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CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR PESTICIDE CUMULATIVE RISK ASSESSMENT & ASSESSMENT OF LIFESTAGE SUSCEPTIBILITY

Series on Testing & Assessment
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IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris 2017

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This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

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FOREWORD

OECD member countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Q)SAR, chemical categories and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches for Testing and Assessment (IATA). There is a need for the investigation of the practical applicability of these methods/tools for different aspects of regulatory decision-making, and to build upon case studies and assessment experience across jurisdictions.

The objective of the IATA Case Studies Project is to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use. The aim is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies.

This case study was developed by the United States for illustrating practical use of IATA and submitted to the 2016 review cycle of the IATA Case Studies project. This case study was reviewed by the project team and revised to consider the comments from reviewers. The document was endorsed at the1st meeting of the Working Party on Hazard Assessment in June 2017.

The following four case studies were also reviewed in the project in 2016 and are published with this case study:

1. CASE STUDY ON THE USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT FOR THE REPEATED-DOSE TOXICITY OF PHENOLIC BENZOTRIAZOLES, ENV/JM/MONO(2017)23, Series on Testing & Assessment No. 271.

2. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT OF 90-DAY RAT ORAL REPEATED-DOSE TOXICITY FOR SELECTED N-ALKANOLS: READ-ACROSS, ENV/JM/MONO(2017)25, Series on Testing & Assessment No. 273.

3. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT OF 90-DAY RAT ORAL REPEATED-DOSE TOXICITY FOR SELECTED 2-ALKYL-1-ALKANOLS: READ-ACROSS, ENV/JM/MONO(2017)26, Series on Testing & Assessment No. 274.

4. CHEMICAL SAFETY ASSESSMENT WORKFLOW BASED ON EXPOSURE CONSIDERATIONS AND NON-ANIMAL METHODS, ENV/JM/MONO(2017)27, Series on Testing & Assessment No. 275.

In addition, a considerations document summarizing the learnings and lessons of the review experience of the case studies is published with the case studies:

REPORT ON CONSIDERATIONS FROM CASE STUDIES ON INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA) -Second Review Cycle (2016)- ENV/JM/MONO(2017)22, Series on Testing & Assessment No. 270.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

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INTRODUCTION

The Federal Food, Drug, and Cosmetic Act (FFDCA), Section 408(b)(2)(D)(v) requires EPA to take into account "available evidence concerning the *cumulative effects* of such [pesticide] residues and other substances that have a *common mechanism of toxicity".* The Office of Pesticide Programs (OPP) has developed two guidance documents that aid in addressing this requirement:

- Guidance For Identifying Pesticide Chemicals and Other Substances that have a Common Mechanism of Toxicity (USEPA, 1999a) which describes the process for establishing CMGs;

- *Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity* (USEPA, 2002a) which describes the steps used in conducting cumulative risk assessment).

This case study provides insight into how the USEPA's Office of Pesticide Programs (OPP) conduct cumulative risk assessments. At EPA-OPP, a cumulative risk assessment begins with the identification of a group of chemicals, called a common mechanism group, that induce a common toxic effect by a common mechanism of toxicity. Pesticides are determined to have a "common mechanism of toxicity" if they act the same way in the body--that is, the same toxic effect occurs in the same organ or tissue by essentially the same sequence of major biochemical events. The organophosphate pesticides (OPs) were established as the first common mechanism group by EPA in 1999 (USEPA, 1999b). EPA-OPP has conducted multiple cumulative risk assessments and has extensive experience applying this guidance.[1]

OPs share the ability to bind to and phosphorylate the enzyme acetylcholinesterase (AChE) in both the central (brain) and peripheral nervous systems, inhibiting acetylcholinesterase, and ultimately leading to cholinergic signs. In 2006, the USEPA-OPP developed a cumulative risk assessment for the OPs which include specific evaluation of lifestage susceptibility using the comparative cholinesterase assay, a study specifically designed to assess various early lifestages (fetal, pregnant females, post-natal), across duration (single dose, repeated dose; USEPA, 2002b), for the molecular initiating event in the OP adverse outcome pathway (AOP). The information contained in this case study was largely taken from:

- U.S. Environmental Protection Agency. 2006. Updated Organophosphorous Pesticide Cumulative Risk Assessment, July 31, 2006. Office of Pesticide Programs, U.S. Environmental Protection Agency. Washington, D.C. https://www.regulations.gov/document?D=EPA-HQ-OPP-2006-0618-0002

The 2006 cumulative risk assessment for the OPs describes the details of the cumulative hazard and exposure assessments. This case study provides limited details on many aspects of the assessment. In brief, OP exposure in food, water, and residential/non-occupational exposure pathways were considered. These exposure assessments evaluated those OPs for registered in the U.S. in 2006 or had import tolerances at that time. The 2006 cumulative risk assessment of the OPs is highly complex, using probabilistic exposure assessment methods and monitoring data, along with consideration of realistic, geographically and demographically explicit co-exposure patterns. Description of the exposure assessments and approach to derive probabilistically-derived margins of exposure (MOEs) are beyond the scope of this case study. The 2006 cumulative risk assessment was used to support tolerance reassessment and reregistration of OP pesticides as required by the FQPA (https://www.epa.gov/pesticide-reevaluation/reregistration-and-other-review-programs-predating-pesticide-registration).

[1] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/cumulative-assessment-risk-pesticides

Tolerance reassessment and reregistration are programs under the FQPA which require EPA to reassess existing uses of pesticides.

1. PURPOSE

The purpose of the case study is to highlight how AOP knowledge can be used to design a testing strategy to focus on potential for life susceptibility for an entire class of pesticides and how the associated data have been used to assess cumulative risk. The case study provides a short description of the protocol and results of comparative cholinesterase studies (CCA studies) for OPs. CCA studies compare AChE inhibition across different lifestages; nearly all OPs registered in the USA have these studies. CCA studies have simple, straightforward study designs, are simple to conduct, use fewer animals and are less expensive compared to developmental neurotoxicity (DNT) guideline. There is a large database of high quality CCA studies. DNT studies are available for >20 OPs. However, none of these studies have provided endpoints or effects at doses lower than those in the CCA studies. Furthermore, the DNT studies are resource intensive requiring > 1100 animals and require a year or more to conduct. The available DNT studies for OPs show no consistent trends for adverse outcome. Moreover, given the lack of consistent apparatus used in the studies, high degree of variability in neurobehavioral measurements, and lack of standardization of morphometric measures, interpretation of DNT studies is challenging.

Target chemical(s)/category definition

Organophosphate pesticides.

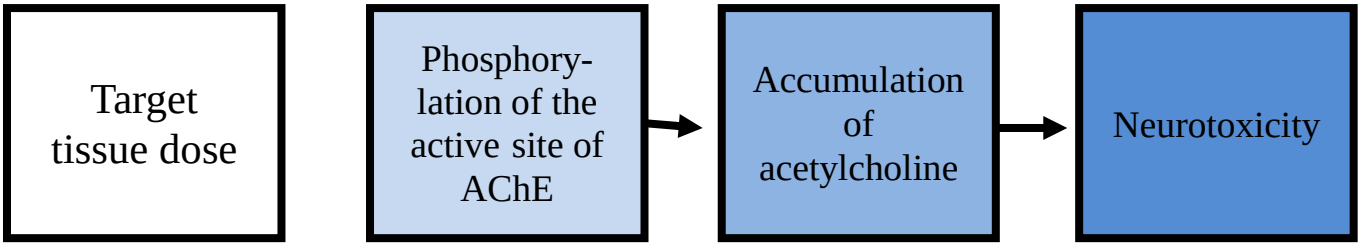
Endpoint(s)

Inhibition of acetylcholinesterase in both the central (brain) and peripheral nervous systems.

2. HYPOTHESIS FOR THE ANALOGUE APPROACH/CATEGORY

The OPs were established as the first common mechanism group by EPA in 1999 (USEPA, 1999b). OPs share the ability to bind and to phosphorylate the enzyme acetylcholinesterase (AChE) in both the central (brain) and peripheral nervous systems. When acetylcholinesterase is inhibited, acetylcholine accumulates and cholinergic toxicity results due to continuous stimulation of cholinergic receptors throughout the central and peripheral nervous systems which innervate virtually every organ in the body. Some OPs are active as the parent compound but some require activation to the oxon metabolite and both are considered in the cumulative risk assessment.

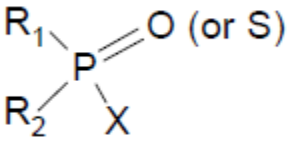
The OECD AOP wiki lists AChE leading to acute mortality in fish as an AOP under development (thehttps://aopwiki.org/wiki/index.php/Aop:16).



3. SOURCE CHEMICALS/CATEGORY MEMBERS

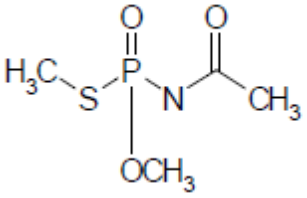
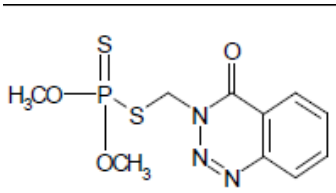
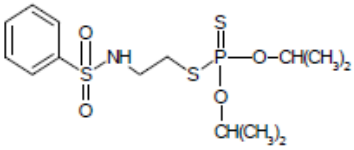
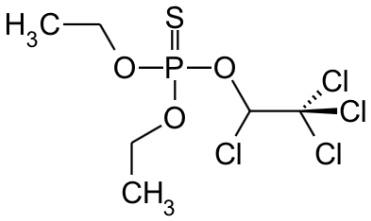
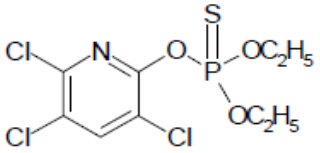
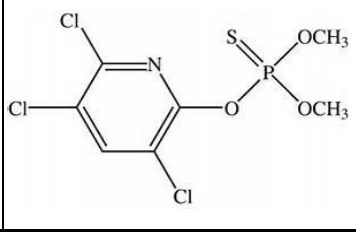
Table 1 provides the list of the OPs included in the USEPA's 2006 cumulative risk assessment of the OPs; the list was derived from registered OPs in the US at that time.

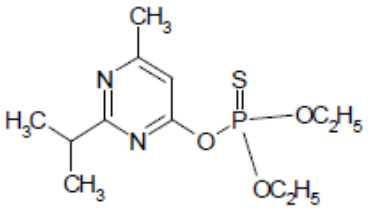
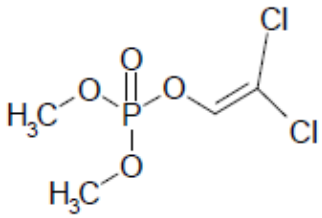
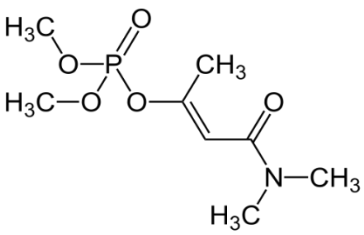
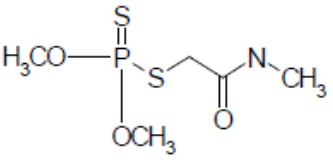
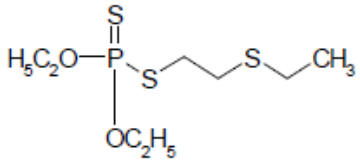
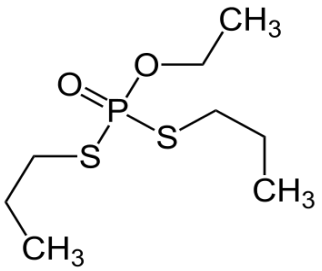
Organophosphorus pesticides are characterized by the general formula:

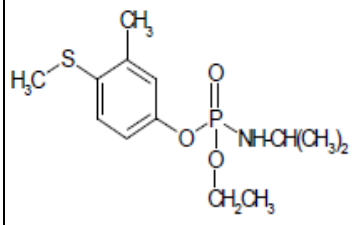
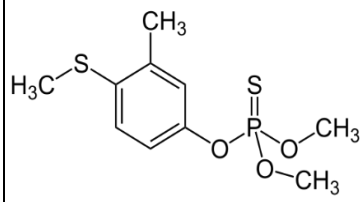
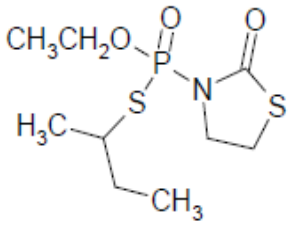
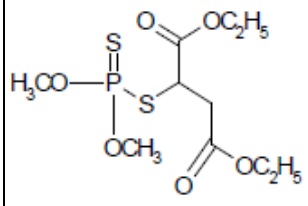
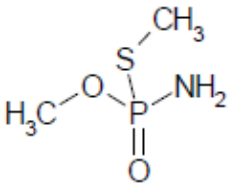


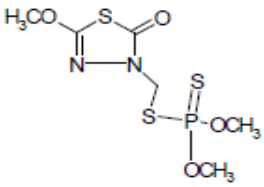
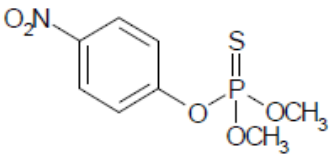
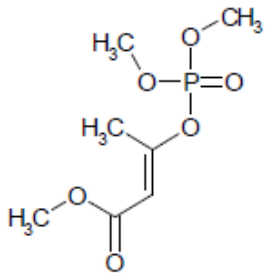
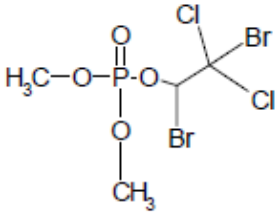
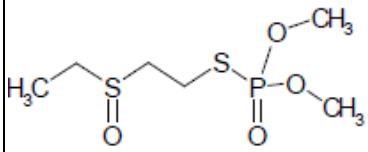
where X is a leaving group of variable structure and R_1 and R_2 are alkoxy, amino, thioalkyl or other substituents Organophosphorus pesticides with a P = O group are direct acting cholinesterase inhibitors, whereas those with a P = S group are relatively poor cholinesterase inhibitors until they undergo metabolic activation by cytochromes P450 or flavin monooxygenases to the oxon form (i.e., with a P = O group). Organophosphorus pesticides vary in their *in vivo* potency due to their structural, steric, and hydrophobicity properties, as well as differences in tissue distribution and detoxification and other toxicokinetic characteristics.

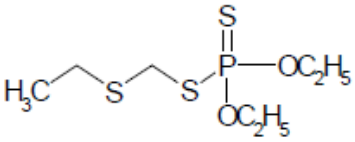
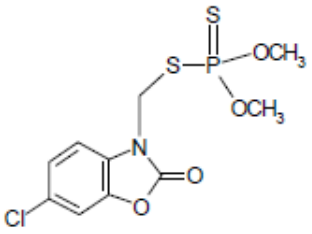
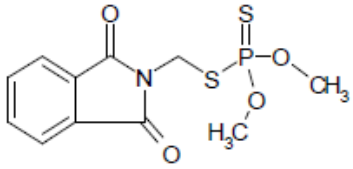
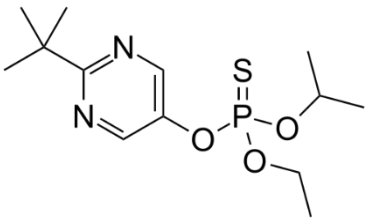
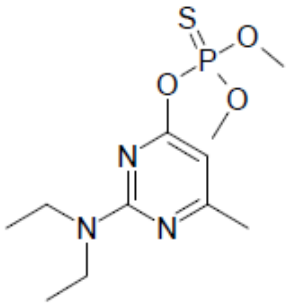
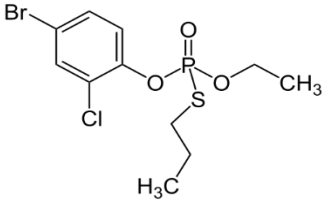
Table 1. Structures of the Organophosphorus Pesticides Included in this Document

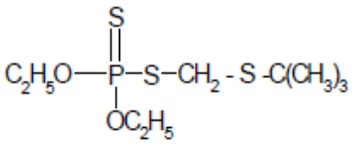
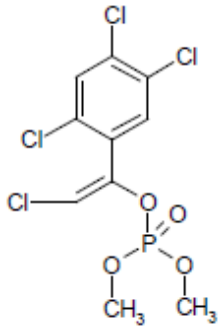
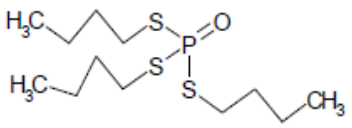
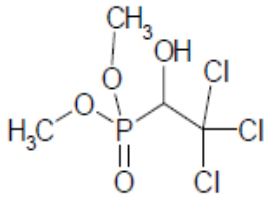
Trade Name	Molecular Structure	Chemical Name	CAS Reg. No.
Acephate		O,S-Dimethyl acetylphosphoramido-thioate	30560-19-1
Azinphos-methyl		O,O-dimethy-S-((4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl)phos-phorodithioate	86-50-0
Bensulide		O,O-diisopropyl phosphorodithioate S-ester with N-(2-mercaptoethyl)-benzenesulfonamide (8CI)	741-58-2
Chlorethoxyfos		Diethoxy-sulfanylidene-(1,2,2,2-tetrachloroethoxy)phosphorane	54593-83-8
Chlorpyrifos		O,O-Diethyl O-(3,5-trichloro-2-pyridinyl) phosphorothioate	2921-88-2
Chlorpyrifos methyl		O,O-dimethyl O-(3,5,6-trichloropyridin-2-yl) phosphorothioate	5598-13-0

Trade Name	Molecular Structure	Chemical Name	CAS Reg. No.
Diazinon		O,O-Diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate	333-41-5
Dichlorvos		2,2-Dichlorovinyl dimethyl phosphate	62-73-7
Dicrotophos		[(E)-4-dimethylamino-4-oxobut-2-en-2-yl] dimethyl phosphate	141-66-2
Dimethoate		O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate	60-51-5
Disulfoton		Phosphorodithioic acid, O,O-diethyl-S-[2-(ethylthio)ethyl] ester (9CI)	298-04-4
Ethoprop		1-(ethoxy-propylsulfanylphosphoryl)sulfanylpropane	13194-48-4

Trade Name	Molecular Structure	Chemical Name	CAS Reg. No.
Fenamiphos		O-Ethyl-O-(3-methyl-4-methyl-thiophenyl)isopropylphosphorami-date	22224-92-6
Fenthion		O,O-Dimethyl O-[3-methyl-4-(methylsulfanyl)phenyl] phosphorothioate	55-38-9
Fosthiazate		(RS)-S-sec-butyl-O-ethyl-(2-oxo-1,3-thiazolidin-3-yl)phosphonothioate	98886-44-3
Malathion		O,O-Dimethyl phosphorodithioate of diethyl mercaptosuccinate	121-75-5
Methamidophos		O,S-Dimethyl phosphoramidothioate	10265-92-6

Trade Name	Molecular Structure	Chemical Name	CAS Reg. No.
Methidathion		O,O-Dimethyl phosphorodithioate, S-ester with 4-(mercaptomethyl-2-methoxy-1,3,4-thiadiazolin-5-one	950-37-8
Methyl parathion		O,O-Dimethyl-O-4-nitrophenyl phosphorothioate	298-00-0
Mevinphos		2-Butenoic acid, 3-((dimethoxyphosphinyl)oxy)-, methyl ester (9CI)	7786-34-7
Naled		1,2-Dibromo-2,2-dichloroethyldimethyl phosphate	300-76-5
Oxydemeton -methyl		S-[2-(Ethylsulfinyl)ethyl] O,O-dimethyl phosphorothioate	301-12-2

Trade Name	Molecular Structure	Chemical Name	CAS Reg. No.
Phorate		O,O-Diethyl S-[(ethylthio)methyl] phosphorodithioate	298-02-2
Phosalone		Phosphorodithioic acid, S-((6-chloro-2-oxo-3(2 H)-benzoxazolyl)methy l) O,O-diethyl ester	2310-17-0
Phosmet		O,O-Dimethyl phosphorodithioate S-ester with N-(mercaptomethyl) phthalimide	732-11-6
Phostebupirim		O-Ethyl O-isopropyl O-[2-(2-methyl-2-propanyl)-5-pyrimidinyl] phosphorothioate	96182-53-5
Pirimiphos-methyl		O-(2-diethylamino-6-m ethylpyrimidinyl) O,O-dimethyl phosphorothioate	29232-93-7
Profenofos		4-bromo-2-chloro-1-[ethoxy(propylsulfanyl) phosphoryl]oxybenzene	41198-08-7

Trade Name	Molecular Structure	Chemical Name	CAS Reg. No.
Terbufos		S-[[(1,1-dimethylethyl)thio]methyl]O,O-diethyl phosphorordithioate	13071-79-9
Tetrachlorvinphos		(Z)-2-Chloro-1-(2,4,5-trichlorophenyl) vinyl dimethyl phosphate	961-11-5
Tribufos		S,S,S-Tributyl phosphorotrithioate	78-48-8
Trichlorfon		Dimethyl (2,2,2-trichloro-1- hydroxyethyl) phosphonate	52-68-6

Before the cumulative risk of exposure to OPs can be quantified, the relative toxic potency of each OP must be determined based on an index chemical. EPA-OPP's cumulative risk assessment guidance defines the index chemical as the chemical used for the point of reference in standardizing the common toxicity of the chemical members of the group. The index chemical should have a clearly defined dose-response, be well defined for the common mechanism of toxicity, and have a toxicological/biological profile for the common toxicity that is representative of the common mechanism effect. The determination of relative toxic potency should be calculated using a uniform basis of comparison, by using, to the extent possible, a common response derived from the comparable measurement methodology, species, and sex for all the exposure routes of interest.

As part of the hazard analysis, all relevant responses were evaluated to identify the most appropriate endpoint pertaining to the common mechanism of toxicity and to determine which endpoint(s) provide(s) a uniform and common basis for determining the relative potency of the cumulative assessment group. OPs exert their neurotoxicity by binding to and phosphorylating the enzyme acetylcholinesterase in both the central (brain) and peripheral nervous systems. There are laboratory animal data on OPs for cholinesterase activity in plasma, red blood cell (RBC) and brain, as well as behavioral or functional neurological effects in submitted guideline studies. Behavioral changes in animal studies usually occur at higher doses compared to doses needed to inhibit cholinesterase. Also, behavioral measures are limited in terms of the scope of effects assessed and the

measurements employed. Plasma, RBC, and brain cholinesterase inhibition were initially considered potential endpoints for extrapolating risk to humans in the OP cumulative risk assessment. Brain AChE data were used as the source of data for relative potency and points of departure (PoDs) as estimates of relative potency based on brain AChE have tighter confidence intervals and therefore will confer less uncertainty on cumulative risk estimates compared to relative potency estimates based on RBC. Also, brain AChE data represent a direct measure of the common mechanism of toxicity as opposed to using surrogate measures. OPP has elected to estimate relative potencies and PoDs using measurements where cholinesterase inhibition in the laboratory animal is not changing with time. OPP defines this point where continued dosing at the same level results in no further increase in enzyme inhibition as steady state. OPP has shown previously that steady state is reached by approximately 21 to 28 days of exposure (USEPA, 2001b). Relative potency should be based whenever possible on data from the same species and sex to provide a uniform measure of relative potency among the chemical members of the cumulative assessment group (USEPA, 2002a). Under FIFRA, toxicology studies in various species (e.g., dog, mouse, rat and rabbit) are submitted to OPP. For the OPs, toxicology studies in the adult rat provided the most extensive cholinesterase activity data for all routes, compartments, and both sexes. AChE data were collected from multiple study types such as subchronic, chronic/cancer, comparative cholinesterase studies, and subchronic neurotoxicity.

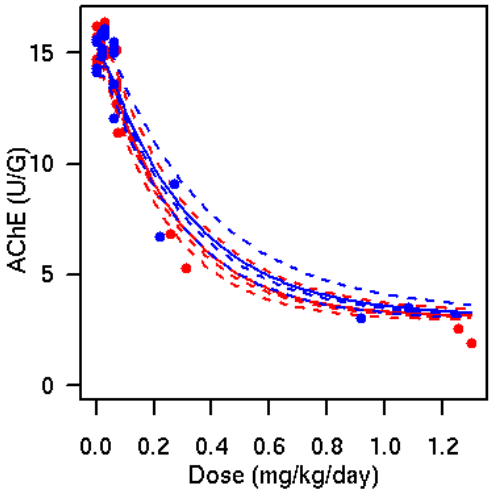
As described in the guidance document for cumulative risk assessment (USEPA, 2002a), dose-response modeling is preferred over the use of NOAEL/LOAELs (i.e., no or lowest observed adverse effect levels) for determining relative toxicity potency. NOAELs and LOAELs do not necessarily reflect the relationship between dose and response for a given chemical, nor do they reflect a uniform response across different chemicals. In the analysis of the oral toxicity data, benchmark dose (BMD) modeling has been used to determine the toxic potency of the OPs. The central estimate on the BMD provides an appropriate measure for comparing chemical potency. In this cumulative risk assessment, the BMD_{10}, the central estimate of a benchmark dose associated with 10% AChE, was selected as the response level for developing RPFs (relative potency factors) and PoDs. In the context of cumulative risk assessment, the PoD is a point estimate on the index chemical's dose-response curve from which risk to the anticipated exposure levels in the human population is extrapolated.

$$RPF = \frac{\text{Index Chemical}_{BMD}}{\text{Chemical n}_{BMD}}$$

The 10% response level is generally at or near the limit of sensitivity for discerning a statistically significant decrease in AChE activity across the brain compartments and is a response level close to the background AChE. As part of EPA's Revised Cumulative Risk Assessment for the OPs, EPA performed a power analysis of brain AChE data available for more than 30 OPs (USEPA, 2002c). The results of the analysis indicated that most studies can reliably detect 10% brain AChE inhibition. Furthermore, in studies available to EPA for the OPs, clinical signs and behavioral effects have not been shown in studies below 10% AChE inhibition.

In the dose-response analysis, the cholinesterase data for various time points for a specific chemical are modeled *together*. Brain AChE for more than 30 OPs were fit to a decreasing exponential model (Example provided in Figure 1 for methamidphos).

Figure 1. Benchmark dose analysis for methamidophos. Extracted from USEPA, 2006



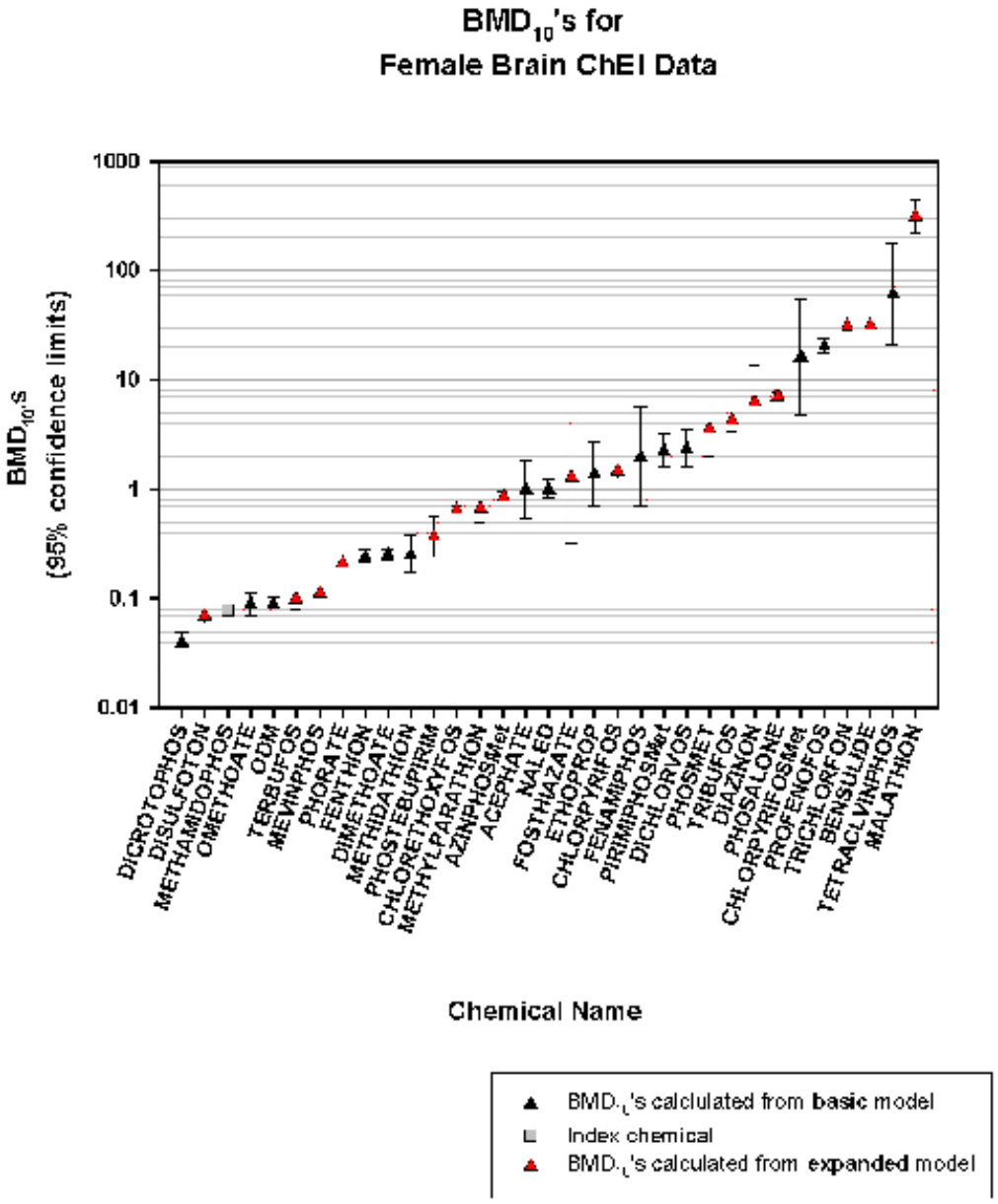
BMD_{10} and $BMDL_{10}$ (lower 95% confidence limit on the BMD_{10}) estimates are provided in Table 2 and Figure 2. EPA's empirical benchmark dose approach to cholinesterase-inhibiting pesticides have been supported by multiple external peer review panels (FIFRA SAP, 2002, 2005a, 2005b, 2005b, 2005c, 2008).

Table 2. Oral BMD_{10}s and BMDLs (mg/kg/day) estimated for adult brain AChE activity

Chemical	Female		Male	
	BMD_{10}	**$BMDL_{10}$**	**BMD_{10}**	**$BMDL_{10}$**
Acephate	0.99	0.53	0.77	0.41
Azinphos-methyl	0.86	0.79	1.14	0.98
Bensulide	31.91	30.44	40.88	37.11
Chlorethoxyfos	0.65	0.61	0.69	0.62
Chlorpyrifos	1.48	1.26	1.50	1.27
Chlorpyriphos-methyl	16.20	4.77	14.26	4.21
Diazinon	6.24	2.89	9.62	5.39
DDVP	2.35	1.61	1.71	0.08
Dicrotophos	0.04	0.04	0.04	0.03
Dimethoate	0.25	0.22	0.35	0.31
Disulfoton	0.07	0.06	0.10	0.09
Ethoprop	1.37	0.70	1.35	0.69
Fenamiphos	1.96	0.69	1.73	0.63
Fenthion	0.24	0.21	0.18	0.15
Fosthiazate	1.28	0.32	1.48	0.38
Malathion	313.91	221.12	212.02	119.31
Methamidophos	0.08	0.07	0.07	0.06
Methidathion	0.25	0.17	0.24	0.16
Methyl-parathion	0.67	0.50	0.70	0.51

Chemical	Female		Male	
	BMD_{10}	$BMDL_{10}$	BMD_{10}	$BMDL_{10}$
Mevinphos	0.11	0.10	0.15	0.13
Naled	1.00	0.82	1.00	0.82
Omethoate	0.09	0.07	0.14	0.12
Oxydemeton-methyl	0.09	0.09	0.07	0.07
Phorate	0.21	0.20	0.29	0.26
Phosalone	6.93	6.27	7.88	7.05
Phosmet	3.56	2.03	4.15	2.25
Phostebupirim	0.37	0.24	0.40	0.26
Pirimiphos-methyl	2.25	1.61	1.58	0.93
Profenofos	20.58	17.64	24.98	21.86
Terbufos	0.10	0.08	0.18	0.17
Tetrachlorvinphos	60.69	20.97	369.27	102.31
Tribufos	4.27	3.31	4.52	3.47
Trichlorfon	31.74	28.62	58.49	45.39

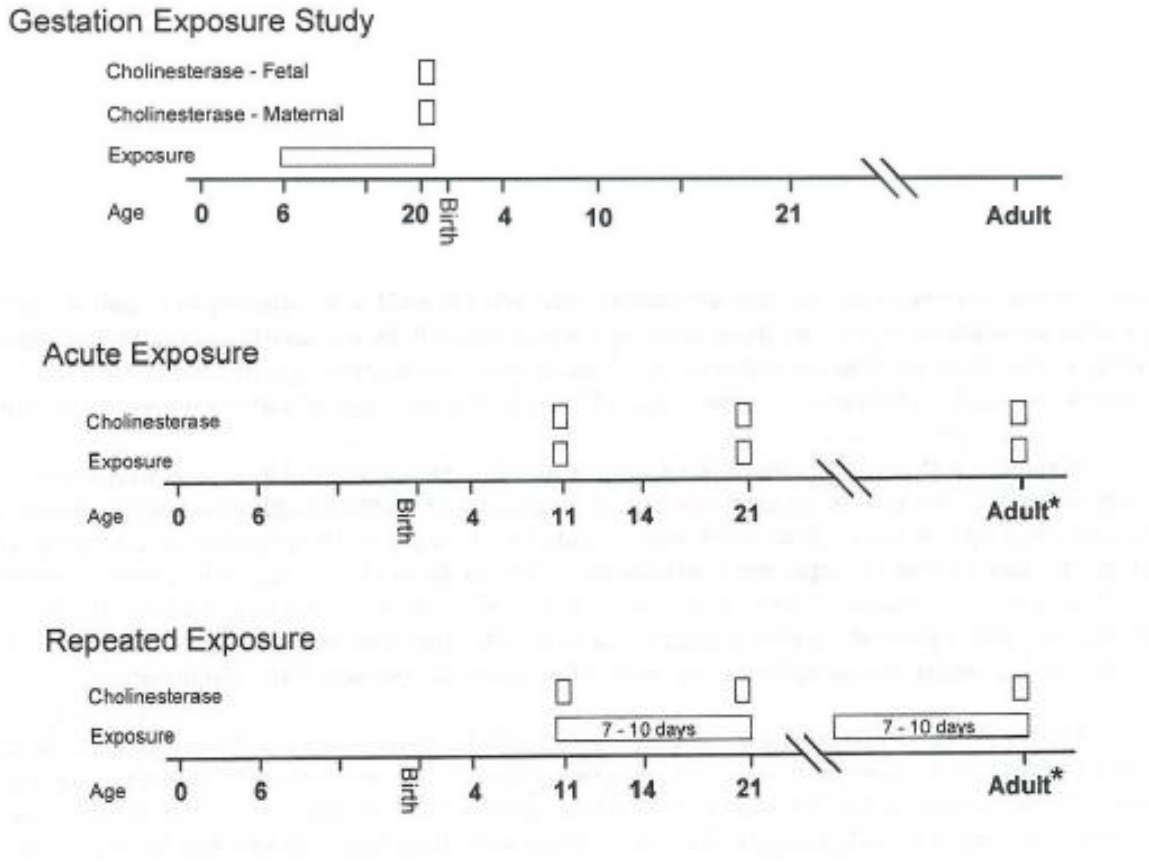
Figure 2. Plot of BMD_{10}s and the 95% confidence limits for female rat brain AChE inhibition for the OPs



With regard to lifestage susceptibility, the comparative cholinesterase study in juvenile and adults—provide the most relevant data for evaluating potential sensitivity to infants and children to OPs. For a number of OPs, DNTs and CCA studies are available and can be used to derive a chemical-specific factor for use in the cumulative risk assessment to reflect the differential sensitivity of children and infants compared to adults. For these OPs, cholinesterase inhibition is a more sensitive endpoint than functional or behavioral effects identified in the DNT. Because the CCA studies provide sensitive results, the data provided from these studies have been judged as reliable and identified for use in the cumulative risk assessment as the most appropriate studies for developing the chemical-specific factors to address the potential susceptibility of infants and children to the effects of OP exposure. Comparative cholinesterase studies can involve acute or repeated dosing to adult and juvenile rats (Figure 3). These studies typical involve time to peak effect and range-finding studies also. USEPA typically reviews the protocol for the definitive dose response studies before they are conducted to ensure the time to peak effect is appropriate and to review proposed dose selection in

juvenile and adult rats. Generally an untreated control and three doses are used. However in some cases, 4-5 doses are used but for these the number of rats/dose are reduced or only 1 sex is used to limit animal use. These studies are of high quality, can be conducted within a few months, and are easily interpretable.

Figure 3. Study design of the comparative cholinesterase study for the OPs (extracted from USEPA, 200b)



To best match the duration of exposure used to derive BMDs, for the OP cumulative risk assessment, the Agency has considered the *repeated dosing exposure* studies only.

4. STRATEGY FOR AND INTEGRATED CONCLUSION OF DATA GAP FILLING

The Food Quality Protection Act (FQPA, 1996) instructs EPA, in making its "reasonable certainty of no harm" finding, that in "the case of threshold effects, **an additional tenfold margin of safety** for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account **potential pre- and postnatal toxicity and completeness of data with respect to exposure and toxicity to infants and children**." Section 408 (b)(2)(C) further states that "the Administrator may use a different margin of safety for the pesticide chemical residue only if, on the basis of reliable data, such margin will be safe for infants and children."

In accordance with the FQPA (1996), the Agency began its analysis of lifestage susceptibility by assigning each OP a FQPA factor of 10X. Next, the Agency used a screening-level approach to identify a subset of OPs considered to be potential contributors to the cumulative risk either from food, water, or residential pathways. Following this screening approach, the Agency searched the scientific literature and pesticide registration databases for toxicity studies which measured brain cholinesterase inhibition in juvenile and adult rats following repeated dosing (Table 3). Table 4 provides the RBC and brain cholinesterase data from gestational dosing with a subset of OPs that further support the conclusion that dams exhibit more inhibition than fetuses during pre-natal exposure.

As such, for purposes of the cumulative risk assessment, data from post-natal exposures in juvenile and adult rats provide the most robust toxicity data for determining the magnitude of the FQPA factor for the OP cumulative risk assessment. For all the OPs except chlorpyrifos, a BMD analysis was then performed on the brain cholinesterase data in juvenile and adult animals extracted from comparative cholinesterase studies; chlorpyrifos was handled differently in 2006 due the quality of the available data[2]. For those OPs who were identified as potential contributors to risk without comparative ChE data in juvenile and adult rats, a FQPA factor of 10X was retained.

Table 3 Summary of BMD_{10}s and $BMDL_{10}$s (mg/kg/day) from comparative cholinesterase studies (repeated dosing only) in juvenile and adult rats for selected OPs. Extracted from 2006 OP cumulative risk assessment[3]

OP	Gender	Age	BMD	BMDL
Acephate	Male	Adult	0.27	0.22
		Pup	0.42	0.30
	Female	Adult	1.25	0.73
		Pup	1.13	0.60
Azinphos methyl	Female	Adult	1.14	1.04
		Pup	0.25	0.22
Diazinon	Male	Adult	40.57	27.87
		Pup	1.08	1.00
	Female	Adult	0.39	0.29
		Pup	0.72	0.68
Dicrotophos	Male	Adult	0.11	0.09
		Pup	0.06	0.05
	Female	Adult	0.09	0.07
		Pup	0.05	0.04
Dimethoate	Male	Adult	0.48	0.22
		Pup	0.39	0.29
	Female	Adult	0.37	0.34
		Pup	0.41	0.26
Disulfoton	Male	Adult	0.11	0.09

[2] Since 2006, additional CCA data available for chlorpyrifos.

[3] Updated BMD estimates have been conducted in 2014-2016 for new risk assessments; these values were extracted directly from the 2006 OP cumulative risk assessment.

OP	Gender	Age	BMD	BMDL
		Pup	0.05	0.05
	Female	Adult	0.07	0.06
		Pup	0.05	0.04
DDVP	Male	Adult	0.72	0.55
		Pup	0.88	0.75
	Female	Adult	0.88	0.71
		Pup	0.95	0.8
Fosthiazate	Male	Adult	1.89	1.65
		Pup	0.74	0.59
	Female	Adult	0.60	0.55
		Pup	0.48	0.44
Methamidophos	Male	Adult	0.10	0.08
		Pup	0.08	0.06
	Female	Adult	0.18	0.11
		Pup	0.09	0.08
	Male	Adult	Data poor quality	
		Pup	0.09	0.07
	Female	Adult	0.66	0.50
		Pup	0.11	0.09
Terbufos	Male	Adult	0.10	0.04
		Pup	0.02	0.01
	Female	Adult	0.02	0.008
		Pup	0.02	0.01

Table 4. RBC and brain AChE activity in dams and fetuses from comparative AChE studies following gestational exposure. Extracted from the 2006 OP cumulative risk assessment.

OP	Cholinesterase & Group	Dose (mg/kg/day)				
Acephate **MRID 46151805**	**Dose**	**0**	**0.5**	**1**	**2.5**	**10**
	GD 21 Dams RBC Brain	1.6360 ± 0.7461 8.6009 ± 1.4779	1.9691 ± 0.7684 7.1673 ± 0.8621 (17)	2.3221 ± 0.5884 7.0441 ± 0.900 (18)	1.4638 ± 0.7615 5.096 ± 0.933 (41)	1.5202 ± 0.6202 3.3112 ± 0.5209 (62)
	GD 21 Fetuses RBC Brain	1.7284 ± 0.5776 1.4688 ±0.0871	1.9883 ±0.7651 1.3613 ± 0.1320	1.4476 ± 0.2403 1.2915 ± 0.1313 (12)	1.0662 ± 0.3121 1.2586 ± 0.1666 (14)	1.3385 ± 0.5334 0.8816 ± 0.1254 (40)
Azinphos methyl **MRID 46291101**	**Dose**	**0**	**0.2**	**0.9**	**1.2**	
	GD 20 Dams RBC Brain	1.43 ± 0.31 11.1 ± 0.5	1.41 ± 0.30 (1) 10.8 ± 0.7 (3)	1.39 ± 0.43 (3) 10.0 ± 1.9 (10)	1.05 ± 0.20 (27) 10.7± 0.7 (4)	
	GD 20 Fetuses RBC Brain	1.36 ± 0.28 2.2 ± 0.1	1.30 ± 0.07 (4) 2.3 ± 0.1	1.31 ± 0.15 (4) 2.3 ± 0.2	1.32 ± 0.17 (3) 2.2 ± 0.1 (0)	

OP	Cholinesterase & Group	Dose (mg/kg/day)			
Chlorpyrifos	**Dose**	**0**	**0.3**	**1**	**5**
MRID 44648102	GD 20 Dams				
	RBC		73.7**±14.5	17.6**±6.7	4.9**±2.8
% activity compared to control	Hindbrain		101.1±7.2	92.0*±2.2	24.0**±4.8
	GD 20 Fetuses				
	RBC		102.2±20.3	106.4±16.7	7.9**±4.3
	Hindbrain		107.0±5.0	99.7±5.6	46.1**±9.3
	Dose	**0**	**0.084**	**0.825**	**26.23**
	GD 20 Dams				
	RBC	1.106± 0.163	1.183 ±0.165	0.719± 0.223 (35)	0.00± 0.00 (100)
Diazinon	Brain	17.272± 1.041	16.925± 1.066	16.675± 0.617	3.228 ±0.229 (81.3)
	GD 20 Male fetuses				
MRID 45842602	RBC	1.188± 0.230	1.392 ±0.183	1.319± 0.230	0.247 ±0.162 (79.2)
	Brain	2.383 ±0.194	2.380± 0.262	2.194 ±0.161	1.689± 0.348 (29.1)
	GD 20 Female fetuses				
	RBC	1.208 ±0.143	1.325± 0.172	1.363± 0.254	0.217± 0.148 (82.0)
	Brain	2.311± 0.198	2.360± 0.395	2.231± 0.234	1.822± 0.372 (21.2)
	Dose	**0**	**0.05**	**0.2**	**1.0**
	GD 20 Dams				
	RBC	2593 ± 218	2342 ± 79 (10)	1638± 120 (37)	1282 ± 226 (51)
Dicrotophos	Brain	4.78 ± 0.99	4.26± 1.06 (10)	2.49± 0.51 (48)	1.03 ± 0.21 (78)
	GD 20 Male fetuses				
MRID 46153201	RBC	2546± 112	2423± 351	1923± 190 (24)	1311± 124 (49)
	Brain	1.75± 0.34	1.51± 0.25 (14)	1.22± 0.28 (30)	0.77± 0.08 (56)
	GD 20 Female fetuses				
	RBC	2523 ± 455	2362± 50	1825± 207 (28)	1414± 142 (44)
	Brain	1.57± 0.18	1.36± 0.13 (13)	1.22± 0.11 (24)	0.72± 0.02 (54)

OP	Cholinesterase & Group	Dose (mg/kg/day)			
Dimethoate **MRID 45529702**	**Dose**	**0.0**	**0.1**	**0.5**	**3.0**
	GD 20 Dams RBC Brain	 1669 ± 180 12,838 ± 1373	 1563 ± 224 (6) 13,044 ± 530 (-2)	 1459 ± 278 (13) 11,563 ± 300 (10)	 709 ± 104 (58) 5094 ± 1081 (60)
	GD 20 Fetuses RBC Brain	 1213 ± 79 1781 ± 175	 1225 ± 98 (-1) 1569 ± 173 (12)	 1181 ± 172 (3) 1600 ± 136 (10)	 834 ± 183 (31) 1188 ± 164 (33)
Disulfoton **MRID 46635901**	**Dose**	**0**	**0.042**	**0.168**	**0.694**
	GD 20 Dams RBC Brain	 2.02±0.34 11.97±0.53	 1.66±0.31 (18) 11.35±0.50 (5)	 1.13±0.37 (44) 8.12±0.44 (32)	 0.20±0.13 (90) 1.76±0.19 (85)
	GD 20 Fetuses RBC Brain	 1.27±0.16 1.81±0.30	 1.21±0.20 1.75±0.28	 1.02±0.19 (20) 1.74±0.26	 0.22±0.11 (83) 1.18±0.21 (35)
Fosthiazate **Not yet assigned**	**Dose**	**0**	**0.1**	**0.7**	**5**
	GD 20 Dams RBC Brain	 3931± 1474.5 49446± 2189.8	 3831 ± 757.3 48974 ± 1364.5	 2193 ± 712.2 (44) 47135 ± 1510 (5)	 20 ± 0.0 (99) 5152± 1718.9 (90)
	GD 20 Fetuses RBC Brain	 2644± 644.1 6612 ± 679.5	 3283 ± 992.4 6328 ± 476.3	 2893± 738.3 6251 ± 649.5 (5)	 1851 ± 593.4 (30) 5182 ± 684.5 (22)
Methamidophos **MRID 46660901**	**Dose**	**0**	**0.10**	**1.03**	**3.12**
	GD 20 Dams RBC Brain	 1.64 ± 0.286 10.82 ± 0.271	 1.68± 0.220 10.40± 1.711	 0.84± 0.117 (49) 4.86 ± 0.416 (55)	 0.45 ± 0.118 (73) 2.32± 0.173 (79)
	GD 20 Fetuses RBC Brain	 1.29 ± 0.196 1.56 ± 0.157	 1.13 ± 0.147 1.51± 0.089	 0.72 ± 0.133 (44) 1.08 ± 0.125 (31)	 0.38 ± 0.075 (55) 0.77 ± 0.061 (51)

OP	Cholinesterase & Group	Dose (mg/kg/day)			
Methyl parathion **MRID 45646501**	**Dose**	**0**	**0.03**	**0.30**	**0.60**
	GD 20 Dams RBC Brain	 1500.1 ± 255.03 13.48 ± 0.807	 1702.3 ± 386.36 13.58 ± 0.428	 979.5± 283.80 (35) 12.26 ± 0.527 (9)	 632.9± 124.52 (58) 9.35± 1.026 (31)
	GD 20 Male fetuses RBC Brain	 1041.3± 145.79 2.10± 0.116	 1082.2 ± 160.9 2.05± 0.095	 1075.0 ± 135.32 2.04 ± 0.173	 808.9 ± 186.38 (22) 1.97± 0.073
	GD 20 Female fetuses RBC Brain	 1090.4 ± 163.7 2.06 ± 0.152	 1118.0 ± 131.13 2.12 ± 0.14	 1010.2 ± 130.36 2.06 ± 0.174	 894.9± 215.77 (18) 2.02 ± 0.092
Phorate **MRID 46241402**	**Dose**	**0**	**0.03**	**0.1**	**0.2**
	GD 20 Dams RBC Brain	 35.98 ± 1.12 2.95 ± 0.54	 33.92 ± 3.76 2.88 ± 0.74	 30.99 ± 4.82 (14) 2.94± 0.70	 27.64 ± 5.16 (23) 1.73 ± 0.67 (41)
	GD 20 Male fetuses RBC Brain	 7.05 ± 0.83 0.57 ± 0.01	 5.72 ± 0.51 (19) 0.58 ± 0.04	 5.69 ± 0.66 (19) 0.56± 0.03	 6.42 ± 0.56 0.60 ± 0.03 (6)
	GD 20 Female fetuses RBC Brain	 6.80± 0.99 0.59± 0.04	 5.81± 0.91 0.57 ± 0.04	 5.48± 0.89 0.58± 0.02	 6.28 ± 0.78 0.59 ± 0.02
Terbufos **MRID 46240802**	**Dose**	**0**	**0.03**	**0.1**	**0.3/0.2**
	GD 20 Dams RBC Brain	 42.30 ± 5.00 3.00 ± 1.12	 40.68 ± 4.00 3.00± 0.79	 14.42± 4.04 (66) 1.96± 0.68 (35)	 4.46± 1.64 (89) 0.69± 0.19 (77)
	GD 20 Male fetuses RBC Brain	 5.16 ± 1.48 0.59± 0.11	 4.63 ± 1.86 0.53± 0.05	 2.51± 0.86 (51) 0.48± 0.04 (19)	 1.62 ± 0.69 (69) 0.36 ± 0.09 (39)
	GD 20 Female fetuses RBC Brain	 4.32 ± 0.85 0.53 ± 0.04	 4.52 ± 0.99 0.57 ± 0.04	 1.99± 1.09 (54) 0.50± 0.05	 1.76 ± 0.75 (59) 0.36± 0.07 (32)

Integrated conclusion

The USEPA-OPP conducts cumulative risk assessments based on groups of chemicals which induce a common toxic effect by a common mechanism of toxicity. The OPs have been assessed for cumulative risk (2006) based on their shared ability to bind and to phosphorylate the enzyme acetylcholinesterase in both the central (brain) and peripheral nervous systems. The 2006 cumulative risk assessment was used to support tolerance reassessment and reregistration of OP pesticides as required by the FQPA (https://www.epa.gov/pesticide-reevaluation/reregistration-and-other-review-programs-predating-pesticide-registration). The OP cumulative risk assessment focuses on inhibition of AChE as the molecular initiating event for deriving BMDs and assessing lifestage susceptibility. This lifestage susceptibility has been evaluated using a specific study protocol called the comparative cholinesterase assay specifically designed to assess various early lifestages (fetal, pregnant females, post-natal) across duration (single dose, repeated dose; USEPA, 2002b). The comparative cholinesterase assay provided high confident data for the specific AOP.

REFERENCES

FIFRA SAP. (2002). "Methods Used to Conduct a Preliminary Cumulative Risk Assessment for Organophosphate Pesticides." Final Report from the FIFRA Scientific Advisory Panel Meeting of February 5-7, 2002 (Report dated March 19, 2002). FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. SAP Report 2002-01.

FIFRA Science Advisory Panel (SAP). 2005a. "Final report on *N*-Methyl Carbamate Cumulative Risk Assessment: Pilot Cumulative Analysis." Final Report from the FIFRA Scientific Advisory Panel Meeting of February , 2005 (Report dated September 2, 1998). Available at: http://www.epa.gov/scipoly/sap/2005/february/minutes.pdf

FIFRA Science Advisory Panel (SAP). 2005b. "Final report on Preliminary N-Methyl Carbamate Cumulative Risk Assessment." Final Report from the FIFRA Scientific Advisory Panel Meeting of July 29-30, 1998 (Report dated September 2, 1998). Available at: http://www.epa.gov/scipoly/sap/2005/august/minutes.pdf

FIFRA Science Advisory Panel (SAP). 2008. "Final report on the Agency's Proposed Action under FIFRA 6(b) Notice of Intent to Cancel Carbofuran." Report from the FIFRA Scientific Advisory Panel Meeting of February , 5-8 2008 (Report dated September 2, 1998). Available at: http://www.epa.gov/scipoly/sap/meetings/2008/february/carbofuransapfinal.pdf

U.S. Environmental Protection Agency. 1999a. "Guidance for Identifying Pesticide Chemicals and Other Substances That Have A Common Mechanism of Toxicity." Environmental Protection Agency, Office of Pesticide Programs. Fed. Reg. 64:5796-5799.

U.S. Environmental Protection Agency. 1999b. US Environmental Protection Agency. Policy on a Common Mechanism of Action: The Organophosphate Pesticides. Federal Register 64(24):5795-5799. February 5.

U.S. Environmental Protection Agency. 2001a. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity." Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC.

U.S. Environmental Protection Agency. 2001b. "Preliminary Cumulative Hazard and Dose Response Assessment for Organophosphorus Pesticides: Determination of Relative Potency and Points of Departure for Cholinesterase Inhibition." Office of Pesticide Programs, US Environmental Protection Agency, Washington, DC. July 31, 2001.

U.S. Environmental Protection Agency. 2002a. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity"

U.S. Environmental Protection Agency. 2002b. "Guidance on Cholinesterase Measures in DNT and Related Studies", Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. October 22, 2002

U.S. Environmental Protection Agency. 2002c. Revised Organophosphorous Pesticide Cumulative Risk Assessment; June 10, 2002. Office of Pesticide Programs, U.S. Environmental Protection Agency. Washington, D.C.

U.S. Environmental Protection Agency. 2006. Updated Organophosphorous Pesticide Cumulative Risk Assessment, July 31, 2006. Office of Pesticide Programs, U.S. Environmental Protection Agency. Washington, D.C.

ACRONYMS USED

Acetylcholinesterase: AChE
Adverse outcome pathway: AOP
Developmental neurotoxicity: DNT
Food Quality Protection Act: FQPA
Office of Pesticide Programs: OPP
Organophosphate pesticides: OPs
U.S. Environmental Protection Agency: US EPA