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A SCIENCE POLICY ON A COMMON MECHANISM OF TOXICITY: THE CARBAMATE PESTICIDES AND THE GROUPING OF CARBAMATE WITH THE ORGANOPHOSPHORUS PESTICIDES

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August 30, 1999

SUMMARY

The 1996 Food Quality Protection Act (FQPA, 1996) directs the US EPA to conduct assessments of potential human risks associated with exposure to pesticides using some fundamentally new approaches. Tolerance assessments and reassessmentsts for pesticides are to consider "available information concerning the cumulative effects of such residues and other substances that have a common mechanism of toxicity....." The Agency is currently developing guidance and a process for performing cumulative risk assessments of this type. Such assessments will play an increasingly important role in the evaluation of risks posed by pesticides, and will improve the Agency's ability to make regulatory decisions that fully protect public health and sensitive subpopulations, including infants and children.

As part of an ongoing effort to implement the requirements of the FQPA, the Health Effects Division (HED) of the Office of Pesticide Programs (OPP) has developed a number of guidance documents on science issues including a guidance document that defines what constitutes a common mechanism of toxicity and how to determine whether two or more chemicals should be grouped based on a common mechanism of toxicity (USEPA, 1999a). Guidance for how to conduct a cumulative risk assessment for pesticides and other substances sharing a common mechanism of toxicity is being developed and a draft of the guidance will be presented to the FIFRA Science Advisory Panel in 1999.

The identification of a candidate group of pesticides and other substances that cause a

common toxic effect by a common mechanism is the first step in the cumulative risk assessment process. Because organophosphorus and carbamate pesticides have been assigned priority for tolerance reassessment, OPP has considered data for identifying whether these pesticides cause common toxic effects by common mechanisms of toxicity under EPA's *Guidance For Identifying Pesticide Chemicals and Other Substances That Have A Common Mechanism of Toxicity* (USEPA, 1999a). The inhibition of acetylcholinesterase (AChE) has been a focal point given that most organophosphorus and many carbamate pesticides cause this effect. The physiological action of AChE is to hydrolyze the neurotransmitter acetylcholine (ACh) so that the activation of the cholinergic receptor is transient. When AChE is inhibited, ACh 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.

OPP already has developed two policy documents which assist the program in identifying a common mechanism of toxicity for cholinesterase inhibiting pesticides: *"Use of Cholinesterase Inhibition for Risk Assessments of Organophosphate and Carbamate Pesticides"* (USEPA, 1998) and *"A Common Mechanism of Toxicity: The Organophosphate Pesticides"* (USEPA, 1999b). The current document provides further guidance as it articulates policies on defining a common mechanism of toxicity for carbamate pesticides and on the grouping of carbamate pesticides with organophosphorus pesticides, based on the potential for members of these two classes of chemicals to act by a common mechanism of toxicity.

OPP approached the issue of grouping carbamate and organophosphorus pesticides for purposes of identifying a candidate set of chemicals for a cumulative risk assessment by asking three questions: (I) Does acetylcholinesterase inhibition provide a sufficient basis for determining a common mechanism of toxicity for grouping carbamate pesticides and a presumption of common toxic effects, (ii) could carbamate pesticides also be subgrouped based on the characteristic of some of these substances to produce effects unrelated to cholinesterase inhibition, and (iii) should the carbamate pesticides that inhibit AChE be grouped with the organophosphorus pesticides that also cause AChE inhibition?

In answering question (i), OPP concluded that acetylcholinesterase inhibition does

provide a sufficient basis for determining that a common mechanism of toxicity exists for the purpose of grouping carbamates whose principal mechanism of toxicity is the inhibition of AChE which results in the overstimulation of cholinergic pathways.

In answering question (ii), OPP concluded that carbamates whose mechanism(s) of toxicity do not involve cholinesterase inhibition should not be grouped with the cholinesterase-inhibiting carbamates and organophosphorus pesticides. These non-AChE-inhibiting carbamates could be grouped, even with certain of the AChE-inhibiting carbamates, if they share one or more mechanisms of toxicity different from a AChEI. However, this grouping exercise would be done separately from that for cholinesterase inhibition.

In answering question (iii), OPP concluded that the cholinesterase-inhibiting carbamates and organophosphorus compounds do act by a common mechanism of toxicity and cause common cholinergic effects. Exposure to a carbamate and a organophosphorus pesticide may lead to cumulative effects if concurrent exposures occur or if the effects following exposure to one or more substances in these two classes persist into the exposure window for additional substances in these classes. Nonetheless, OPP recognizes that the grouping of chemicals based on a common mechanism of toxicity is just one step in the cumulative risk assessment process and will not necessarily lead to a cumulative risk assessment involving all members of the group. Other factors, to be discussed in detail in the *Guidance on Conducting Cumulative Risk Assessments of Pesticide Chemicals That Have a Common Mechanism of Toxicity* (US EPA, in preparation, 1999b), will be addressed prior to the final selection of chemicals for a cumulative risk assessment. These include the potential for concurrent exposures, the critical window for expression of effects, duration of toxic effects, reversibility of acetylcholinesterase inhibition, and significance of other potentially critical toxic effects (e.g., neuropathic effects of some thio- and dithiocarbamates) that may be produced in the absence of AChEI.

Questions for the FIFRA Scientific Advisory Panel

The purpose of this presentation is to solicit advice from the FIFRA Scientific Advisory Panel (SAP) regarding OPP's effort described above. Specifically, OPP would like the SAP to answer the following questions.

Question 1. Does the Panel agree with OPP's conclusion that acetylcholinesterase inhibition provide a sufficient basis for determining a common mechanism of toxicity for grouping carbamate pesticides?

Question 2. Does the Panel agree with OPP's conclusion that carbamate pesticides that inhibit AChE should be grouped with the organophosphorus pesticides that also cause AChE inhibition?

1. PURPOSE, SCOPE, AND ORGANIZATION OF DOCUMENT

1.1. Purpose & Scope

The purpose of this science policy document is to present the scientific basis and criteria for selecting and grouping some or all carbamate pesticides by a common mechanism. The focus of the policy is on the state of the science regarding the biochemical sequence of events that result in the expression of cholinergic effects for the carbamate pesticides that might cause a common toxic effect by a common mechanism. This paper also discusses toxicity endpoints that are considered as common toxic effects. The science policy addresses toxicological features of subgroups of carbamate pesticides that require further evaluation before a candidate group of specific chemicals among the subgroups can be definitively identified as being toxic by a common mechanism.

Toxicological characteristics that the organophosphorus compounds and carbamate pesticides share are also briefly reviewed in order to answer the general question: should all, or some, carbamate pesticides be grouped with all, or some, organophosphorus pesticides based on a common mechanism of toxicity?

Identification of each substance that causes a common toxic effect by a common mechanism and the actual selection and grouping of those substances that share a common mechanism of toxicity is beyond the scope of the current document. Guidance provided in the EPA document *"Guidance for Identifying Pesticide Chemicals and Other Substances that Have a Common Mechanism of Toxicity"* (USEPA, 1999a) will be followed when a defined candidate set of carbamate and organophosphorus pesticides is selected for a cumulative risk assessment. The current document is intended to provide an outline of the data needed and a general framework for selecting a common mechanism and a common toxic effect for each chemical of a common mechanism of toxicity group. There is also a brief discussion of some additional biological, toxicological, and exposure information that should be considered before combining

carbamate or carbamate and organophosphorus pesticides in a cumulative risk assessment.

The document concludes by identifying data on carbamate and organophosphorus pesticides which could be obtained from laboratory investigations that could lead to refinements in cumulative risk assessments.

1.2. Organization

This science policy document describes the approaches that OPP will take when determining whether or not carbamate pesticides have a common mechanism of toxicity, and whether or not carbamate and organophosphorous pesticides also should be grouped on the basis of a common mechanism of toxicity.

- Section 1 (this section) describes the the purpose and scope of the science policy and also summarizes additonal information, discussed briefly in the body of this science policy document, that must be considered before making a final selection of chemicals that are to be included in a cumulative risk assessment (part 1.1) and the organization of the document (part 1.2.).

- Section 2 presents the historical background to the development of a policy on how to determine if a common mechanism of toxicity exists for the carbamate pesticides. This section also includes a brief summary of the development of science policies which address issues of common mechanism of toxicity of the organophosphorus compounds and for identifying chemicals that have a common mechanism of toxicity, in general.

- Section 3 provides a very brief description of how many of the carbamates exhibit their pesticidal action and mammalian toxicity by a biochemical mechanism that begins with the inhibition of acetylcholinesterase.

- Section 4 presents structural similarities and differences among various carbamates that may account for their differing potencies with which they inhibit cholinesterase.

- Section 5 describes effects other than cholinesterase inhibition that should be considered before reaching a decision whether or not a carbamate should be included in a group of cholinesterase-inhibiting carbamates identified as candidates for a cumulative risk assessment. Many carbamates are used to kill insects by inhibiting acetylcholinesterase but may have other toxicities. Others, such as the thiocarbamates and dithiocarbamates are generally used as herbicides or fungicides, because their ability to inhibit cholinesterase is lacking or minimal.

- Section 6 describes some of the evidence to support that it is appropriate to group the ChEI-carbamates and organophosphorus pesticides. The mechanism of toxicity for the two classes is indistinguishable, toxicologically, despite biochemical differences (e.g., carbamylation versus phosphorylation). Both classes of chemicals serve as pseudo-substrates for acetylcholinesterase and lead to a common spectrum of toxic effects associated with cholinesterase inhibition.

- Section 7 provides the policy guidance that OPP will when making initial group selections for a cumulative risk assessment for carbamates, as a group, and for ChEI-carbamates and organophosphorus chemicals.

- Section 8 presents a general discussion of some additional considerations that will influence if a chemical initially included in a common mechanism of toxicity grouping will be assessed for potential cumulative effects and risks.

- Section 9 concludes the document with a brief outline of research that could lead to refinements in the approaches to the qualitative and quantitative aspects of a cumulative

risk assessment.

2. INTRODUCTION

The Food Quality Protection Act (FQPA) of 1996 specifies, among other things,[1] that when determining the safety of a pesticide chemical, EPA shall base its assessment of the risk on: aggregate (i.e., total dietary, residential, and other non-occupational) exposure and available information concerning the cumulative effects to human health that may result from dietary, residential, or other non-occupational exposure to pesticides and other substances that have a common mechanism of toxicity. The Act accounts for the possibility that low-level exposures to multiple substances that cause a common toxic effect by a common mechanism could lead to the same adverse health effect as would a higher level of exposure to any of the chemicals individually. Individuals, including infants and children, exposed to a pesticide at a level that is considered safe may in fact experience harm if that person is also exposed to other substances that cause a common toxic effect by a mechanism common with that of the subject pesticide, even if the individual exposure levels to the other substances are also considered safe.

Both the organophosphorus and carbamate classes of pesticides have been given high priority by the Office of Pesticide Programs for the reassessment of their tolerances in accordance with the mandates of FQPA. The organophosphorus pesticides have been assigned the highest priority for tolerance reassessment; preliminary risk assessment documents for many have been completed or are nearing completion. Revised risk assessments have been prepared for several chemicals, based upon comments received during a public comment period. The need to complete such reassessments in a timely manner led to the development of several science policy

[1] For details see *The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Federal Food, Drug, and Cosmetic Act (FFDCA) As Amended by the Food Quality Protection Act (FQPA) of August 3, 1996;* U.S. Environmental Protection Agency, Office of Pesticide Programs, document # 730L97001, March, 1997.

documents to be used when performing hazard and risk assessments on the organophosphorus compounds. These policies apply, as well, to the carbamate pesticides. These papers have been reviewed by the FIFRA Scientific Advisory Panel (SAP) and also were published in the Federal Register for broader public comment. These science policies include *Guidance for Identifying Pesticide Chemicals and Other Substances that have a Common Mechanism of Toxicity (USEPA, 1999a), Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphate and Carbamate Pesticides* (USEPA, 1998) and *A Common Mechanism of Toxicity: The Organophosphate Pesticides* (USEPA, 1999b). The essence of the science policy on the organophosphorous pesticides is to regard those that express toxicity through a common biochemical interaction with acetyl cholinesterase, which may lead to a variety of cholinergic effects, as a common mechanism group for consideration in a cumulative risk assessment.

As had been done for the organophosphorus pesticides, an expert panel was convened through a cooperative agreement between the EPA and the Risk Sciences Institute (RSI) of the International Life Sciences Institute (ILSI) to address whether carbamate pesticides act by a common mechanism of toxicity. This panel applied the same basic principles developed by the earlier RSI panel for addressing whether the organophosphorus pesticides shared a common mechanism of toxicity (Mileson et. al., 1998). Briefly, the RSI panel evaluated the potential for two or more carbamate pesticides to act by the same mechanism by applying three principles. The principles were: 1) do they cause the same critical effect(s), 2) do they act on the same molecular target at the same target tissue, and 3) do they act by the same biochemical mechanism of action, perhaps because they share a common toxic intermediate? The RSI panel focused on acetylcholinesterase inhibition as a scientifically accepted mechanism of action for the ChEI-carbamates and found that the three principles were met. The workgroup concluded that the ChEI carbamates should be considered to act by a common mechanism of toxicity. RSI also pointed out that some carbamates also produce effects that may not be related to AChEI.

The information reviewed in the sections following provided the scientific basis considered by the Office of Pesticide Programs in reaching conclusions regarding:

(i) whether acetylcholinesterase inhibition is sufficient evidence of a common mechanism

of toxicity for grouping carbamate pesticides,

(ii) whether carbamate pesticides could also be grouped based on the ability of some members of the class to produce effects unrelated to cholinesterase inhibition, and

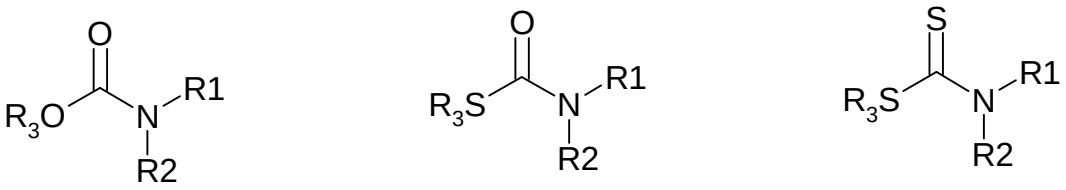
(iii) whether the carbamate pesticides that inhibit AChE should be grouped with the organophosphorus pesticides that inhibit AChE.

3. GENERAL DESCRIPTION OF ACETYLCHOLINESTERASE (AChE) INHIBITION BY CARBAMATES

The toxicity of the acetylcholinesterase-inhibiting carbamates is manifested primarily through their interaction with, and carbamylation of, AChE in the peripheral and central nervous systems. Acetylcholinesterase is the enzyme which breaks down acetylcholine in the synapses between neurons. Inhibition of AChE leads to an accumulation of acetylcholine and a prolongation of its action at the nerve endings. This results in cholinergic responses of smooth muscle contractions (e.g., abdominal cramps), glandular secretions (e.g., sweating and lacrimation), and skeletal muscle twitching or even paralysis. Depending on the dose, a broad range of effects may be seen affecting most bodily functions, and these effects can be serious or fatal. Like the organophosphorus pesticides (OPs), individual carbamates may produce different patterns of cholinergic effects, depending on both pharmacokinetic (absorption, distribution, metabolism) and pharmacodynamic factors (e.g., substrate affinity, binding potency, rate of reversal). Reviews of the toxicity of carbamates have been prepared by Baron (1991), Ecobichon (1982), WHO (1986, 1988a,b) and provide general reviews of the cholinergic effects and the AChE inhibition common to many members of this class.

4. STRUCTURAL CATEGORIES, USES AND CHOLINESTERASE INHIBITION AMONG THE CARBAMATES

Figure 1. Schematic backbone structures of Carbamates, Thiocarbamates, and Dithiocarbamates.



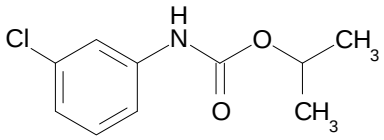
I. Carbamates II. Thiocarbamates III. Dithiocarbamates

Carbamate pesticides may be divided into three major structural categories, shown in Figure 1 that differ with respect to whether oxygen (I) or sulfur (II, III) linkages are seen at R_3; and whether oxygen (I, II) or sulfur (III) is doubly bonded to the carbamate carbon.
For any of these categories, R_3 esters may be alkyl groups, oxime derivatives, aryl groups, or other more complex moieties.

For **Carbamates (I)** , R_1 and R_2 may both be methyl groups (methyl carbamates), or R_1 may be hydrogen and R_2 a methyl group (N-methyl carbamates). Pesticides with these substituents are generally potent acetylcholinesterase (AChE) inhibitors and are commonly used

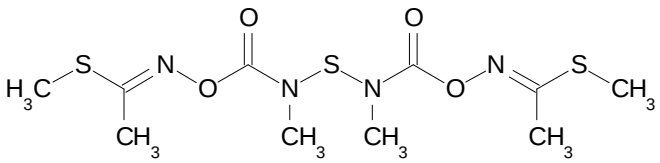
as insecticides. When R_1 and R_2 are groups larger than hydrogen or a methyl group, the substituted carbamates generally have minimal or no anti-cholinesterase activity. They are typically marketed as herbicides or fungicides, or for other uses. Chlorpropham, a phenylcarbamate herbicide, where R_2 is a phenyl group, is in this category (Figure 2).

Figure 2. Chlorpropham



Procarbamates are another sub-category of carbamate insecticides in which the R_1 substituent is an oxygen or sulfur ester moiety and in which the parent is transformed to a pesticidal carbamate. They are designed to be more readily metabolized to carbamates in insects than in mammals. Thiodicarb is an example of a procarbamate in which the R_1 sulfur ester is another carbamate (Figure 3).

Figure 3. Thiodicarb



Thiocarbamates (II) are used as herbicides and have less **(**if any) potency then Group I substances as AChE inhibitors. Nevertheless, they meet the general structural requirements for inhibition of AChE of this class, i.e., an R_3 ester, which may carbamylate the

AChE serine site. Some have been shown to cause AChE inhibition in animals at higher doses. The critical, or most sensitive effect for this and other thiocarbamates, may not be a consequence of AChEI.

Dithiocarbamates (III) are generally used as fungicides, though some have other uses and they also may have R_3 esters that, in theory, could inhibit AChE. At least one, Thiram, has been shown to do so, but again, at doses higher than those inducing other critical effects of concern.

In summary, it is expected that the potential for AChE inhibition will be a critical effect and primary concern for most of the ChEI-carbamate insecticides. While some thiocarbamates and dithiocarbamates may also inhibit AChE, for most, this is probably not their most sensitive or critical effect.

Table 1 lists the carbamate pesticides and their primary uses for each of the three structural categories. Chemicals currently not registered in the U.S. are noted with an asterisk *

Table 1. Carbamates Registered under FIFRA by EPA

PC Code	Carbamates	PC Code	Thiocarbamates	PC Code	Dithiocarbamates
098301	Aldicarb I	041405	Butylate H	014504	Mancozeb F
106901, 2,3	Asulam H	041301	Cycloate H	014505	Maneb F
105201	Bendiocarb I	041401	EPTC H	014601	Metiram F
056801	Carbaryl I	041402	Molinate H	014506	Zineb F
090601	Carbofuran I	041403	Pebulate H	039003	Metam, Na, K. F,I
018301	Chlorpropham H	041404	Vernolate H	034804	NaDMDTC F, I
104801	Desmidipham H	078801	Diallate H	079801	Thiram F,H,I
097301	Formetanate HCl I	078802	Triallate H	034801	Ferbam F
100501	Methiocarb I	108401	Thiobencarb H	034805	Ziram F
090301	Methomyl I				
103801	Oxamyl I				
098701	Phenmedipham H				
106101	Pirimicarb A				
119301,2	Propamocarb HCl F				
047601	Propham H SI				
047802	Propoxur I				
114501	Thiodicarb I				
103401	Thiophanate F				
102001	Thiophanate methyl F				
107801	IPBC WP				
102400(1,2)	Trimethacarb I				
110801	Aldoxycarb I				
125301	Fenoxycarb GR				
115001	2-EEEBC F				
017601	Barban H				
59301,2	Bufencarb I				
044201	Mexacarbate I				

A = aphicide; I = insecticide; H = herbicide; F= fungicide; GR= growth regulator; SI = sprout inhibitor; WP = wood preservative; *= not registered.

5. OTHER CRITICAL TOXIC EFFECTS OF CARBAMATE PESTICIDES

The critical or most sensitive effects produced following exposure to carbamate pesticides are not limited to the consequences of inhibition of acetylcholinesterase. Depending on the particular carbamate, other effects may result including reproductive or developmental effects, thyroid toxicity and neuropathic effects. Specific effects associated with treatment of laboratory animals with carbamates and reported in studies submitted to the Office of Pesticide Programs include:

- Reproductive Toxicity - effects on fertility such as decreased spermatogenesis, testicular toxicity, increased resorptions and postimplantation losses, and increases in spontaneous abortions;
- Developmental Toxicity - cerebral and ocular malformations, morphometric alterations of the brain and skeletal anomalies;
- Neuropathic Effects - sciatic nerve degeneration and neuronal cell necrosis;
- Thyroid Toxicity - disruptions in thyroid function associated with decreases in T3 and T4, increases in TSH, hypertrophy of thyroid tissue, thyroid hyperplasia and thyroid tumors.

The additional toxicities reported for members of this class of pesticides should not be overlooked when considering the grouping of compounds that inhibit cholinesterase. This is because effects other than those associated with AChEI may have a common underlying mechanism among several members of the carbamate pesticides. These other effects may occur below, near, or above doses that are associated with AChEI. In particular, the dithiocarbamates and thiocarbamates are two subgroups of carbamates whose toxicities are characterized principally by effects other than cholinesterase inhibition. Effects reported for these two groups include dose-dependent reproductive and thyroid toxicity, developmental toxicity including cleft palate, hydrocephaly, and skeletal malformations, and neurotoxicity expressed as muscular and

peripheral nerve atrophy, demyelination and degeneration of peripheral nerves, ataxia and paralysis (WHO, 1988a,b). These effects are likely to occur as a result of biochemical mechanisms not involving AChEI and which may operate in the absence of AChEI. It is beyond the scope of this paper to conduct a thorough evaluation of all available data to determine if carbamate pesticides act by a common mechanism of toxicity for these other effects, and if so, how they would be grouped for a cumulative risk assessment.

6. COMPARISON OF AChE -INHIBITING CARBAMATES WITH ORGANOPHOSPHORUS CHEMICALS: DO THEY SHARE A COMMON MECHANISM?

While Fukuto (1990) has described the inhibition of AChE by carbamates and organophosphorus esters as "virtually identical", he also describes two distinct differences at the biochemical level.

The major difference is the regeneration rate of the carbamylated AChE enzyme, which is spontaneous and much faster than for phosphorylated AChE enzyme, and may take only about 30 minutes. The regeneration of the phosphorylated enzyme may be on the order of hours to days, or even longer. Ecobichon (1982), in reviewing the mechanism of action for carbamates, also notes that "In effect, the only distinctive difference between carbamate and organophosphorus esters lies in the rate at which the decarbamylation and dephosphorylation takes place". He also notes the importance of recognizing that the rate of carbamylation and decarbamylation with acetylcholine is thousands of times faster than with carbamate pesticides.

The time course of enzyme inhibition generally differs among either carbamates or organophosphorus chemicals. The duration of effects for any carbamate (or organophosphorus compound) will depend on both pharmacokinetic and pharmacodynamic factors, and may vary considerably within and between classes. Baron (1991) notes large differences in the duration of AChE inhibition between two carbamates, propoxur and promecarb, which appear to be a function of different rates of absorption.

The duration of effect of a carbamate may last much longer than 30 minutes, as a function of the dose administered. In February, 1999, the FIFRA Scientific Advisory Panel concluded, based on evaluation of human data following acute exposures to aldicarb, that while the effects of aldicarb (clinical signs or AChEI in blood) "are fully reversed for many adult

subjects within 8 hours of exposure", "8 hours may not be long enough for full recovery in a significant fraction of healthy adults, in the very young, or in the very old" (US EPA, 1999d). So, even though the binding times of carbamates to the AChE are generally short compared to organophosphorus compounds, there may be a such a wide range of durations of effects for members of these two classes that a variety of potential interactions across different, particularly acute, time frames may occur. This possibility needs to be considered.

In conclusion, while the biochemical reaction of the different chemical structures of these classes is different, i.e., carbamylation versus phosphorylation, and the general duration of inhibition of the enzyme is much longer for organophosphorous compounds, the physiological consequence, inhibition of acetylcholinesterase activity, is essentially the same. Because the duration of inhibition and effects may overlap between these two classes, there is no clear means of separating these two groups with respect to potential interactions. In the context of a common mechanism of toxicity and the criteria defined in EPA's *Guidance For Identifying Pesticide Chemicals and Other Substances That Have A Common Mechanism of Toxicity* (USEPA, 1999a), OPP knows of no means to meaningfully differentiate between the consequences of AChEI by these carbamate and organophosphorus compounds.

7. SCIENCE POLICY ON GROUPING OF CARBAMATE PESTICIDES AND GROUPING OF AChEI-CARBAMATE AND ORGANOPHOSPHOROUS PESTICIDES

The approach used by the Office of Pesticides Programs to reach decisions regarding the grouping of the carbamate pesticides and the grouping of ChEI-carbamate and organophosphorus pesticides based on a common mechanism of action lies in the answers the following questions:

1. Does acetylcholinesterase inhibition provide sufficient evidence of a common mechanism of toxicity for grouping of carbamate pesticides?
2. Can carbamate pesticides be subgrouped based on the characteristic of some to produce effects unrelated to cholinesterase inhibition?
3. Should the carbamate pesticides that inhibit AChE be grouped with the organophosphorus pesticides that inhibit AChE ?

1. Does acetylcholinesterase inhibition provide sufficient evidence of a common mechanism of toxicity for grouping of carbamate pesticides?

OPP has concluded that acetylcholinesterase inhibition is sufficient evidence of a common mechanism of toxicity for the grouping of AChEI carbamate pesticides. The common mechanism of such compounds is the carbamylation of the hydroxy serine of acetylcholinesterase followed, sequentially, by an accumulation of acetylcholine at a nerve synapse or neuromuscular junction and, with continued accumulation of acetylcholine, overstimulation of cholinergic pathways in the central and peripheral nervous systems. The accumulation of acetylcholine can lead to the expression of cholinergic signs and symptoms such as nausea, gastrointestinal distress, vomiting, tremors, paralysis and depression of respiratory function. The common toxic effects of the ChEI carbamate pesticides are acetylcholinesterase inhibition (blood, central nervous system, and/or peripheral nervous system)[1] and/or cholinergic effects.

[1]See "*Science Policy on the Use of Cholinesterase Inhibition for Risk Assessments of Organophophate and Carbamate Pesticides"* (USEPA, 1998) for details on selecting cholinesterase inhibition endpoints as indicators of toxicity.

2. Can carbamate pesticides be subgrouped, based on the characteristic of some to produce effects unrelated to cholinesterase inhibition?

The distinctive toxicity profiles of individual members of subgroups of the carbamate pesticides (e.g., carbamates, thiocarbamates, and dithiocarbamates) should be considered during identification of chemicals that belong to a common mechanisms of toxicity group. A carbamate pesticide should not be grouped with carbamates whose common mechanism of toxicity is AChEI unless reliable data are available that show its potential to cause cholinesterase inhibition. Carbamates whose critical toxic effect(s) are other than cholinesterase inhibition may be grouped with other carbamates only if they share some other common mechanism of toxicity.

Although a number of carbamate pesticides have been shown to produce toxicities or effects that are not attributable to AChEI, careful evaluations are required to determine if the chemical also expresses toxicity via cholinesterase inhibition. In particular, if the chemical is a weak cholinesterase inhibitor **(**e.g., marginal depression of cholinesterase activity in one compartment and at relatively high dose-levels when compared to other effects produced by the chemical) and ChEI does not appear to be associated with the expression of clinical effects or neurotoxicity, a weight of evidence approach should be observed before concluding the chemical should be grouped with cholinesterase-inhibiting pesticides.

3. Should the carbamate pesticides that inhibit AChE be grouped with the organophosphorous pesticides that inhibit AChE?

OPP has concluded that cholinesterase inhibiting carbamate and organophosphorus pesticides share a common mechanism of toxicity and should be grouped during the initial phase of determining the appropriate scope of the cumulative risk assessment for the group. The common mechanism of toxicity for both groups of pesticides is interaction with a specific molecular site, a serine hydroxyl of acetylcholinesterase, followed by a cascade of effects including the accumulation of acetylcholine at a neuromuscular junction or nerve synapse leading to overstimulation of cholinergic pathways in the central and peripheral nervous systems. Cholinesterase inhibiting carbamate and organophosphorus compounds can each be considered

to act as pseudo substrates for acetylcholine, act on target pests and vertebrates by the same biochemical mechanism, and produce the same clinical and neurobehavioral effects in animals and humans.

8. CONSIDERATIONS FOR CONDUCTING A CUMULATIVE RISK ASSESSMENT

Identification of a common mechanism of toxicity for a group of carbamates or a series of carbamate and organophosphorus compounds is an initial step in identifying candidate chemicals for a cumulative risk assessment. General policy guidance for cumulative risk assessment is now being developed by the Agency. The goal of the cumulative risk assessment is to characterize the magnitude of the potential effects following known or anticipated exposures to multiple substances that are toxic by a common mechanism. Pesticides within a common mechanism grouping must undergo further evaluation to determine which ones to include in the group selected for the cumulative risk assessment. In other words, the number of chemicals in the group that "survives" the common mechanism decision process may not be the same as the number remaining after these additional factors are considered. Some of these additional factors are:

1. The relationshhips of endpoints comprising the common toxic effect (AChEI);
2. The relationship between AChEI and other toxicities elicited by each member of the group;
3. Exposure considerations
4. Temporal factors
5. Pharmacodynamics
6. Environmental degradation or metabolism in plants
7. Subpopulations for which exposures are anticipated; and
8. Susceptibility and sensitivity of exposed individuals or subpopulations to the common toxic effect.

A detailed discussion of how these factors may affect cumulative toxicity of carbamate and organophosphorus pesticides is beyond the scope of this document. Nonetheless, a brief

discussion of several key factors is provided below to illustrate how these factors may affect groupings.

1. **The relation of the endpoints comprising the common toxic effect (AChEI)**

Cholinesterase data should be analyzed using a weight-of-evidence approach, as delineated in the Office of Pesticide Programs' policy document entitled "Use of Cholinesterase Inhibition Data in Risk Assessments of Organophosphate and Carbamate Pesticides" (US EPA, 1998). The weight-of-evidence approach includes consideration of the cholinesterase-containing compartments affected (blood, brain, peripheral tissues), the presence or absence of clinical signs, and results of neuorbehavioral tests. A comparison of the pattern of doses required to produce these effects for each chemical in the grouping should be made. The analysis should also include identification of the most relevant endpoint/parameter for the group. This would entail the development of several parallel analyses, one for each of the cholinesterase-containing compartments (e.g., plasma, RBC, brain, peripheral tissues) one for clinical signs, and one for neurobehavioral effects, etc., perhaps for more than one species, if the data allowed. Informed by these parallel analyses, a decision would be made as to which of these endpoints/parameters would be the most appropriate for use in the cumulative hazard assessment. The point of departure (i.e., NOAELs or Benchmark Doses) for that endpoint/parameter for each chemical would then be integrated into a hazard value for the group.

2. The relation between AChEI and other toxicities elicited by each member of the group.

The doses at which critical non-AChEI toxic effects of each chemical occur should be compared to the doses associated with AChEI to determine the extent to which these effects may overlap on the dose response curve. Where no overlapping effects are anticipated, i.e., the critical effect not related to AChEI occurs at substantially lower doses than does AChEI, the exclusion of that chemical from the ChEI grouping may be appropriate.

3. Exposure considerations.

The potential for the toxicities of individual chemicals to accumulate depends on the opportunity for overlapping exposures and durations of effects as well as the patterns of toxicity

of the chemicals. Exposure data that should be analyzed before reaching a decision to include or exclude a specific chemical from a cumulative risk assessment includes geographic patterns of usage, temporal use patterns (e.g., Spring, Summer, continuous, etc.), inclusion of two or more carbamate and/or organophosphorus pesticides in a formulation, and presence of parent and active (AChEI) metabolites as residues.

4. Temporal factors.

Cholinesterase inhibition following exposure to a carbamate pesticide generally has a rapid onset and recovery (e.g., the half-life for recovery of N-methyl carbamylated AChE is approximately 30 minutes, (Fukuto, 1990). Total recovery generally occurs over a 8-24 hour period (US EPA, 1999d). Toxic effects also subside and disappear during the recovery period. In contrast, AChEI and toxic effects following exposure to an organophosphorous pesticide may persist for several hours to days or longer. Even if there is recovery from AChEI and its consequences, sensitivity of the organism to a subsequent exposure to the same or a different AChEI-carbamate or organophosphorous compound may be altered. Data regarding onset, recovery and persistence of effects following acute, intermediate, subchronic, and chronic exposures should be included in the common mechanism assessment as background information for a cumulative risk assessment. This will allow comparisons between the onset and persistence of toxic effects and the patterns of exposure. A determination can then be made whether or not overlapping exposures to multiple chemicals are anticipated to occur in a pattern that could lead to cumulative toxicity.

5. Pharmacodynamics.

Combining of cholinesterase inhibiting carbamate and organophosphate compounds in a cumulative risk assessment should be based on the use of cholinesterase data from the same compartment(e.g., blood, brain or nervous system) and preferably from the same species. The fact that the common effect selected for an initial grouping of candidate chemicals, for example RBC AChEI, does not represent the most sensitive response observed for an individual chemical should not serve as a basis for excluding the chemical from consideration for grouping with other RBC AChEI chemicals.

6. Environmental degradation or metabolism in plants.

A potential route of human exposure for carbamate pesticides is via ingestion of residues in treated crop plants. Both oxidative and hydrolytic detoxification mechanisms are known to be important in plants. The metabolite derivatives in plants tend to be glucoside, phosphate and amino acid conjugates. Further metabolism in humans would liberate unconjugated metabolites which may also be acetylcholinesterase inhibitors.

9. RESEARCH CONSIDERATIONS

The Office of Pesticide Programs has identified several areas where additional data would expand our understanding of toxicological characteristics of the carbamate pesticides that should be considered when preparing cumulative risk assessments. Examples of research areas that would allow the preparation of more comprehensive and refined cumulative risk assessments of carbamate and organophosphorus pesticides are:

1. **Combination studies** - there is an absence of data regarding the cumulative effects, or lack thereof, when two or more carbamate pesticides are administered concurrently to animals. Similarly, data are lacking on interactions that may be expected from concurrent administration of carbamates and organophosphorus pesticides. The default assumption in the absence of such data is to assume additivity of exposure (dose addition) from exposure to two or more carbamate and/or organophosphorus compounds. Studies with combinations of carbamate and organophosphorus compounds would provide improved understanding for developing the appropriate approach to accumulating toxicities in a cumulative risk assessment.

2. **Temporal studies** - there is a paucity of data regarding whether or not toxicities will accumulate when single, acute exposures to multiple, cholinesterase-inhibiting pesticides are separated by hours, days, weeks, or months. There are also virtually no data available that show the extent to which toxicities accumulate in an individual exposed, acutely, to an AChEI pesticide when the same individual is chronically exposed to a second AChEI. Data are also absent regarding toxicity that may result from repeated acute exposures that are separated by

several days. A cumulative risk assessment likely will include exposures which occur sporadically for some chemicals and continuously for other chemicals. Combination studies involving administration of individual chemicals for varying durations would provide insights regarding potential interactions of groups of chemicals when exposures to individual chemicals in the group do not overlap.

3. **Pharmacokinetic/pharmacodynamic studies** - it is the policy of the Office of Pesticide Programs to assume that inhibition of blood AChE serves as a surrogate for potential inhibition in peripheral nervous tissues, in the absence of specific data on AChEI in peripheral tissues. Well designed PK/PD studies would provide data to determine if a AChEI-pesticide affects only the CNS or PNS, or both systems. PK/PD data could also provide information on the time required to reach a steady state of AChEI and time to recovery of cholinesterase activities following acute, subchronic, and/or chronic exposures. Such information would aid in defining maximum cumulative effects that may occur from concurrent exposure to two or more chemicals (e.g., if the time to reach a steady state of AChEI is the same or different for two or more AChEI-pesticides) and insights regarding the potential for interactions to occur if exposures are not overlapping (e.g., complete recovery from the effects of the first AChEI-pesticide occurs before exposure to a second AChEI-pesticide occurs).

4. **Low dose interactions** - there are uncertainties regarding the accumulation of effects with low dose exposures to multiple chemicals. Studies involving administration of pesticides to animals using dose-levels that are representative of residue levels that are anticipated to be encountered in food, water, and in residential settings would provide data needed to define cumulative effects anticipated to occur from exposures to environmental levels of multiple pesticides. Such studies should include doses that represent NOAELs and LOAELs of the individual chemicals so that data can be obtained on interactions that may occur when no-effect doses and low-effect doses from multiple chemicals are administered together. In the absence of data on interactions that may occur at low dose-levels, additivity of doses from exposure to multiple chemicals will be assumed.

It should be recognized that qualitative and quantitative procedures used for a

cumulative risk assessment are in the developmental stage. As the Agency gains more experience in this type of risk assessment, research needs will be more fully delineated.

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