DEVELOPMENT OF HUMAN HEALTH TOXICOLOGICAL CRITERIA FOR DMPT AND DEPT

Prepared for

Syngenta Crop Protection
P.O. Box 18300
Greensboro, NC 27419-8300

Prepared by

Riva 400 Office Park
2661 Riva Road, Bldg. 500, Ste. 520
Annapolis, MD 21401

October 31, 2006

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

ACh	acetylcholine
AChE	acetylcholinesterase
ATSDR	Agency for Toxic Substances and Disease Registry
bw	body weight
CAS #	Chemical Abstract Service number
CDC	Center for Disease Control
DEDTP	O,O'-diethyl dithiophosphate
DEPT	diethyl phosphorodithoic acid
DIMP	diisopropyl methylphosphonate
DMDTP	O,O'-dimethyl dithiophosphate
DMPT	dimethyl phosphorodithoic acid
HPV	high production volume
IMPA	isopropyl methylphosphonic acid
IRIS	Integrated Risk Information System
LC50	median lethal concentration
LD50	median lethal dosage
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
MRL	minimum risk level
MW	molecular weight
NDEP	Nevada Division of Environmental Protection
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OP	organophosphate pesticide
ppm	parts per million
RfD	reference dose
RTECS	Registry of Toxic Effects of Chemical Substances
TCLo	lowest published toxic concentration
UF	uncertainty factor
USEPA	U.S. Environmental Protection Agency

1. INTRODUCTION

On August 29, 2006, the Nevada Division of Environmental Protection (NDEP) wrote a letter recommending the use of toxicological surrogates to evaluate the potential human toxicity of dimethyl phosphorodithioic acid (DMPT) and diethyl phosphorodithioic acid (DEPT). The toxicological surrogates recommended for DMPT and DEPT by NDEP were dimethoate and phosalone, respectively. Both of these chemicals inhibit acetylcholinesterase (AChE) and disrupt neural transmission. AChE inhibition is the most sensitive toxic endpoint for these and other organophosphate pesticides (OP) and, therefore, is the basis for toxicological criteria developed for them.

In a communication dated September 7, 2006, Syngenta Crop Protection, Inc. (Syngenta) stated that DMPT and DEPT (metabolites of OPs) were not AChE inhibitors and, in fact, were classified as nontoxic by the U.S. Center for Disease Control (CDC) (Crouse 2006). Syngenta therefore took the position that the surrogates proposed by the NDEP were not appropriate surrogates for DMPT and DEPT. In the reply dated September 12, 2006, NDEP agreed that there is mixed information regarding the ability of dialkyl phosphates such as DMPT and DEPT to inhibit AChE (Rakvica 2006). NDEP suggested that Syngenta document the biological insignificance of AChE inhibition by the chemicals of interest and develop alternative toxicological surrogates for these chemicals based on other toxicological endpoints (Rakvica 2006).

This report is organized around these two requests. Section 2 of this report presents toxicological evidence to support the assertion that DMPT and DEPT are not AChE inhibitors. Section 3 of this report presents a summary of what is known regarding the potential toxicity of DMPT and DEPT to other endpoints and develops human health toxicological criteria. Section 4 summarizes the reports findings and recommendations. Section 5 list references cited in this report and Section 6 provides citations for other literature reviewed.

2. ANALYSIS OF ACHE INHIBITION POTENCY OF DMPT AND DEPT

To understand the AChE inhibition potential of DMPT and DEPT, or lack thereof, it is necessary to understand the chemical structure of organophosphate compounds, the role of AChE in biological systems, the mechanism of action by which organophosphate chemicals may inhibit AChE, and the attributes of organophosphate chemicals that contribute to AChE inhibition and how they compare to the attributes of DMPT and DEPT. This section briefly addresses each of these topics.

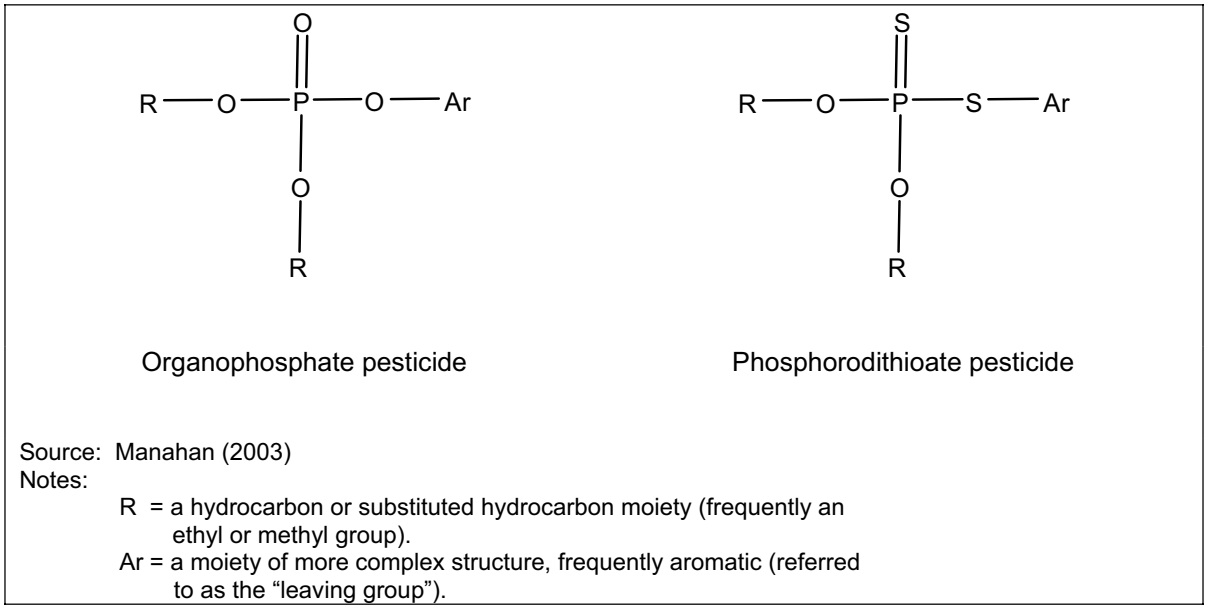
2.1 CHEMICAL DEFINITION AND STRUCTURE

DMPT, CAS No. 756-80-9, is also referred to as O,O'-dimethyl dithiophosphate (DMDTP); dimethyldithiophosphoric acid; and dimethyl phosphorodithioate. DEPT, CAS No. 298-06-6, is also referred to as O,O'-diethyl dithiophosphate (DEDTP); diethyl acid; and diethyl phosphorodithioate.

DMPT and DEPT belong to the family of organophosphate chemicals. The general structure of an OP consists of a phosphorous atom double-bonded[1] either to an oxygen or a sulfur atom surrounded by two alkyl/alkoxy groups and a third group, termed the leaving group (Science Group 2004) (see Figure 1). The leaving group is so named because it is the portion of the molecule that is removed during initial *in vivo* metabolism.

OPs that contain a P=S bond and an additional sulfur, such as malathion, phosalone, and methyl parathion, are categorized as phosphorodithioate pesticides. Figure 1 shows the general structure of OPs with P=O bonds and those with P=S bonds.

[1] In this report, double bonds between atoms are designated in text and figures by double lines (=).



Source: Manahan (2003)
Notes:
R = a hydrocarbon or substituted hydrocarbon moiety (frequently an ethyl or methyl group).
Ar = a moiety of more complex structure, frequently aromatic (referred to as the "leaving group").

Figure 1. General Structures of Organophosphate and Phosphorodithioate Pesticides.

The chemical structure of OPs determines their AChE inhibition potential. The chemical structures of DMPT and DEPT are shown in Figure 2. DMPT and DEPT are similar in that both have a P=S bond and a thiol (-SH) leaving group. They differ in that DMPT has two methyl groups ($-CH_3$) attached to the P atom, while DEPT has two ethyl groups ($-C_2H_5$) attached to the P atom.

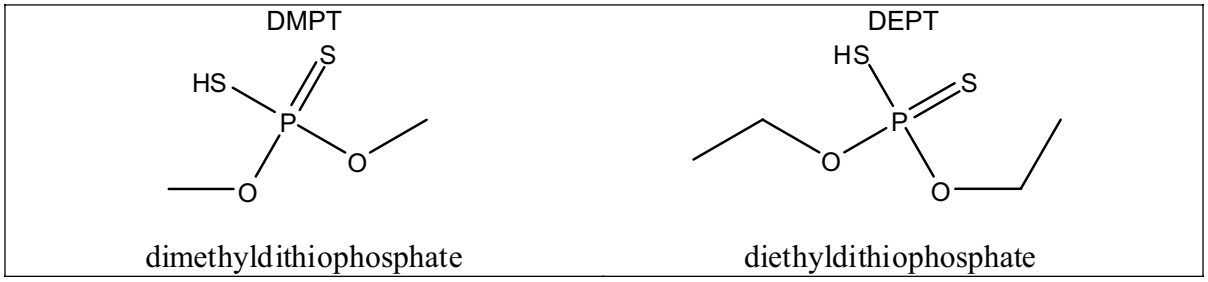


Figure 2. Chemical Structure of DMPT and DEPT.

DMPT and DEPT are not manufactured as OPs, but rather are metabolites of OPs (parent compounds). They are commonly detected in human urine and are considered biomarkers of general OP exposure. They are not specific to a particular OP (CDC 2005; ATSDR 2001). According to the CDC, about 75% of registered OPs will be metabolized to measurable dialkyl phosphate metabolites (including DMPT and DEPT). Figures 3 and 4 depict examples of how DMPT and DEPT may be metabolized from different parent

compounds. In general, the dialkyl phosphate metabolites have much smaller and less reactive leaving groups than the parent compounds. This is apparent from the example in the figures below.

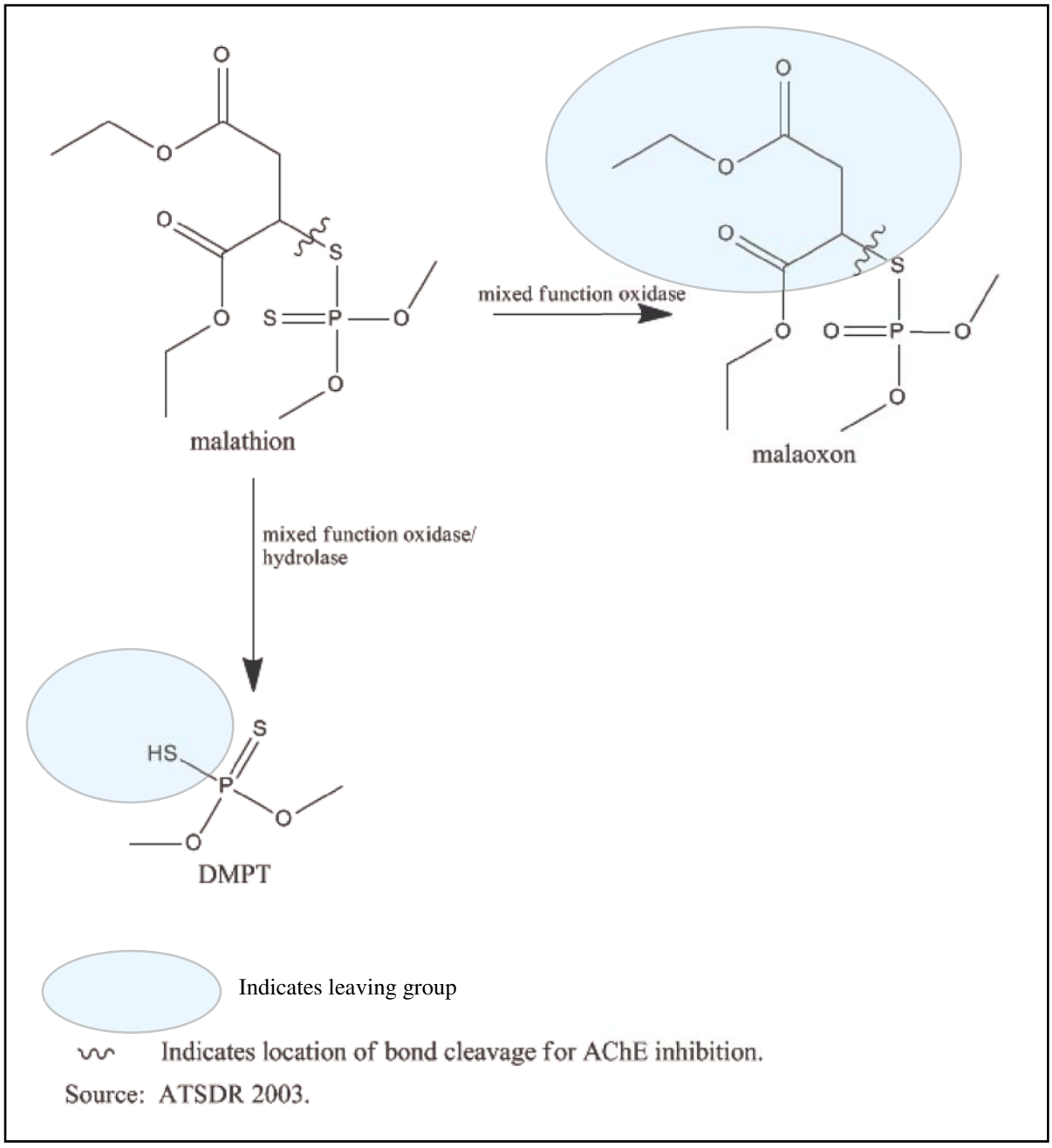


Figure 3. Metabolic Origin of DMPT from Malathion.

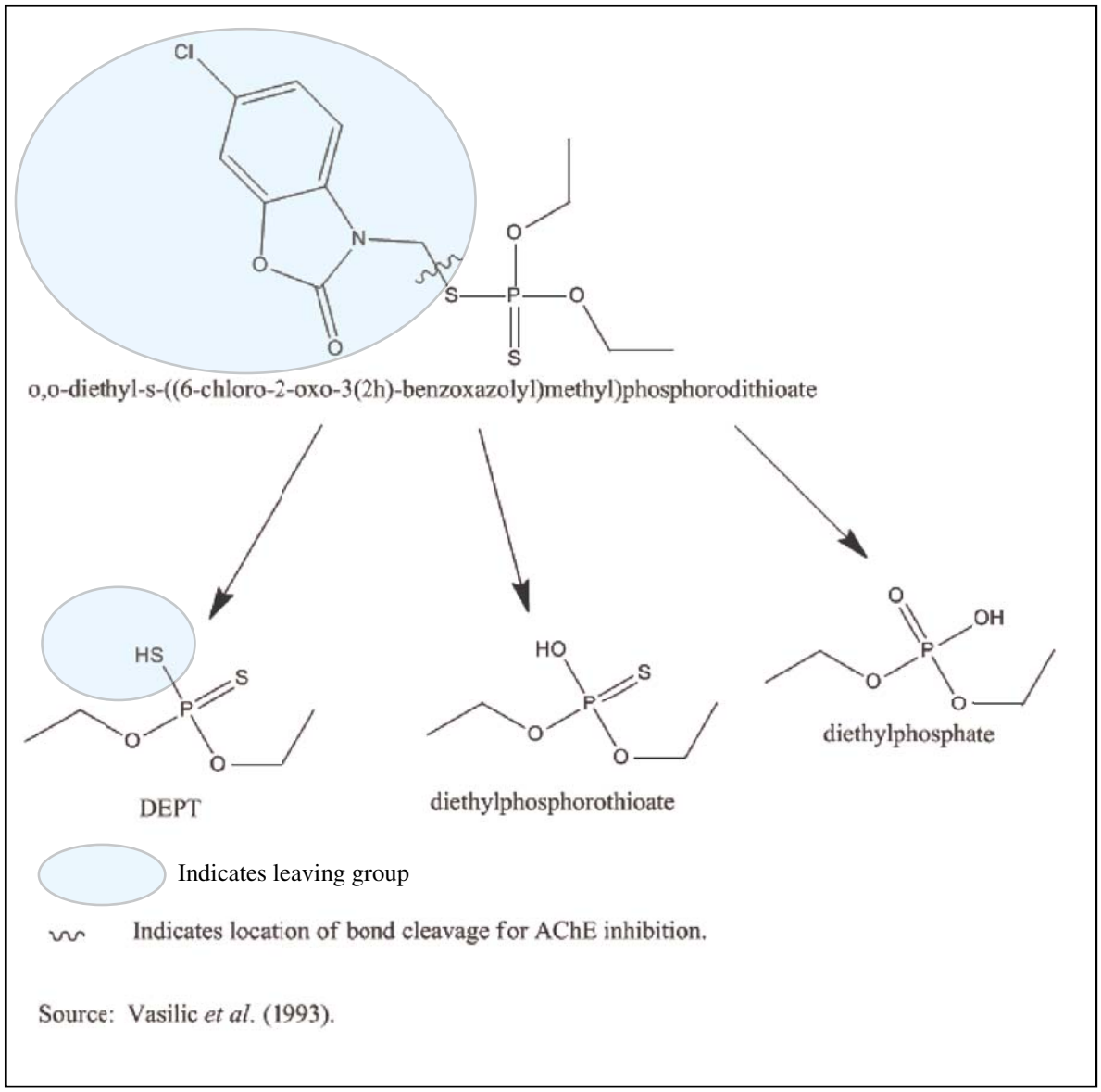


Figure 4. Metabolic Origin of DEPT from o,o-diethyl-s-((6-chloro-2-oxo-3(2h)-benzoxazolyl)methyl)phosphorodithioate (Phosalone).

2.2 BIOCHEMICAL AND MOLECLUAR MECHANISMS FOR ACHE INHIBITION

To evaluate the potential inhibition of AChE by the chemicals of interest, it is first necessary to understand the role of AChE in biological systems. AChE is a ubiquitous enzyme expressed in a wide range of mammals and invertebrates. Its role is to hydrolyze acetylcholine (ACh) as part of the regulation of cholinergic neurotransmission in both the central and peripheral nervous systems. ACh is a neurotransmitter that activates two types of cholinergic receptors, muscarinic and nicotinic (Science Group 2004). AChE

normally rapidly degrades acetylcholine in the synapse. The inhibition of AChE allows accumulation of ACh with subsequent excessive stimulation of ACh receptors in postsynaptic cells/end organs (ATSDR 2001; Pope 1999). The resulting effect on cholinergic transmission can result in autonomic dysfunction (excessive secretions of the airways, excretory systems, salivary glands, and lacrimal glands), involuntary movements (tremors, convulsions) muscle fasciculations, and respiratory depression (Pope 1999; ATSDR 1997, 2001).

The U.S. Environmental Protection Agency (USEPA) has grouped most OPs into a class exhibiting a common mechanism of toxicity, AChE inhibition (Science Group 2004; Mileson et al. 1998). AChE inhibition by an OP is a two-step process; the first being the formation of an enzyme inhibitor complex and the second being the phosphorylation of the serine hydroxyl group located in the active site of the enzyme (Science Group 2004; Mileson et al. 1998) (see Figure 5).

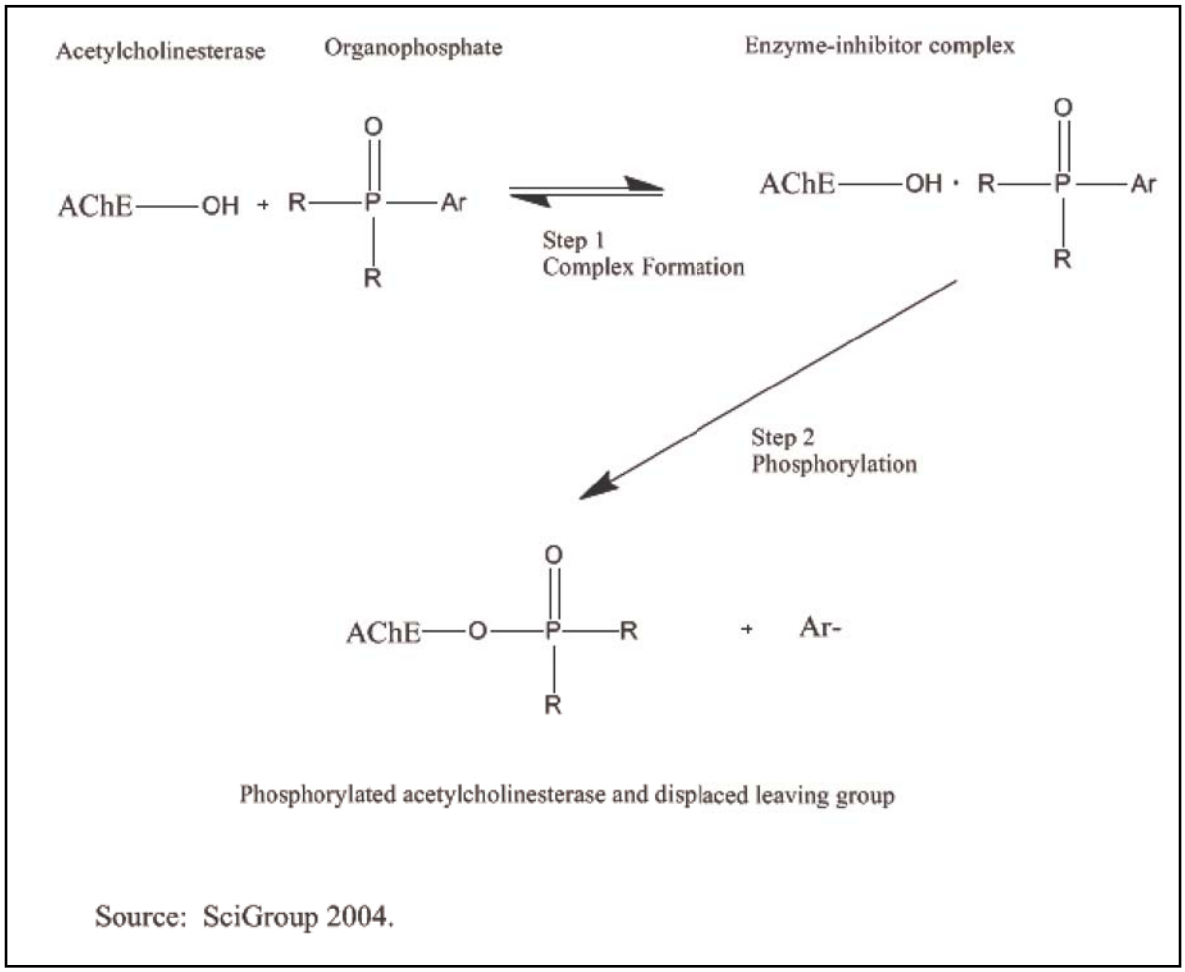


Figure 5. AChE Phosphorylation.

The phosphorylation reaction parallels the acetylation that would normally occur during the hydrolysis of ACh (ATSDR 2003). The phosphorylated enzyme then may undergo a second process, called aging, by loss of an alkyl group (dealkylation). After aging has occurred, the phosphorylated enzyme is resistant to cleavage or hydrolysis and can be considered irreversibly inhibited (ATSDR 2001; Manahan 2003; IOM 2004).

The reactivity of OP compounds varies depending upon the chemical structure. To phosphorylate the nucleophilic serine hydroxyl residue located in the active site of the AChE enzyme, the P atom of the OP has to be highly electrophilic (Science Group 2004). Electrophilicity of the P is crucial for the biological actions of OP compounds. The P atom is not intrinsically reactive and is dependant on two key attributes to be converted to a highly electrophilic state which enhances the reactivity of the overall compound. These key attributes concern the nature of the bonds between the phosphorous atom and the other atoms/groups present in the molecule (Science Group 2004). For the overall molecule to act as an AChE inhibitor, the bond between the phosphorous atom and the leaving group must be the most labile of the four bonds to phosphorous in the molecule. The greater the lability of this bond, the greater the potential reactivity with AChE (Maxwell and Lenz 1992). Chemical structure therefore plays a predictable role in determining AChE inhibition potential (Science Group 2004).

The first key attribute is the nature of the P double bond. OP compounds that have a double bond between P and O (phosphorate esters) are highly electrophilic at the P atom and are highly reactive. In contrast, the phosphorodithioate pesticides that have a double bond between P and S are generally not AChE inhibitors because the sulfur atom double bonded to the phosphorous atom is far less electronegative than the oxygen atom. This makes the P=S bond less polarized than the P=O bond, and results in a phosphorous atom with low reactivity and minimal, if existent, capacity to inhibit AChE (Science Group 2004).

Phosphorodithioate pesticides, such as ethion, malathion and methyl parathion, are not themselves AChE inhibitors, rather they require metabolic activation to confer the capacity to inhibit AChE. These pesticides are activated by oxidative desulfuration (replacement of the P=S with a P=O), mediated by cytochrome P450 isoforms, resulting in an oxygen analog of the parent compound (Mileson et al. 1998). The oxygen analog (e.g., mala-oxon, para-oxon) contains a P=O bond (ATSDR 2003, 2001).

The second key attribute is the electronegativity of the leaving group (Science Group 2004). In general, the smaller and more electronegative the group, the greater the reactivity of the phosphorous atom (and the lability of the leaving group bond), and the greater the resultant AChE inhibition (Science Group 2004; Mileson et al. 1998). If the leaving group of an OP is electropositive or very weakly electronegative (as is the case for

DEPT and DMPT), it will not be able to withdraw the electron from the phosphoryl atom and the atom will not be sufficiently reactive to form the bond required to inhibit AChE (Science Group 2004). Leaving groups that enhance the reactivity of the phosphorous are nitro, cyano, halogen, ketone, and carboxylic ester. Leaving groups that deactivate the phosphorous atom, resulting in a P atom with low reactivity, include hydroxyl and carboxylic acid (Mileson et al. 1998). The leaving group on both DEPT and DMPT is a thiol group. The thiol group is less electronegative than a hydroxyl group (Considine 2005).

Evidence that supports the deactivating role of a hydroxyl leaving group includes a study of potential cholinesterase inhibition by the organophosphate metabolite O,S-dimethyl phosphorothiolate. O,S-dimethyl phosphorothiolate contains a P=O bond and has a hydroxyl group as the leaving group. Though containing a P=O, no cholinesterase inhibition was found to occur by this chemical when tested *in vitro* using mouse erythrocyte enzyme (Chukwudebe et al. 1984). It can be concluded from this study that the electrophilicity of the P atom was insufficient to result in AChE inhibition, despite the presence of the P=O bond; thus, supporting the role of deactivation by the hydroxyl leaving group. Since a thiol leaving group is less electronegative than a hydroxyl leaving group, it would be expected to confer even less reactivity to the overall molecule.

This finding is consistent with the rest of the literature. Toxicity studies on metabolites of similar size and structure to DEPT and DMPT, including O,S-dimethyl phosphorothiolate, diisopropyl methylphosphonate (DIMP), and isopropyl methylphosphonic acid (IMPA) indicate no potential for AChE inhibition (Chukwudebe et al. 1984; USEPA 2006a; ATSDR 1998). For example, toxicity studies with DIMP in mink indicated no signs of AChE inhibition at dietary concentrations of 2,700, 5,400 or 8,000 ppm (400, 827 or 1,136 mg/kg-day) for 90 days (ATSDR 1998). USEPA has reviewed existing toxicity data for DIMP and IMPA and has established chronic toxicity thresholds for both chemicals that are not based upon AChE inhibition (USEPA 2006a). Additional detail on the toxicity of DIMP and IMPA is provided in Section 3.2 of this report.

2.3 CONCLUSIONS REGARDING ACHE INHIBITION BY DMPT AND DEPT

In summary, OP compounds that have been shown to inhibit AChE share the following key attributes:

- P=O bond in parent compound or active metabolite (oxon) with P=O bond,
- Two alkyl or alkoxy groups, and
- Strongly electronegative leaving group.

DMPT and DEPT do not have any of these characteristics. First, the P=S bond found in the chemicals of interest is a nonpolar covalent bond that does not confer sufficient electrophilicity to the phosphorous atom to render it an AChE inhibitor. Secondly, the thiol leaving group possessed by DMPT and DEPT behaves in a deactivating manner similar to a hydroxyl leaving group, further reducing the potency for AChE inhibition. In addition, toxicity studies on similar metabolites, including O,S-dimethyl phosphorothiolate, DIMP, and IMPA indicate no potential for AChE inhibition (Chukwudebe et al. 1984; USEPA 2006a; ATSDR 1998). Therefore, on the basis of structural activity, the evidence indicates that DEPT and DMPT are not AChE inhibitors.

This conclusion is consistent with the general consensus for an overall lack of AChE inhibition by chemicals with P=S bonds and small leaving groups such as the dialkyl phosphates DMPT and DEPT that is presented in the literature (Maxwell and Lenz 1992; Mileson et al. 1998; Pope 1999; Science Group 2004). It also is consistent with a review by CDC (2005) which states that "[i]n contrast to the organophosphates, the dialkyl phosphate metabolites do not inhibit acetylcholinesterase enzymes."

3. EVALUATION OF TOXICITY OF DMPT AND DEPT

Although DMPT and DEPT are not AChE inhibitors, that alone does not preclude them from causing other toxic effects. An evaluation of other potential toxic endpoints of DMPT and DEPT was performed and is summarized in this section.

A literature search was conducted to identify studies addressing toxicity of DEPT and DMPT to humans or animals. The literature search consisted of two major steps. The first step was to search online and published toxicity databases maintained by government entities for both chronic and acute toxicity data. The databases searched included the Integrated Risk Information System (IRIS) (USEPA 2006a), the Health Affects Assessment Tables (USEPA 1997), the Registry of Toxic Effects of Chemical Substances (RTECS) (CCOHS 2006a, b) and the High Production Volume (HPV) Chemical Challenge Program (USEPA 2006b). Pesticide re-registration documents and Agency for Toxic Substance and Disease Registry (ATSDR) toxicological profiles were also reviewed for parent OPs to identify any pertinent toxicological information on metabolites.

The second step was to search the primary literature for both review documents of dialkyl phosphate toxicity in general and for toxicity studies of DEPT and DMPT in particular. Primary literature was searched using the general online search engine Google Scholar©, and also by searching PubMed and TOXLINE databases maintained by the National Library of Medicine and the National Institute of Health. Searches were made using the various synonyms of the chemicals of interest and by CAS Number. General terms, such as "organophosphate metabolites" and "dialkyl phosphates" were also used as keywords for primary literature searches.

3.1 TOXICITY DATA FOR DMPT AND DEPT

There is little information on the direct toxicity of dialkyl phosphates themselves. Acute toxicity data (median lethal dosages -LD50, or concentrations -LC50) for both DEPT and DMPT were found in the RTECS database, and for DEPT in a HPV submittal, as previously submitted to NDEP by Syngenta. These acute toxicity results are summarized in Table 1.

Table 1. Summary of Acute Toxicity Data.

Chemical of Interest	Toxicity Threshold	Administration Route	Test Species	Dose	Toxic Effects
DMPT	LD50	Oral	Rat	694 mg/kg	
	LC50	Inhalation	Rat	1,700 mg/m^3/4H	
	LD50	Oral	Mouse	1,550 mg/kg	
	LD50	Parenteral	Mouse	68.5 mg/kg	Enzyme inhibition
	LD50	Oral	Mammal (unspecified)	1,400 mg/kg	
	Reprod. TCLo	Inhalation	Rat	161 mg/m^3/6H	Male fertility
DEPT	LD50	Oral	Rat	4,510 mg/kg	
	LC50	Inhalation	Rat	1,640 mg/m^3/4H	
	LD50	Dermal	Rabbit	> 2,000 mg/kg	

Source: CCOHS (2006a,b); Graham (2003).

DEPT and DMPT were not found in the chronic/subchronic toxicity databases compiled by USEPA, such as the IRIS (USEPA 2006a) or the Health Effects Assessment Tables (USEPA 1997). They do not have toxicological profiles published by ATSDR. Further, the primary literature search did not identify any subchronic or chronic studies of toxicity of DMPT or DEPT in humans or animals.

Based upon the analysis of structural activity, DMPT and DEPT are not expected to be particularly reactive. Additionally, they are rapidly excreted in mammalian systems. Therefore, they are not likely to exhibit strong toxicity in animals or humans. It is not surprising, therefore, that there are limited toxicological data available on these compounds.

3.2 IDENTIFICATION OF TOXICOLOGICAL SURROGATES

Given the absence of chronic or subchronic toxicity data for DMPT and DEPT, the longer-term toxicity of other compounds that are likely to have *in vivo* behavior similar to DMPT and DEPT was explored. These other compounds are termed toxicological surrogates. Potential toxicological surrogates were selected based upon chemical structural similarities to DMPT and DEPT, physical/chemical properties, biochemical attributes, and the availability of chronic toxicity data. Based on these factors, two potential toxicological surrogates were identified for further evaluation: DIMP and IMPA.

DIMP and IMPA are similar in size to DMPT and DEPT with molecular weights of 180.18 and 138.1, respectively. Both of these compounds are metabolites of sarin, an organophosphate nerve agent and a potent AChE inhibitor. Toxicity studies of DIMP indicate that it does not inhibit AChE (USEPA 2006a; ATSDR 1998). DIMP is metabolized

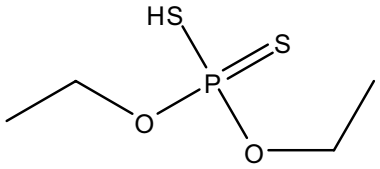
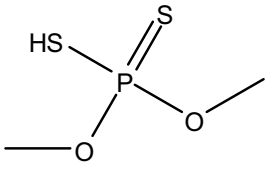
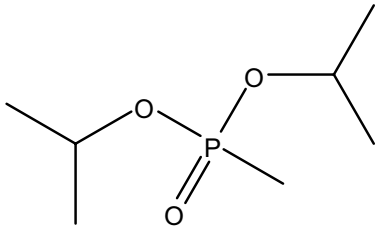
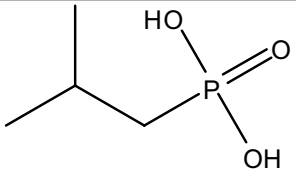
to IMPA in humans and other mammals, and the absence of AChE effects for DIMP is considered to indicate a lack of AChE effects for IMPA, as well.

For other toxic effects, a review of the literature revealed that more toxicity data were available for DIMP as compared to IMPA, however available data for these two chemicals were considered together in order to add weight of evidence to the relative levels of toxicity for chemicals of this type/ basic structure.

Although the chemical structures of DIMP and IMPA are similar to those of DMPT and DEPT, there are important differences that likely affect toxicity. DIMP and IMPA are both expected to exhibit greater *in vivo* reactivity due to the presence of a P=O bond (see Table 2). This is because, as discussed previously, the P=O is highly polarized and therefore more reactive. The presence of bonds or functional groups that are particularly prone to react with biomolecules (such as P=O) can be used to predict the likelihood and severity of toxicological effects. In general, greater reactivity is associated with greater toxicity (Manahan 2003).

Table 2 presents key attributes of the two chemicals of interest (DMPT and DEPT) and the potential toxicological surrogates (DIMP and IMPA) for comparison.

Table 2. Comparison of Chemicals of Interest to Potential Surrogates.

Chemicals of Interest			
DEPT CAS # 298-06-6 MW 186.22	 diethyldithiophosphate	**DMPT** CAS # 756-80-9 MW 158.17	 dimethyldithiophosphate
Oral RfD:	NA	Oral RfD:	NA
Toxicity Studies:	No subchronic or chronic studies. Oral LD50, rat = 4,510 mg/kg Inhal LC50, rat = 1,640 mg/m^3	Toxicity Studies:	No subchronic or chronic studies. Oral LD50, rat = 694 mg/kg Oral LD50, mice = 1,550 mg/kg Oral LD50, mammal = 1,400 mg/kg Inhal LC50, rat = 1,700 mg/m^3/4H
Potential Surrogates			
DIMP CAS # 1445-75-6 MW 180.18	 diisopropyl methyl phosphonate	**IMPA** CAS # 1832-54-8 MW 138.1	 isopropyl methyl phosphonic acid
Oral RfD:	0.08 mg/kg-day	Oral RfD:	0.1 mg/kg-day
Oral RfD Basis:	NOEL = 3,000 ppm diet (75 mg/kg-day) based on 90-day study in dogs. Study evaluated body weight, hematological parameters, clinical chemistry and cholinesterase inhibition. UF = 1,000 (10 each for inter- and intra-species, and use of subchronic data for chronic RfD). None of the studies evaluated by USEPA demonstrated a reliable LOAEL.	Oral RfD Basis:	NOAEL = 3,000 ppm (279 mg/kg-day) based on 90-day drinking water study in rats. Study evaluated body weight, clinical chemistry, hematology, and histology. UF = 3,000 (10 each for intra-and inter-species variability and use of subchronic data for chronic RfD and 3 for lack of supporting toxicity studies). None of the studies evaluated by USEPA demonstrated a LOAEL.

Notes:

CAS # = Chemical Abstract Service Number
LOAEL = Lowest-observed-adverse-effect level
mg/kg = Milligram per kilogram
MW = Molecular weight
NOAEL = No-observed-adverse-effect level
NOEL = No-observed-effect level
ppm = Parts per million
RfD = Reference dose
UF = Uncertainty factor

USEPA has derived oral reference doses (RfD) to be used to assess the potential for toxicity in humans exposed chronically for both of these toxicological surrogates. The RfDs are estimates of the daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (USEPA 1997).

The oral RfDs for DIMP and IMPA are 0.08 and 0.1 mg/kg-day, respectively. The oral RfD for DIMP was based on a no-observed-effect level (NOEL) of 75 mg/kg-day in a subchronic (90-day) dietary study in dogs (see Table 2). Toxic endpoints evaluated included body weight, hematological parameters, clinical chemistry and cholinesterase inhibition, as well as reproductive and teratogenic endpoints (USEPA 2006a). USEPA applied an uncertainty factor (UF) of 1,000 (10 each to account for inter- and intra- species extrapolation, and use of subchronic data for establishing a chronic threshold) to the NOEL in establishing the RfD.

The oral RfD for IMPA was based on a no-observed-adverse-effect level (NOAEL) of 279 mg/kg-day in a subchronic drinking water study in rats. Toxic endpoints evaluated included body weight, clinical chemistry, hematology and histology (USEPA 2006a). An UF of 3,000 (10 each for intra- and inter- species extrapolation, and use of subchronic data for establishing a chronic threshold, and 3 for lack of supporting toxicity studies) to the NOAEL in establishing the RfD. These studies are consistent with findings from other studies that indicate that neither of these surrogates are AChE inhibitors (ATSDR 1998).

None of the studies used by USEPA to derive RfDs for DIMP or IMPA identified a lowest-observed-adverse-effect level (LOAEL). The no-effect levels identified are therefore termed "unbounded" An unbounded NOAEL/NOEL provides an indication of a dose at which no toxic effects would be expected to occur, but does not identify the dose at which toxic effects may begin to occur, which may be at a significantly greater level than the doses tested. Therefore, it is possible that the RfDs established by USEPA for these chemicals are overly protective. In addition, due partially to the sparseness of the dataset, the UFs applied by USEPA to derive oral RfDs for DIMP and IMPA were 1,000 and 3,000, respectively, resulting in RfD values well below the documented no-effect levels.

ATSDR proposed a chronic minimum risk level (MRL) for DIMP of 0.6 mg/kg-day based upon a NOAEL of 57 mg/kg-day in a 13-month dietary study of mink. The same study established a LOAEL (for less serious effects) of 330 mg/kg-day. At this level, Heinz body counts (measures of blood toxicity) were increased in first generation (F1) females. Other effects included a statistically significant increase in plasma cholinesterase (noted by the investigators to be biologically insignificant) and an increase in ovarian follicles (this endpoint was only tested at this concentration). In the ATSDR evaluation, an UF of 100 (10 each for intra- and inter- species extrapolation) was applied to the NOAEL of 57

mg/kg-day (ATSDR 1998). Because the basis of the MRL is a NOAEL which was considerably lower than the level at which adverse effects (noted as less serious by ATSDR) were measured, the MRL is likely to be a conservative toxicity threshold.

ATSDR's subchronic MRL for DIMP of 0.8 mg/kg-day was based on the 90 day study in dogs that was used to establish USEPA's oral RfD. In this case, a UF of 100 (10 each for inter- and intra- species extrapolation) was applied to the NOAEL of 75 mg/kg-day. Although no LOAEL was reported for this study, a subchronic LOAEL in minks of 262 mg/kg-day for males and 330 mg/kg-day for females was noted in ATSDR's review. Exposures at these levels resulted in significant decreases in plasma cholinesterase. This endpoint is usually considered to be a marker of exposure rather than an adverse effect. ATSDR noted this effect as a LOAEL for "less serious effects" (ATSDR 1998).

In summary, DIMP and IMPA were the most appropriate toxicological surrogates found to estimate potential toxicity of DMPT and DEPT. They are not considered AChE inhibitors and they are structurally similar to the chemicals of interest. However, on the basis of structural activity, they are likely to be more reactive and therefore more toxic than DMPT and DEPT. In addition, the RfDs established by USEPA for DIMP and IMPA are well below documented no-effect levels and therefore are likely conservative estimates of actual toxicity thresholds.

3.3 TOXICITY CRITERIA FOR DMPT AND DEPT

Two different approaches were applied to estimate chronic RfDs for DMPT and DEPT using the available toxicity information. The first approach was to base the RfDs for the chemicals of interest upon the oral RfDs already established by USEPA for the toxicological surrogates. The oral RfDs for DIMP and IMPA are expected to be conservative estimates of toxicity thresholds for the chemicals of interest, because (1) they were based on an unbounded NOAEL/NOEL, and (2) of expected higher toxicity of DIMP and IMPA due to an increased reactivity of the P=O bond compared to the P=S bond of DMPT and DEPT. Therefore, a modifying factor was applied to the oral RfDs for DIMP and IMPA to derive oral RfDs for DEPT and DMPT. As part of this approach, the MRLs developed by ATSDR were considered to provide perspective to the proposed numbers.

The second approach was to develop a ratio for acute to chronic toxicity for each of the surrogate chemicals and then to estimate potential chronic toxicity of the chemicals of interest by applying this ratio to the available acute toxicity values. This approach was based upon the assumption that the chemicals of interest act similarly to the identified surrogates with respect to both acute and chronic toxicity. This assumption is more likely to be true for chemicals that cause toxicity by similar mechanisms of action, which is expected for DMPT, DEPT, and the proposed surrogates. Additionally, the approach

depends on having a fairly robust dataset, including both acute and longer term chronic (or subchronic) toxicity thresholds in the same animal species so that ratios may be more scientifically justifiable. Potential toxicity values for DEPT and DMPT were developed using both approaches, as described in the paragraphs below.

3.3.1 Modified RfD Approach

The modification factor approach utilized the oral RfDs established by the USEPA for DIMP and IMPA as a starting point. As previously described, the oral RfDs for the surrogates are expected to be conservative estimates of toxicity for DMPT and DEPT because they rely on the use of an unbounded NOAEL/NOEL and because DIMP and IMPA are expected to be more toxic due to the reactivity of the P=O bound. Therefore, an upward adjustment of the surrogates' RfDs is considered warranted.

As previously described, an assessment of structural activity indicates that DEPT and DMPT would be expected to have lesser reactivity, and, thus, lesser toxicity than both surrogates, and than DIMP in particular. Given that the oral RfDs established for the surrogates are already well below documented no-effect levels, it is reasonable to expect that actual toxicity thresholds for DEPT and DMPT would be substantially less than the surrogate RfDs.

A modifying factor of 0.1 was assigned to approximate the potential difference in toxicity of DMPT and DEPT due to the significantly lesser reactivity of the P=S bond in the chemicals of interest, as compared to the P=O bond in the surrogates. Though conceptually grounded in a general understanding of the relative difference in toxicity due to differences in reactivity, the quantitative factor of 0.1 was based upon the near order of magnitude difference in electronegativity between the P=O and the P=S bonds estimated using the Sanderson scale of electronegativity (Considine 2005).

On the basis of structural similarity and molecular weight, DIMP was selected as the surrogate for DEPT, and IMPA was selected as the surrogate for DMPT.

The proposed RfD for DEPT was derived by applying a modifying factor of 0.1 (dividing the RfD by 0.1) to the established DIMP oral RfD of 0.08 mg/kg-day, resulting in a proposed RfD for DEPT of 0.8 mg/kg-day for DEPT. The RfD for DMPT was derived by applying a modifying factor of 0.1 to the established IMPA oral RfD of 0.1 mg/kg-day, resulting in a proposed RfD of 1.0 mg/kg-day for DMPT.

These proposed RfDs are considered conservative. Both of these values are near the MRLs of 0.6 to 0.8 mg/kg body weight (bw) developed by ATSDR for DIMP. Conceptually, the modifying factor of 0.1 could also be applied to ATSDR's MRLs, and used as the basis of the toxicity values for DMPT and DEPT. If this was done, the

resultant toxicity values would be between 6 and 8 mg/kg-day. This is an order of magnitude higher than the RfDs proposed here for DMPT and DEPT. Therefore, the RfDs proposed for DMPT and DEPT are considered conservative.

3.3.2 Acute-to-Chronic Ratio Approach

As a further exploration of potential RfDs for DMPT and DEPT, the acute to chronic ratio approach was also applied to develop RfDs for the chemicals of interest. A review of the acute, subchronic, and chronic toxicity thresholds included in ATSDR's toxicological profile for DIMP provided comprehensive scientific data for this approach (ATSDR 1998). Although the studies included in their review were extensive, many of the studied doses did not result in any measured effects (i.e. a LOAEL, or lowest-observed–effect level (LOEL)). NOAELs and NOELs from toxicity studies were not included for consideration in the derivation of ratios due to the fact that these levels do not constitute a level of biological effect.

A ratio of acute to chronic toxicity was developed for DIMP using only data for oral exposure. Acute toxicity data were available for several species, but subchronic and chronic toxicity data were only available for mink. Table 3 presents a brief summary of toxicity thresholds identified for DIMP by exposure period and species.

Table 3. Summary of Data Used to Establish Acute/Chronic Toxicity Ratios for DIMP.

Acute Exposure			Subchronic Exposure			Chronic Exposure		
Test Species	Toxicity Threshold (mg/kg-day)		Test Species	Toxicity Threshold (mg/kg-day)		Test Species	Toxicity Threshold (mg/kg-day)	
Rat	LD50	1,125 (m); 826 (f)	Mink	LOAEL (less serious effects)	201	Mink	LOAEL (less serious effects)	330
Mouse	LD50	1,041 (m); 1,363 (f)						
Mink	LD50	503 (f)						
Duck	LD50	1,490						

Source: ATSDR (1998).

Ratios of acute toxicity to longer-term effects were calculated for mink as follows:

Acute LD50/chronic LOAEL = 503/330 = 1.5

Acute LD50/subchronic LOAEL = 503/201 = 2.5

The ratios derived from the DIMP toxicity data were then applied to the limited acute toxicity data available for DMPT and DEPT (see Table 1 for summary of acute toxicity data). Acute oral toxicity LD50s for DMPT ranged from 694 mg/kg-day in rats to 1,400 mg/kg-day in unspecified mammal. Application of the ratio of 1.5 to the range of acute oral LD50s resulted in a chronic range of lowest effect of 462 – 933 mg/kg-day. Applying a UF of 1,000 (to account for inter-and intraspecies variability and paucity of data) to this value resulted in a toxicity threshold range of 0.4 – 0.9 mg/kg-day.

The only available acute oral toxicity LD50 for DEPT was 4,510 mg/kg in rats. Application of a ratio of 1.5 to the acute LD50 resulted in a chronic level of lowest effect of 3,006 mg/kg-day. Applying a UF of 1,000 to this value resulted in a toxicity threshold of 3 mg/kg-day.

The limited number of measured effects for chronic exposure to DIMP in multiple species does not allow for an in depth, comprehensive exploration of the relative ratios of acute to chronic toxicity for this compound that can be readily applied to other chemicals. Although the results of this preliminary analysis must be used with caution, they do indicate toxicity values in the range of those derived using the RfD approach. This is considered to positively add to the overall weight of evidence supporting these values.

3.3.3 Recommended RfDs for DMPT and DEPT

The RfDs derived for DEPT using both the modification factor approach and the ratio approach were 0.8 mg/kg-day and 3 mg/kg-day, respectively. The RfDs derived for DMPT using both the modification factor approach and the ratio approach were 1.0 mg/kg-day and a range of 0.4 – 0.9 mg/kg-day, respectively. Both approaches resulted in RfDs of similar magnitude. Therefore, the RfDs developed using the simplest approach, the modification factor approach, were selected as the proposed oral RfDs for DEPT and DMPT.

The proposed oral RfD for DEPT is 0.8 mg/kg-day. The proposed oral RfD for DMPT is 1 mg/kg-day. These RfDs are based upon oral exposure over a chronic toxicity period. Given that the proposed RfDs are modified from oral RfDs that rely on the use of unbounded NOAEL/NOEL levels at which no adverse effects were observed and to which large uncertainty factors of 1,000 and 3,000 were applied, the proposed RfDs are considered conservative. The conservative nature of the proposed RfDs can be

demonstrated by considering ATSDR MRLs for DIMP. Conceptually, the modifying factor of 0.1 could also be applied to ATSDR's MRLs for DIMP, and used as the basis of the toxicity values for DMPT and DEPT. If this was done, the resultant toxicity values would be between 6 and 8 mg/kg-day. This is an order of magnitude higher than the RfDs proposed here for DMPT and DEPT.

4. FINDINGS AND RECOMMENDATIONS

There is sufficient evidence in the literature to support the conclusion that DEPT and DMPT are not AChE inhibitors. Although they may engender other toxic effects, there is insufficient direct evidence in the literature to identify what those toxic effects may be, particularly under subchronic and chronic exposure scenarios. Given the lack of direct toxicity data for the chemicals of interest, potential toxicological surrogates were identified based upon chemical structure, physical/chemical properties, biochemical attributes, and the availability of chronic toxicity data. DIMP was selected as a toxicological surrogate for DEPT and IMPA was selected as a toxicological surrogate for DMPT. As a result of the review of the derivation of oral RfDs for the surrogates and the structural activity comparison between the surrogates and the chemicals of interest, it was concluded that the RfDs established by USEPA for the surrogates proposed by NDEP were overly conservative for the chemicals of interest. Two approaches were used to develop more appropriate RfD values for DMPT and DEPT.

The first approach used a modifying factor to account for the structural activity differences between the surrogates and the chemicals of interest. The second approach applied a ratio of acute toxicity threshold to chronic toxicity threshold developed from toxicity data for the surrogates, to estimate potential chronic toxicity thresholds for the chemicals of interest.

Both approaches resulted in RfDs of similar magnitude. Therefore, the RfDs developed using the simplest approach, the application of a modifying factor, are proposed as the most appropriate RfDs for evaluating potential risk associated with DMPT and DEPT.

The proposed RfD for DMPT is 1 mg/kg-day.

The proposed RfD for DEPT is 0.8 mg/kg-day.

5. REFERENCES

ATSDR. 1997. Toxicological profile for chlorpyrifos. Available at: www.atsdr.cdc.gov/toxprofiles/tp84.pdf. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Washington, DC. pp. 217.

ATSDR. 1998. Toxicological profile for diisopropyl methylphosphonate. Available at: www.atsdr.cdc.gov/toxprofiles/tp119.pdf. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Washington, DC. pp. 169.

ATSDR. 2001. Toxicological profile for methyl parathion. Available at: www.atsdr.cdc.gov/toxprofiles/tp48.pdf. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Washington, DC. pp. 267.

ATSDR. 2003. Toxicological profile for malathion. Available at: www.atsdr.cdc.gov/toxprofiles/tp154.pdf. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Washington, DC. pp. 327.

CCOHS. 2006a. Registry of Toxic Effects of Chemical Substances (RTECS): phosphorodithioic acid, O,O-diethyl ester web page. www.ccohs.ca/products/databases/rtecs.html. Last updated on February 2004. Canadian Centre for Occupational Health & Safety, Ontario.

CCOHS. 2006b. Registry of Toxic Effects of Chemical Substances (RTECS): phosphorodithioic acid, O,O-dimethyl ester web page. www.ccohs.ca/products/databases/rtecs.html. Last updated on February 2004. Canadian Centre for Occupational Health & Safety, Ontario.

CDC. 2005. Third national report on human exposure to environmental chemicals. Available to www.cdc.gov/exposurereport/3rd/pdf/thirdreport.pdf. Centers for Disease Control and Prevention, National Center for Environmental Health, Division of Laboratory Sciences, Atlanta, GA. pp. 475.

Chukwudebe, A.C., M.A. Hussain, and P.C. Oloffs. 1984. Hydrolytic and metabolic products of acephate in water and mouse liver. *J. Environ. Sci. Health*, B19 (6): 501-522.

Considine, G. D. (ed). 2005. *Van Nostrand's encyclopedia of chemistry, fifth edition*. John Wiley & Sons, Inc., Hoboken, NJ.

Crouse, G. 2006. Personal communication (letter to B. Rakvica, Nevada Department of the Environment, Las Vegas, NV, dated September 7, 2006, regarding toxicological surrogates for DMPT and DEPT). Syngenta Crop Protection, Inc., Greensboro, NC.

Graham, C. 2003. Personal communication (email to A. Nguyen, U.S. Environmental Protection Agency, Washington, DC, dated November 26, 2003, regarding cover letter, test plan and IUCLID document on O,O-diethyl dithiophosphate (CAS # 298-06-6) for submission to the US EPA HPV Challenge Program). Bayer Polymers LLC, Pittsburgh, PA.

IOM. 2004. *Gulf War and health, update literature review of sarin*. Available at: www.nap.edu. Board on Health Promotion and Disease Prevention, Institute of Medicine of the National Academies, The National Academies Press, Washington, DC.

Manahan, S. E. 2003. *Toxicological chemistry and biochemistry, third edition*. CRC Press LLC, Boca Raton, Florida.

Maxwell, D.M., and D.E. Lenz. 1992. Structure-activity relationships and anticholinesterase activity. pp. 47-58. In: Clinical and Experimental Toxicology of Organophosphates and Carbamates. B. Ballantyne and T. C. Marrs (eds). Butterworth-Heinemann Ltd, Oxford.

Mileson, B.E., J.E. Chambers, W.L. Chen, W. Dettbar, M. Ehrich, A.T. Eldefrawi, D.W. Gaylor, K. Hamernik, E. Hodgson, A.G. Karczmar, S. Padilla, C.N. Pope, R.J. Richardson, D.R. Saunders, L.P. Sheets, L.G. Sultatos, and K.B. Wallace. 1998. Common mechanism of toxicity: a case study of organophosphorus pesticides. *Toxicol. Sci*. 41:8-20.

Pope, C.N. 1999. Organophosphorus pesticides: do they all have the same mechanism of toxicity? *J. Toxicol. Environ. Health*. Part B. 2: 161-181.

Rakvica, B.A. 2006. Personal communication (letter to M. Paris, Basic Remediation Company, Henderson, NV, S. Crowley, Tronox LLC, Henderson, NV, S. Chamberlain, Pioneer Companies, Inc., Houston, TX, J. Kelly, Montrose Chemical Corp of CA, Bainbridge Island, WA, G. Crouse, Syngenta Crop Protection, Inc., Greensboro, NC, and C. Wilkinson, Titanium Metals Corporation, Henderson, NV, dated August 29, 2006, regarding BMI plant sites and common areas projects, Henderson, NV). Nevada Division of Environmental Protection, Las Vegas, NV.

Science Group. 2004. A critique of the United States Environmental Protection Agency's (US EPA) grouping of insecticidal organophosphates and N-methyl carbamates into common mechanism groups. 39 pp.

USEPA. 1997. Health affects assessment summary tables, FY-1997 update. U.S. Environmental Protection Agency, Office of Research and Development, Office of Emergency and Remedial Response, Washington DC.

USEPA. 2006a. Integrated Risk Information System (IRIS) web page. www.epa.gov/iris/. Accessed on October 20, 2006. Last updated on March 8, 2006. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2006b. High production volume (HPV) challenge program. www.epa.gov/chemrtk/. Accessed on October 20, 2006. Last updated August 29, 2006. U.S. Environmental Protection Agency, Washington, DC.

Vasilic, Z., V. Drevenkar, B. Stengl, Z. Frobe, and V. Rumenjak. 1993. Diethylphosphorus metabolites in serum and urine of persons poisoned by phosalone. *Chem.-Biol. Interactions*. 87:305-313.

6. OTHER LITERATURE REVIEWED

Ali, H.M. and A.A. Mostafa. 1999. Quantitative structure-activity relationship of a series of N-aryl O-aryl phosphoramidate insecticides. *Environ. Toxicol. Chem*. 18(2):167-171.

ATSDR. 1995. Toxicological profile for disulfoton. Available at: www.atsdr.cdc.gov/toxprofiles/tp65.pdf. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Washington, DC. pp. 239.

Barr, D.B., R. Bravo, G. Weerasekera, L.M. Caltabiano, R.D. Whitehead, Jr., A.O. Olsson, S.P. Caudill, S.E. Schober, J. L. Pirkle, E.J. Sampson, R.J. Jackson, and L.L. Needham. 2004. Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the U.S. population. *Environ. Health Perspect*. 112(2):186-200.

Bradbury, S.P., C.L. Russom, G.T. Ankley, T.W. Schultz, and J.D. Walker. 2003. Overview of data and conceptual approaches for derivation of quantitative structure-activity relationships for ecotoxicological effects of organic chemicals. *Environ. Toxicol. Chem*. 22(8):1789-1798.

Breau, A.P., W.M. Mitchell, J. Swinson, and L. Field. 1985. Mutagenic and cell transformation activities of representative phosphorothiolate esters in vitro. Abstract. *J. Toxicol. Environ. Health*. 16(3-4):403-413.

Comber, M.H.I., J.D. Walker, C. Watts, and J. Hermens. 2003. Quantitative structure-activity relationships for predicting potential ecological hazard of organic chemicals for use in regulatory risk assessments. *Environ. Toxicol. Chem*. 22(8):1822-1828.

Davies, J.E., and J.C. Peterson. 1997. Surveillance of occupational, accidental, and incidental exposure using urine alkyl phosphate and phenolic metabolite measurements. *Annals NY Academy of Sciences*. 837:257-268.

Edwards, F.L., and P.B. Tchounwou. 2005. Environmental toxicology and health effects associated with methyl parathion exposure – a scientific review. *Int. J. Environ. Res. Public Health*. 2(3):430-441.

FAO and WHO. 1997. Pesticides residues in food, part II: toxicological assessment. Draft. Available at: www.inchem.org/documents/jmpr/jmpmono/v96pr05.htm. York, United Kingdom. 60 pp.

Guizzetti, M., S. Pathak, G. Giordano, L.G. Costa. 2005. Effect of organophosphorus insecticides and their metabolites on astroglial cell proliferation. *Toxicology*. 215:182-190.

Hernandez, O. 2004. Personal communication (letter to C. Graham, Bayer CropScience LP, Pittsburgh, PA, dated May 20, 2004, regarding EPA's comments on summaries and test plan for chemical RTK HPV challenge submission: O,O-Diethyl Dithiophosphate). U.S. Environmental Protection Agency.

Holmstedt, B. 1963. Structure-activity relationships of the organophosphorus anticholinesterase agents. pp. 428-485. In: Handbuch der Experimentellen Pharmakologie: Cholinesterases and Anticholinesterase Agents. O. Eichler and A. Farrah (eds). Springer-Verlag, Berlin.

Holmstedt, B. 1959. Pharmacology of organophosphorus cholinesterase inhibitors. *Pharmacol. Rev.* 11: 567-688.

Kovacic, P. 2003. Mechanism of organophosphates (nerve gases and pesticides) and antidotes: electron transfer and oxidative stress. *Current Medicinal Chem*. 10:2705-2709.

Kwong, T.C. 2002. Organophosphate pesticides: biochemistry and clinical toxicology. *Therapeutic Drug Monitoring*. 24:144-149.

Lima, A., and L. Vega. 2005. Methyl-parathion and organophosphorus pesticide metabolites modify the activation status and interleukin-2 secretion of human peripheral blood mononuclear cells. *Toxicol. Letters*. 158:30-38.

Main, A.R. 1964. Affinity and phosphorylation constants for the inhibition of esterases by organophosphates. *Science*. 144: 992-993.

Maxwell, D.M., and K.M. Brecht. 1992. Quantitative structure-activity analysis of acetylcholinesterase inhibition by oxono and thiono analogues of organophosphorus compounds. *Chem. Res. Toxicol*. 5: 66-71.

Menzer, R.E., and W.C. Dauterman. 1970. Metabolism of some organophosphorus insecticides. *J. Agr. Food Chem.* 18(6):1031-1037.

Taylor, P., L. Wong, Z. Radic, I. Tsigelny, R. Bruggemann, N.A. Hosea, and H.A. Berman. 1999. Analysis of cholinesterase inactivation and reactivation by systemic structural modification and enantiomeric selectivity. *Chem.-Biol. Interactions*. 119-120:3-15.

Worek, F., H. Thiermann, L. Szinicz, and P. Eyer. 2004. Kinetic analysis of interactions between human acetylcholinesterase, structurally different organophosphorus compounds and oximes. *Biochemical Pharmacol*. 68: 2237-2248.