplet size. The optimal droplet size from the relationship was calculated to be 10 – 15 µm. Thus, to obtain optimal efficacy for mosquito control, nozzles used in ULV applications of malathion typically have volume median diameters (i.e., VMDs) less than 100 µm, and are angled at 45^{o}, both of which help maximize the time that malathion droplets linger in the air (Mickle et al., 2005; Schleier et al. 2012). In turn, this has the effect of maximizing the potential for drift and thereby minimizing the total quantity of sprayed material that will deposit in any one area. By design, the majority of the malathion sprayed in an agricultural setting will deposit onto the sprayed field. Mosquito control applications are designed to drift. Adulticide clouds have a very low settling velocity and transport characteristics that are similar to aerisols (Schleier et al. 2012). This is the

opposite of malathion products intended for crop use where malathion is deposited in close proximity to the application equipment. Because of the unique characteristics of adulticide clouds, the standard regulatory spray drift models (AGDISP and AgDRIFT) are generally not well suited for use in modeling adulticide applications (Schleier et al. 2012).

Johnson (2014) conducted a review of available field studies (e.g. Mickle et al. 2005; Moore et al. (1993); Tietze et al. (1994, 1996); Knepper et al. 1996) and information on deposition of malathion mosquito adulticide applications in support of a request for information from the Canadian Pest Management Regulatory Agency (PMRA). Of particular interest was a study by Mickle (2005) that examined deposition of Fyfanon ULV (96.5%) malathion from ground and aerial applications. Mickle (2005) conducted field trials in Florida to compare deposition of Fyfanon ULV (96.5%) malathion from ground and aerial applications. For ground applications, a Clarke Grizzly nozzle was mounted 1.85 m above the ground and angled at 45 degrees. The chemical was injected at 6 psi and dispersed using a blower. The flow of chemical was dependent on the speed of the spray truck, with the intent to deliver 60.8 g a.i./ha. For aerial applications, nozzles mounted on the application plane were set to deliver 8.18 L/min flow at 1520 psi. The rate of application was 260 g a.i./ha over the same spray line width. Spray height using the aerial method was set to be 60 m. The study site for ground spray was a vacated sod field with 500 m of upwind fetch. Ground samplers were placed every 10 m from the spray line to 500 m downwind, with an impinger placed at every second sample site to measure drop density and size of the cloud. The study site for the aerial trials took place at a different location. Samplers were placed every 100 m along the roadway, up to 5000 m from the flight line. Impingers were placed every 200 m to determine droplet size and density. For ground applications, peak deposit levels were higher in lower wind conditions, up to 20 gm/ha. Drop densities were greatest 50-150 m from the application site and ranged from 300-500 drops/cm^2. For both aerial and ground applications, four passes were made for each trial, occurring in opposite directions along a 10 km spray line. An application rate that was four times greater than permitted by the label was also used to ensure the chemical would reach the 5000 m samplers. Results suggest that up to 50% of the spray was deposited within the 500 m downwind sample area. Deposition from aerial application ranged from 6-20 g/ha, similar to that for ground applications, with maximum deposit occurring within 500 -1000 m of the flight line. Peak drop density occurred 1 km downwind from the deposit peak. Additionally, drop size was found to decrease with distance away from the flight line and up to 55% of the total emitted malathion was recovered within 5 km. The vast majority of recovered chemical collected within the first 2000 m downwind of application for all aerial trials. It was determined that ground applications produced greater drop densities than did aerial applications when normalizing for malathion released per unit length of spray line.

One additional field study was reviewed here. Schleier et al. (2012) conducted three ground-based ULV field trials, one in each of Elk Grove, CA, Bozeman, MO, and Baton Rouge, LA. The sites chosen had little vegetative structure (i.e. low interception) and a flat topography. The sites were 200 m long and horizontal drift collectors were placed 25 m left and right of center or the plot. Applications were initiated in the evenings (no earlier that 18:00) with most applications later to maximize the number of adult mosquitoes present. Deposition was collected using

ground-level petri dishes and a fluorescent tracer was used to track the presence of the droplets using a fluorometer. The data points collected from the three field trials were combined (N=1067) and used for statistical analysis. Regression analysis of the data yielded a relationship between numerous variables (e.g., wind speed, application rate, flow rate, density VMD, and others). The model describing these relationships was recommended for use in evaluating deposition and potentially as a tool for regulatory decision making. However, subsequently the findings by Schleier et al. (2012) have come under scrutiny. Teske et al. (2015) reviewed the Schleier et al. (2012) paper that summarized the workings of a regression model used to predict dispersion of ground-based ultra-low volume (ULV) pesticide applications used for mosquito control. The MULV-Disp model was developed as an improvement to the widely-used AgDISP model for ULV application. The MULV-Disp was developed from field tests that evaluated deposition of seven ULV formulations up to 180 m downwind of application. Pesticides were applied from a spray gun mounted on a pickup truck, which is typical for application of mosquito adulticides, to flat land with little vegetation. Teske et al. (2015) stated that application to flat, non-vegetated surfaces was a model flaw, as it reduced applicability for most application scenarios and decreased deposition. Teske et al. (2015) was most critical of the data collection methods used by Schleier et al. (2012) for meteorological data. The Hobo Micro Station Data Logger has very low accuracies and high data collection thresholds (Teske et al., 2015). Therefore, data like wind speed, which was reported as lower than the stall speed for the data capturing device, were likely incorrect and skewed the results (Teske et al., 2015).

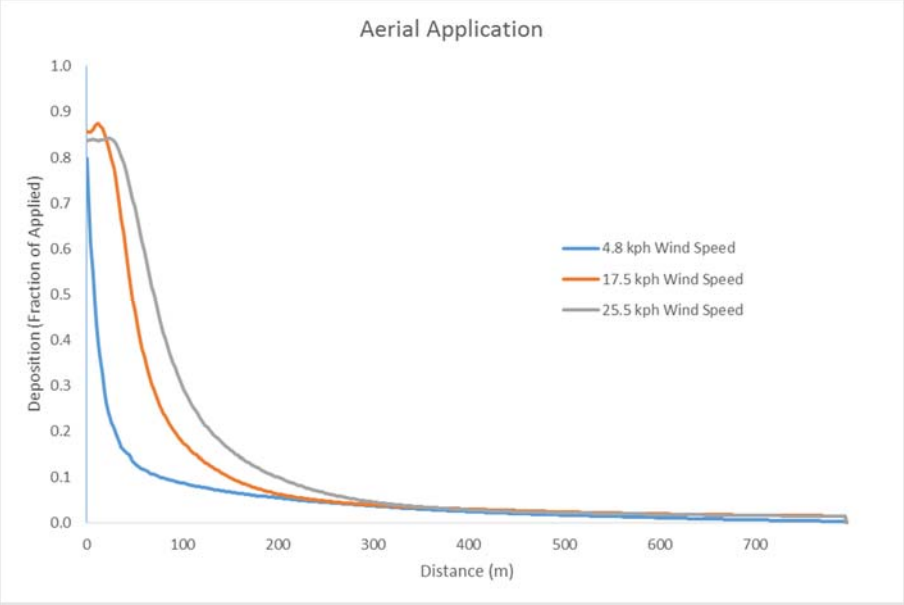
Teske et al., (2015) was also highly critical of the collection methods for pesticide deposition used by Schleier et al. (2012). In fact, Schleier et al. (2012) used flat, glass Petri dishes for collection, which reportedly have less than 20% collection accuracy when compared to rough dishes (Thistle et al., 2009; Teske et al., 2015). Compounding matters, Schleier et al. (2012) failed to correct for collection efficiency, which contributes a significant amount of uncertainty to calculations. Schleier et al. (2012) measured an average deposition of 10.4% within the 180 m field. Considering the application of aerosols, a deposition field of 180 m is extremely short (Teske et al., 2012). Additionally, the 90% of mass that is unaccounted for may have been the result of analytical errors, lack of correction for collection efficiency, incorrect calculations of wind speed and other meteorological conditions, or any number of other reasons. Basing all calculations on a deposition of 10.4% is highly uncertain.

Teske et al. (2015) also found a number of issues with regards to the regression model used in MULV-Disp. For example, when applied from a truck with a gun pointed upward at 45º, one would expect minimal deposition adjacent to the truck, with increasing deposition at increasing distance then decreasing deposition once the peak has been reached (Teske et al., 2015; Mickle, 2005). Instead, the regression used in MULV-DISP has peak deposition at the point of release and decreasing deposition at increasing distance from the site of application.

In terms of model sensitivity, Teske et al. (2015) found that the MULV-Disp model incorrectly predicts deposition when the application rate is adjusted. For example, Schleier et al. (2012) showed a 59.5% decrease in deposition within 180 m of application when the application rate was increased, while in actual fact deposition should increase if the application rate is increased

(Teske et al., 2015). Furthermore, an increase in flow rate would increase the droplet size and in turn, increase deposition, but MULV-Disp predicts a decrease in deposition when flow rate is increased. Therefore, the model described in Schleier et al. (2012) was not used to estimate ground application of ULV malathion as an adulticide.

Johnson (2014) determined that the data generated by Mickle, et al. (2005) constitute the best available information to estimate deposited residues of malathion following aerial and ground ULV applications for mosquito control. Based on Mickle et al. (2005), deposited residues of malathion following aerial and ground ULV applications at 260 g ai/ha and 60.8 g ai/ha, respectively, can be estimated for risk assessment purposes in a few different ways. First, maximum average deposited residues were the same (approximately 0.070-0.075 µg/cm²), regardless of application method. Second, over the first 93 m (swath width) downwind from the spray line, average deposition is approximately 0.03 µg/cm² following ground applications and practically negligible following aerial applications. Third, based on integrated deposition, average deposited residues are approximately 0.028 µg/cm² across the 500 m downwind from a ground application and approximately 0.022 µg/cm² across the 5 km downwind from an aerial application. A protective yet representative exposure estimate would result when potential post-application residential exposures are estimated using one or all of the measured deposition values from Mickle et al. (2005). In addition, Mickle, et al. (2005) successfully parameterized the regulatory spray drift model AgDISP v8.13 to simulate ULV mosquito control application conditions. The input parameter values for equipment, application and nozzles are used to predict deposition for different application rates, conditions or chemicals malathion ULV mosquito control applications using AgDISP v8.27 in this assessment. The spray drift curve for both aerial and ground application from Mickle et al. (2005) is depicted in Figure M-4.



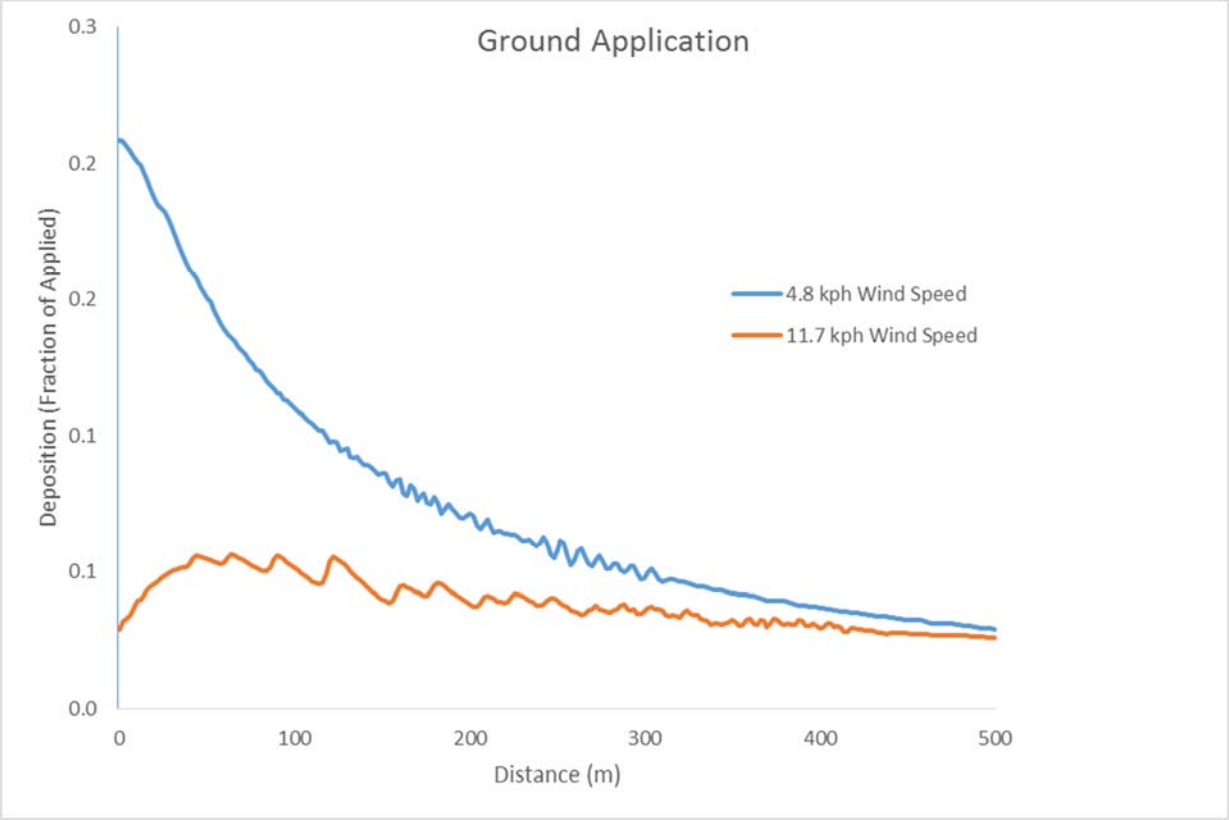


Figure M-4 Aerial and ground deposition spray drift curves from Mickle et al. (2005)

The Mickle et al (2005) spray drift curves were used in the terrestrial ESA assessment to evaluate potential exposure of plants and wildlife to mosquitocide spray.

Aquatic Modeling

For aquatic taxa, environmental exposure concentrations (EECs) were calculated for illustration purposes, for the three flowing and three static habitat bins as characterized in Table M-3. The EPA tool, Pesticide Water Calculator (PWCv1.5) was used to estimate EECs for the aquatic bins 2-7 for malathion adulticide applications. In these simulations, malathion was applied six times annually at a rate of 0.067 lbs ai/A per application applied three days apart. This represents a higher than average annual application frequency as captured in the AMCA survey of AMCA members (Table M-1)

Table C-3 Characteristics of aquatic habitat bins (EPA, 2016a)

Generic Habitat	*BIN#*	*Depth (m)*	*Width (m)*	*Length (m)*	*Flow (m^3/s)*	*Velocity (m/s)*
Low flow	2	0.1	2	Length of field	0.001 - 1	0.005
Medium flow	3	1	8	Length of field	1 - 100	0.14
High flow	4	2	40	Length of field	>100	1.4
Low volume	5	0.1	1	1	Static	Static
Medium volume	6	1	10	10	Static	Static
High volume	7	2	100	100	Static	Static
Nearshore intertidal	8	0.5	50	Length of field	-	-

Table C-3 Characteristics of aquatic habitat bins (EPA, 2016a)						
Generic Habitat	***BIN#***	***Depth (m)***	***Width (m)***	***Length (m)***	***Flow (m^3/s)***	***Velocity (m/s)***
Nearshore subtidal	9	5	200	Length of field	-	-
Offshore marine	10	200	300	Length of field	-	-

The same application efficiency (29%) and aquatic bin specific drift fractions listed in BE Appendix 3-3 (Table B3-3.3)(EPA, 2016a) were used in the modeling. Runoff curve numbers were lowered so that no runoff was simulated. Adulticides are applied to remain in the air to maximize the potential for contact with flying mosquitoes. The Mickle et al. (2005) study described above indicated that deposition does occur slowly over fairly large distances depending upon wind speed (Figure M-4). The water half-life was set to 3.27 days, hydrolysis half-life of 6.21 days, benthic half-life of 7.64 days, and a K_{OC} of 151 L/kg.

The predicted aquatic EECs using PWCv1.5 are provided in Table M-4. One day EECs ranged from 0.00066 µg/L (Bin 4) to 8.82 µg/L (Bin 5). These EECs are used in the NESA to evaluate risks to aquatic organisms (or terrestrial organisms with an aquatic life-stage) in Step 1.

Table M-4 Aquatic EECs for malathion ULV adulticide application at the maximum applications rate (0.234 lb/A) and 6 applications with one day interval.

Bin#	*Generic Habitat*	*Drift Fraction**	*Depth (m)*	*Width (m)*	*Length (m)*	*Flow (m^3/s)*	*Water EECs (ppb)*				
							Peak	*1-Day*	*4-Day*	*21-day*	*6-Day*
2	Low flow	0.076	0.1	2	356.8	0.001	5.75	2.86	1.60	1.01	0.36
3	Moderate flow	0.072	1	8	356.8	1	0.54	0.018	0.0089	0.0051	0.0018
4	High flow	0.053	2	40	356.8	100	0.20	0.00066	0.00033	0.00019	0.000066
5	Low volume	0.077	0.1	1	1	0	10.10	8.82	7.27	5.54	2.12
6	Moderate flow	0.071	1	10	10	0	1.11	1.00	0.85	0.65	0.25
7	High volume	0.035	2	100	100	0	0.28	0.25	0.21	0.16	0.06

Uncertainty

Spatial

- The AMCA member survey collected responses from 21% of the members surveyed. Therefore, there could be more malathion adulticide use across the U.S. than the AMCA survey results depict. However, the combined AMCA survey results, Florida and California dataset, and Cheminova sales data help to reduce this uncertainty source and combined these data represent the best currently available data.

Terrestrial Modeling

- Mickle et al. (2005) conducted five aerial and five ground spray tests in open field conditions. Thus, it is one of the most extensive, conservative drift studies available for ULV adulticide applications. All meteorological conditions were typical of standard operating conditions. The open field applications also minimized spray drift interception (by vegetation) and maximized drift distances. Therefore, the drift curves are considered conservative for use in the risk assessment. The presence of vegetation could alter estimates and thus there is uncertainty associated with topography.

- Mickle et al. (2005) optimized AGDISP to predict maximum deposits drift deposits that were equivalent to those measured in the field. By adjusting AGDISP to account for Mickle et al. (2005), it is possible to use AGDISP to model ULV adulticide application. There are uncertainties with this process including potential differences in results due to wind speeds, for example. This uncertainty is not expected to overly influence the risk assessment as multiple wind speeds were evaluated for both ground and aerial application in the Mickle et al. (2005) study.

Deposition

- In the terrestrial assessment, application efficiency was assumed to be 0.29 as per the approach used by EPA in the biological evaluation (EPA, 2016a). There is some uncertainty with respect to this value. However, it is highly unlikely that adulticide application efficiency would exceed 1 at any time given the equipment and delivery methods used. The US Forestry Service indicated that point deposition fractions could exceed 1 (EPA, 2016a – Appendix 3-3). This is true for crop ground applications at the edge of field when multiple swaths are considered. It is not true for mosquitocide applications where equipment is angled at 45^{o} for ground applications and one swath. The application efficiency of 0.29 is considered a reasonable estimate for use in the evaluation of adulticide applications at screening. In a refined assessment this variable could be made into a distribution to capture uncertainty about the variable and its impact on exposure.

- Topography of the area in which an adulticide is sprayed as well as the method used (ULV or thermal fog aerosol) can have a significant impact on deposition and the effectiveness of the application (Britch et al. 2010). Therefore, rate of deposition may be an excellent variable to address with a distribution of rates in a probabilistic model in future assessments to capture uncertainty and its impact on exposure.

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