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Biomimetic Chemistry as a Useful Tool for Studying Reactive Metabolites of Pesticides†

Yoffi Segall*

Department of Organic Chemistry, Israel Institute for Biological Research, P.O. Box 19, Ness Ziona 74100, Israel

ABSTRACT: Most organophosphate (OP) pesticides require metabolic activation before attacking the target site, as opposed to chemical nerve agents, such as VX and sarin, which inhibit the enzyme directly. The majority of OP pesticides exhibit weak anticholinesterase activity in vitro compared to their In Vivo activity. Biooxidation is probably the principal route by which these pesticides are activated or detoxified. The oxidized product, usually a short-lived intermediate, may either hit the target directly or hydrolyze rapidly or, following a rearrangement reaction, convert to another species with enhanced reactivity (metaphosphate) or lose its phosphorylation or carbamoylation properties. Biomimetic studies of these processes, using various model systems, have important advantages: in some cases they allow for identifying short-lived intermediates, formed metabolically, and direct monitoring of the systems' properties by NMR. Once identified, they may be synthesized in large amount to investigate their adverse effects, if any. Biomimetic studies allow for monitoring reactions at low temperature seeking transient intermediates and evaluation of activation and detoxification mechanisms as well as mode of action based on chiral isomers. This, in turn, allows for determination of whether certain compounds act directly, on preactivation, or both, and the possible design of safer pesticides. This paper covers over three decades of extensive fundamental and applied research that has been carried out at the Environmental Chemistry and Toxicology Laboratory (ECTL) at the University of California at Berkeley under the supervision of Prof. John E. Casida.

KEYWORDS: biomimetic model systems, oxidative bioactivation, N-oxidation, S-oxidation, transient intermediates, *S*-oxide and *N*-oxide rearrangements, stable thiophosphorus *S*-oxides, toxic industrial phosphorus intermediates

INTRODUCTION

Organisms may be adversely affected by a large number of synthetic chemicals. Long accumulation of xenobiotics will inevitably lead to damage. Biotransformation of chemicals, initiated by drug-metabolizing enzymes (DME), is usually characterized by the generation of a new functional group, such as hydroxyl, that allows for the metabolite to further conjugate to endogenous substrates, such as glutathione, glucuronic acid, and cysteine. Therefore, the overall process is conversion of a lipophilic molecule to a highly hydrophilic molecule that may be readily excreted.[1]

Some of the biologically active organophosphorus (OP) compounds are among the most powerful synthetic poisons. Those of greatest toxicological concern are the chemical warfare agents and the widely used OP pesticides, acting as AChE inhibitors.[2,3]

OP pesticides exhibit weak anticholinesterase activity in vitro compared to their In Vivo activity. They require metabolic activation before attacking the target site.[4,5] In contrast, chemical warfare agents, such as sarin (*O*-isopropyl methylphosphonofluoridate) and VX [*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate], inhibit the enzyme directly.

Biooxidation, involving various DME depending on the S and N moieties present in the molecule, is probably the principal route by which pesticides are activated or detoxified. Considerable efforts have been directed toward using synthetic iron porphyrins,[6−9] cyclodextrin-based peroxidase systems,[10] and artificial enzymes,[11] to biomimic cytochrome P450. Typical approaches are summarized in Table 1. Fabrication of such synthetic molecules is complicated due to the lack of sufficient data related to the enzyme oxidation mode of action. Rather than designing enzymes with emphasis on accelerating reaction rates, the staff at the Environmental Chemistry and Toxicology Laboratory at the University of California at Berkeley (ECTL) has used low molecular weight organic oxidants to mimic the oxidation step of the metabolizing enzyme. The research goals were focused primarily on mimicking reaction pathways and identifying and examining transient intermediates for their relevance to the overall toxicity and mode of action.

The oxidized molecule, obtained on *m*-chloroperoxybenzoic acid (MCPBA) oxidation, is usually a short-lived transient intermediate. Its fate depends primarily on its reactivity and the environment. It may hit the target directly, undergo conjugation, hydrolyze rapidly, or, following rearrangement, convert to another species with enhanced reactivity or lose its phosphorylation or carbamoylation properties.[12] Optically active intermediates may hit different targets or the same one with different reaction rates. This may have a major effect on their selectivity. Most of the oxidizing systems studied shed light on the mode of action of pesticides, but some systems were found not to be suitable models for biomimetic activation. Thus, without biomimetic experiments, highly reactive intermediates may be overlooked. In general, modeling DME-mediated oxidations may lead to prediction of a pesticide's potential to cause adverse reactions.

The current paper is a critical review that analyzes the usefulness of various approaches in biomimetic chemistry as

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Table 1. Typical Biomimetic Approaches for Studying Oxidative Bioactivations

biomimetic system	pesticides or chemicals applied to	results	refs
m-chloroperoxybenzoic acid (MCPBA)	*S*-alkyl phosphorothioates (profenophos, prothiophos, chlorpyriphos, sulprophos, methamidophos, phospholan, VX, and related chemicals and starting materials), dialkyl hydrogenphosphonates, phosphorothioic acids	formation of highly reactive *S*-oxide intermediates (activation), followed by rearrangement to phosphoroxysulfonates (detoxification), thioperoxoic acids	5, 12, 13, 15, 19, 23, 24, 33−35, 40, 41, 44, 47, 49
	phosphoramidates (methamidophos, phospholan, mephospholan, HMPA, isofenphos, phosphordiamidothioates, glyphosate)	highly reactive products, formation of *N*-oxides followed by dealkylation and rearrangement to potent mutagens	13, 30−38, 42
	thiocarbamates, *S*-(2-chloropropenyl) thio- and dithiocarbamates (diallate, triallate, sulfallate)	potent thiocarbamate sulfoxides, rearrangement-elimination reactions to highly mutagenic haloacroleins	13, 20−22
	potential prodrugs (*S*-alkyl dioxaphosphorinane)	formation of highly potent γ-aminobutyric acid antagonists	17, 47
synthetic iron porphyrins	primary and N-substituted amine functions, C−H bond oxidation, modeling oxidative metabolism (carbofuran, carbaryl, pirimicarb), acetaminophen and ellipticine derivatives, polycyclic aromatic hydrocarbons	N-oxidative transformations, aminium radicals, specific C−H bond oxidations, hydroxylated carbamate metabolites, pyrene quinones and phenols	6−9
cyclodextrin-based peroxidase	glutathione peroxidase mimic by reducing alkyl peroxides and thiols, oxidation of amines to nitro derivatives and alcohols to ketones	reduction of alkyl peroxides to alcohols, thiols to disulfides (GSSG, DTNB)	10
chemzymes (artificial enzymes)	oxone epoxidation of alkenes, cleavage of glycosidic bonds, H_2O_2 oxidation of benzyl alcohols, cerium oxidation of luminal	enantiomeric selectivity for alkene epoxidations, high catalytic rate values for oxidation of benzyl alcohols	11

basic tools for studying pesticides mode of action and exploiting the chemical and toxicological properties of the active intermediates formed on oxidative activation.

RESULTS AND DISCUSSION

Observations at ECTL indicate that most pesticides, in particular those comprising S and N heteroatoms, require metabolic preactivation because very few of them are active in vitro.

Two types of rearrangement reactions were observed on oxidation of various S- or N-containing OP pesticides (Figure 1).

It was noted that highly reactive intermediates, obtained from metabolic preactivation, are often identical to those generated from MCPBA oxidation of many pesticides.[13]

Phosphorus *S*-Oxide Intermediates: Synthesis and Chemical Properties. Initial oxidation of *S*-alkyl phosphorothioate pesticides leads to a short-lived highly reactive *S*-oxide intermediate[13] that may hit the target directly (activation), hydrolyze, undergo conjugation and excretion (deactivation), or otherwise rearrange so as to insert the oxygen between the phosphorus and the sulfur atoms (Figure 1). The rearranged phosphinyloxysulfenate anhydride, formed via a novel rearrangement reaction, is further oxidized to the appropriate oxysulfonate. The latter is a sulfonylating rather than a phosphorylating agent (detoxification). Thus, its hydrolysis in $H_2{}^{18}O$ gives the appropriate phosphoric and sulfonic acids in which the ^{18}O is incorporated only in the sulfonic acid[12] (Figure 2). Because the formation of ^{18}O-enriched sulfonic acid is strongly favored, it is assumed that the mechanism of solvolysis does not involve separation into ion

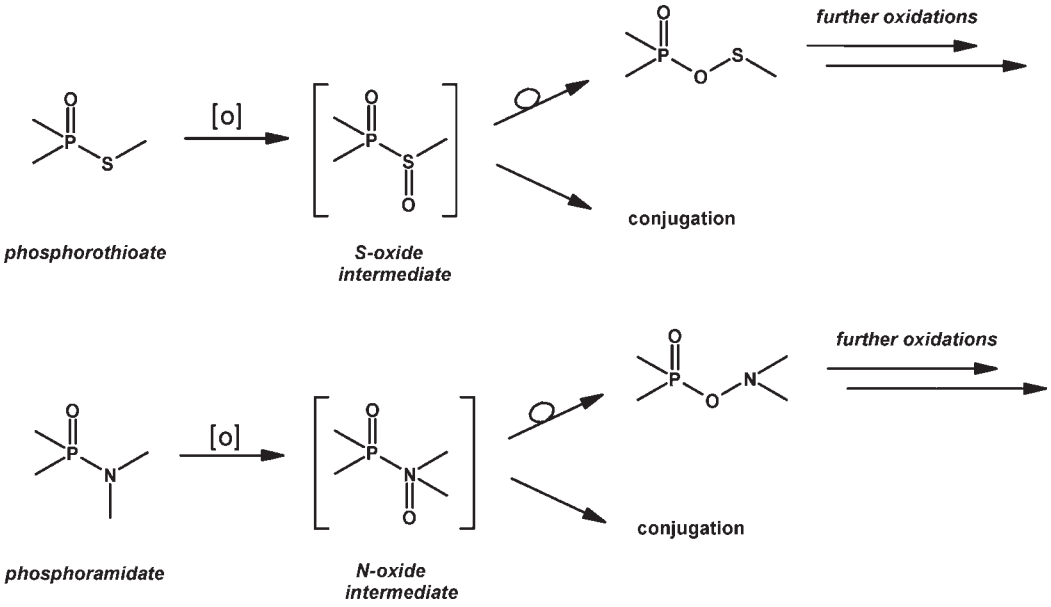

Figure 1. Proposed intermediates and rearrangement reactions obtained on MCPBA oxidation of phosphorothioate and phosphoramidate pesticides as possible biomimetic models.

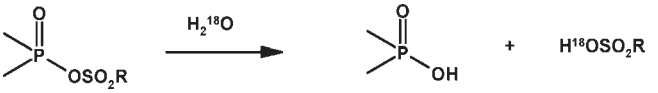

Figure 2. Hydrolysis of phosphorus oxysulfonate esters in ^{18}O-labeled water indicating its sulfonylating properties.

1 2

Figure 3. MCPBA oxidation of profenophos insecticide **1** in 0.75% EtOH-stabilized $CHCl_3$.

pairs but rather a nucleophilic attack at sulfur; otherwise, the ^{18}O would equally label both acids. Similar evidence for S−O rather than P−O bond cleavage was found from hydrolysis of ^{18}O-enriched thiophosphorus−sulfonic mixed anhydrides.[14]

Excluding phosphoramidothioate derived *S*-oxides (see below), most *S*-alkyl phosphorosulfoxide intermediates were not observed spectroscopically due to their high reactivity and short-lived nature. In the absence of nucleophiles, they rearrange rapidly and spontaneously to the oxysulfenate ester, followed by further oxidation to the oxysulfonate (Figures 1 and 4), lacking phosphorylation properties.[12,15,16] The latter product is strongly favored because on oxidation with an equivalent amount of MCPBA little of the starting material is consumed. Therefore, the rate-limiting step appears to be the initial oxidation leading to the *S*-oxide intermediate, rather than its rearrangement to phosphoroxysulfenate.

The phosphorylation potency of the proposed phosphorus *S*-oxides was observed serendipitously. When profenophos insecticide **1** was oxidized with excess MCPBA in $CHCl_3$, the only product isolated was its diethyl ester analogue **2** (Figure 3).

The unexpected formation of diethyl ester **2**, obtained as a major product, is due to the phosphorylation by profenophos *S*-oxide intermediate of the 0.75% ethanol normally added to $CHCl_3$ as a stabilizer.[12,17] That small amount of ethanol was sufficient to quantitatively convert the *S*-oxide to **2**. It appears that the alkyl−*S*-oxide group (Figure 1) is one of the best known leaving groups attached to a tetracoordinated phosphorus.[16]

The high reactivity of the intermediate was further established on its formation in various alcohols as solvents and nucleophiles.[18] Studies indicate that its phosphorylation rate versus rearrangement to oxysulfenate is almost independent of the other substituents attached to phosphorus. The reaction is rather sterically controlled by the nature of the nucleophile. Thus, alcohol phosphorylation is the exclusive route for the *S*-oxide intermediate in primary alcohols, but not in benzyl alcohol, as opposed to rearrangement followed by quantitative sulfonylation of the alcohol in tertiary alcohols, whereas both routes are nearly equally favored in secondary alcohols (Figure 4).

Transformation of the *S*-oxide intermediate to the oxysulfenate anhydride (Figure 1) may proceed either by (a) internal sulfoxide attack at phosphorus, involving a phosphoraneoxide anion as the transition state, (b) dissociation of the intermediate into ion pair followed by recombination, or (c) radical dissociation and recombination. MCPBA oxidation of optically active *S*-ethyl O-isopropyl methylphosphonothioate led to the appropriate phosphonyloxysulfonate with complete retention of the configuration at phosphorus. Assuming the initial sulfoxidation does not alter the phosphorus configuration, this supports a mechanism involving a phosphoraneoxide anion transition state.[19]

Thiocarbamate *S*-Oxide Intermediates. In a pioneering study, Casida et al. found that MCPBA oxidation of thiocarbamate herbicides yields thiocarbamate sulfoxides, a new class of compounds with increased herbicidal activity but decreased stability compared to the parent thiocarbamates.[20] Mice treated with thiocarbamates gave urinary sulfoxide metabolites similar to those obtained on MCPBA oxidation, suggesting they are transient intermediates in the mammalian metabolism of

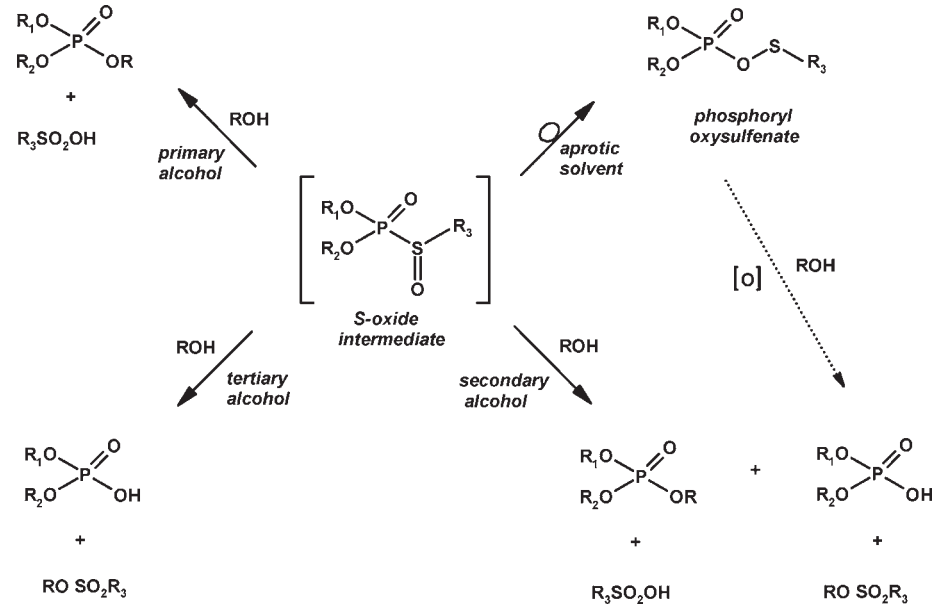

Figure 4. Reactions of the pesticide-derived *S*-oxide intermediates with primary, secondary, and tertiary alcohols.

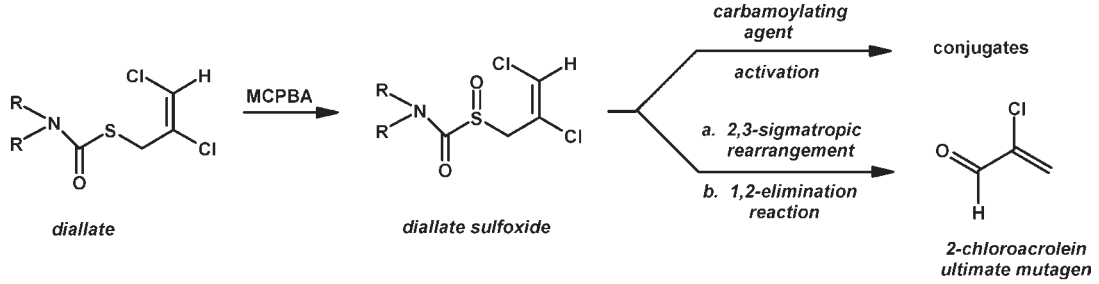

Figure 5. MCPBA biomimetic oxidation of diallate (R = C_2H_5) herbicide indicating herbicidal activation, followed by sequential rearrangement−elimination reactions, leading to highly mutagenic 2-chloroacrolein.

thiocarbamate herbicides. Compared with thiophosphorus *S*-oxide intermediates, thiocarbamate sulfoxides are much more stable, their structures are easily elucidated by spectroscopy, and sometimes they are isolable. Depending on the *S*-alkyl moiety, their half-life at room temperature may vary from a few minutes to a few hours (see below).

Phosphoramide *N*-Oxides. In analogy to thiophosphorus *S*-oxide intermediates, phosphoramide *N*-oxides, obtained from MCPBA oxidation of the corresponding phosphoramidates, are also unstable and were not observed spectroscopically. Once formed, these compounds usually undergo diverse metabolic rearrangements generating a variety of metabolites. This leads to uncertainty in pesticide development and predictions on its potential to cause adverse reactions.

Following are selected examples of activation of individual pesticides with emphasis on their suitability as biomimetic models, their stability, mode of action, and relevance to toxicity.

INDIVIDUAL PESTICIDES

Oxidative Biomimetic Activation of Carbamothioate and Phosphorothioate Pesticides: Suitable Biomimetic Models. Carbamothioate and phosphorothioate pesticides require metabolic preactivation, in contrast to chemical warfare agents that hit the target enzyme with extremely high reaction rates. Schuphan et al. found that diallate herbicide undergoes similar oxidation both with MCPBA and on incubation with microsomal monooxygenases, yielding the bacterial potent mutagen 2-chloroacrolein[21,22] (Figure 5).

The mixed-function oxidase (MFO) conversion of diallate to 2-chloroacrolein was established by isolating its 2,4-dinitrophenylhydrazone derivative directly from mutagenicity assays. This supports diallate sulfoxide (Figure 5) as the reactive metabolic intermediate and the system as a suitable biomimetic model.

Incubation of chlorpyriphos and sulprophos with MCPBA produced active metabolites that were exploited to distinguish between AChE enzymes conferring OP susceptibility and resistance in diverse insect strains.[23] This system is in general a suitable biomimetic model. The major drawback was the inconsistency of the activation process. However, no attempt was made to identify intermediates, or their concentration and stability, in the biological assays.

The MFO system converts profenophos insecticide to a potent inhibitor of AChE.[24] The activation is stereospecific for the two chiral isomers (Figure 6).[25] The (−)-isomer is activated to a 34-fold better inhibitor of AChE in vitro, whereas the (+)-isomer is deactivated by a factor of 2. MCPBA is a suitable biomimetic model for profenophos because evidence points to profenophos *S*-oxide as the reactive intermediate formed both enzymatically and with MCPBA[15] and acting as

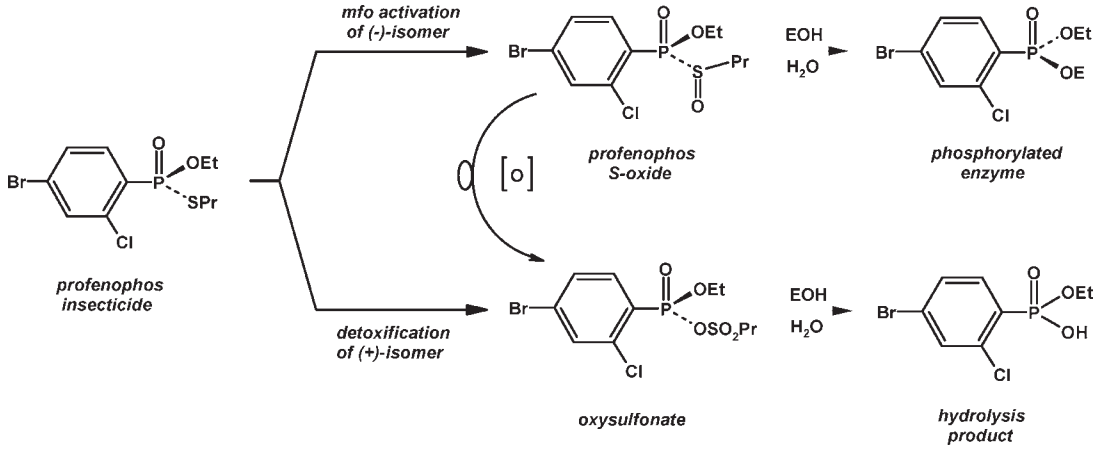

Figure 6. Mouse liver MFO bioactivation of profenophos insecticide (−)-isomer and detoxification of its (+)-isomer.

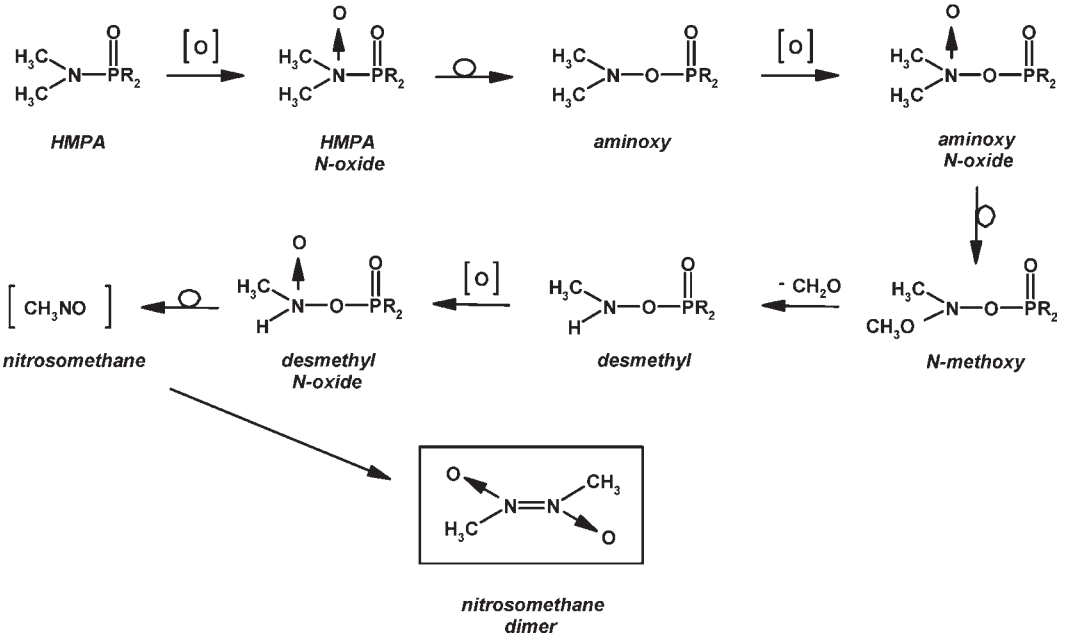

Figure 7. Peracid-mediated N-oxidation and rearrangement of hexamethylphosphoramide [R = $N(CH_3)_2$].

the phosphorylating agent. The difference between the toxicity of the two chiral isomers of profenophos may explain the discordance between the red cell AChE activity and the clinical picture in patients exposed to profenophos.[26] In these patients there was no reactivation of red cell AChE when loaded with a high dose of pralidoxime antidote. This may be attributed to the very different postinhibitory nature of the (+)- and (−)-profenophos-inhibited AChE. Whereas AChE inhibited by the (+)-enantiomer undergoes spontaneous reactivation, that inhibited by the (−)-enantiomer ages rapidly and is resistant to reactivation.[27] Because the rearrangement of profenophos *S*-oxide to the appropriate oxysulfenate is an intramolecular first-order reaction, both chiral isomers are detoxified rapidly if no enzymatic reaction takes place.

Oxidative Biomimetic Studies with Phosphoramidates: HMPA and Methamidophos. N-Substituted amines may undergo P450 catalyzed N-oxidative transformations leading to highly reactive metabolites. Frequently this is a starting point for a variety of metabolites because the *N*-oxide may undergo further rearrangement reactions, such as N-dealkylation (rarely found with alkyl sulfoxides) or oxidation of the adjacent phosphorus, in phosphoramidate pesticides. On the one hand, these transformations may produce mutagenic metabolites; on the other hand, they may equally yield water-soluble products that are readily excreted, therefore promoting detoxification. Schradan (octamethylpyrophosphoramide) insecticide is metabolically activated to an unidentified potent AChE inhibitor and also undergoes N-demethylation presumably via *N*-hydroxylamine intermediate.[28]

Hexamethylphosphoramide (HMPA), an extensively used solvent, has been long known as an experimental animal carcinogen and anticipated human carcinogen. With *Salmonella typhimurium* mutation assays it showed mutagenic activity only on activation with a high level of rat liver S9 protein.[29] Metabolism of HMPA involves N-demethylation with liberation of formaldehyde.[28] HMPA and related dimethylaminophosphoramidates react with MCPBA, leading to a new dimethylaminoxyphosphorus derivative probably via N-oxidation and rearrangement

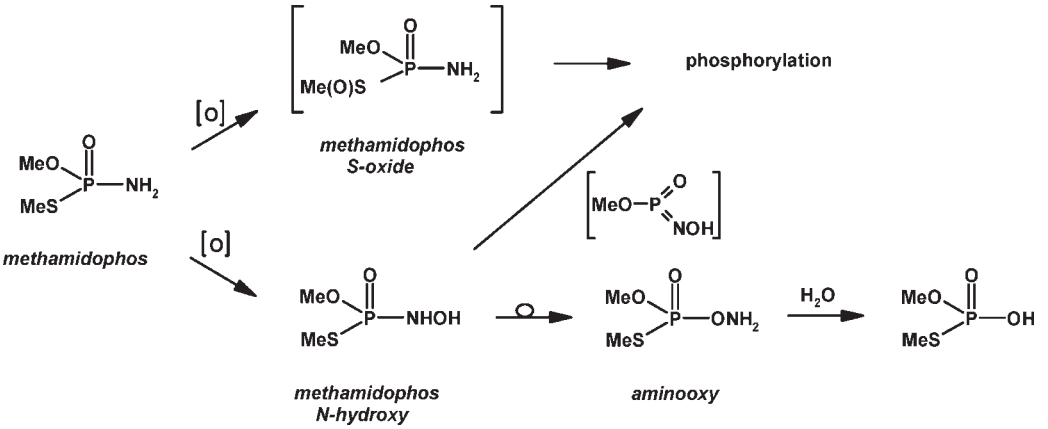

Figure 8. Methamidophos: alternative routes for oxidative biomimetic activation.

(Figure 7), in analogy to phosphorus *S*-oxide intermediates (see above). Studies with ^{1}H and ^{31}P NMR indicate the collapse of the appropriate signal from a doublet in ^{1}H to a singlet (δ ^{1}H 2.81 ppm) and from a multiplet in ^{31}P to a 7-line signal (δ ^{31}P +3.48 ppm) due to the lack of coupling between proton and phosphorus.[30] This suggests the formation of an aminoxy derivative, identified on its independent synthesis, rather than the *N*-oxide intermediate. Despite its importance as a metabolite, it was not isolated or structurally elucidated due to its short-lived nature.

The reaction sequence, depicted in Figure 7, comprises several oxidation rearrangement pathways. The aminoxy derivative is further oxidized to the aminoxy-*N*-oxide and converts to HMPA-desmethyl by loss of formaldehyde (identified by ^{1}H NMR at 8.15 ppm and by derivatization). Further oxidation, followed by rearrangement, leads to the ultimate nitrosomethane mutagen, isolated as a *trans*-nitrosomethane dimer. The identity of the dimer was confirmed by ^{13}C NMR (δ 47.2) and by chemical ionization mass spectrometry ($M + 1^{+}$, 91).

As indicated,[28,29] formaldehyde is a metabolite of HMPA and a carcinogen, but its potency is insufficient to account for the carcinogenicity of HMPA. Later studies identified [*E*]-azoxybis-(methylene) bis(3-chlorobenzoate) as a potent direct acting mutagen from a reaction mixture of HMPA and MCPBA.[31] However, because the derivatizing oxidant is a part of the bioactive product, MCPBA oxidation of HMPA provides only partial proper biomimetic model for its metabolic activation as a mutagen and carcinogen.

In analogy to the proposed oxidation pathway for Schradan pesticide, MCPBA activation of *N*-alkyl-substituted phosphoramidothioate insecticides was also suggested to proceed by oxidation at a site close to the nitrogen atom, forming a potent inhibitor of AChE.[32]

Biomimetic Oxidation of a Pesticide Comprising both P−S and P−N Bonds. Methamidophos and acephate provide examples of pesticides comprising both sulfur and nitrogen moieties directly bonded to phosphorus. Early studies proposed that MCPBA oxidation takes place primarily at sulfur to yield the *S*-oxide, but not at nitrogen to yield *N*-oxide, because ^{15}N NMR revealed no signals in the region expected for *N*-oxides (at least 70 ppm downfield of the ^{15}N signal of acephate).[15,33] Claim for the synthesis of methamidophos *S*-oxide was based on its ^{13}C-enriched NMR spectroscopy,[34] but later was refuted.[35]

Methamidophos N-hydroxylation was then examined as an alternative to S-oxidation, proposed earlier for its bioactivation.[36] Both alternatives are depicted in Figure 8.

N-Hydroxymethamidophos, synthesized separately to study its relevance to toxicity, was found to be less potent than methamidophos and inactive in vitro as an enzyme inhibitor. Its further rearrangement to the aminoxy involves cleavage of the P−N bond followed by hydrolysis (deactivation pathway). However, *N*-hydroxymethamidophos may undergo fragmentation via an iminometaphosphate intermediate, which is proposed to be the active species causing phosphorylation. It is assumed that both routes are involved in the bioactivation pathway of methamidophos (Figure 8).

Biomimetic Studies with Pesticides Comprising S and N in a Remote Position from Phosphorus: Sulprophos, Phospholan, Mephospholan, and Glyphosate. Monitored by ^{31}P NMR, the biomimetic MCPBA system made it possible to evaluate the S-oxidation preference rate in a multifunctional S-containing pesticide, such as sulprophos (Figure 9).

The first and second oxidations take place at *S*-methyl thioether, forming the sulfoxide and sulfone. Further oxidations give in sequence phosphorothioate (sequence 3, Figure 9), *S*-oxide intermediate (not observed), rearrangement, and finally the ultimate oxysulfonate derivative.[15]

Phosfolan and mephosfolan insecticides are similarly activated by microsomal MFO and by MCPBA to potent AChE inhibitors.[37] Phosfolan-*S*,*S*-dioxide and mephosfolan-*S*,*S*-dioxide, both obtained from MCPBA oxidation of the parent insecticides, are activated by almost 10000- and 50000-fold (Figure 10). This is an attractive example representing good suitability of a peracid system as a model for oxidative bioactivation.

Glyphosate herbicide comprises a nitrogen atom, at β-position from phosphorus, which is susceptible to peracid oxidation. MCPBA oxidation gives initially the appropriate hydroxylamine intermediate.[38] Further dehydration followed by hydrolysis leads to aminomethylphosphonic acid, formylphosphonic acid that originates from C−N bond cleavage, and glycine. Formylphosphonic acid, on further hydrolysis, involving a rare cleavage of a P−C bond, converts to phosphoric acid and formaldehyde. These products, obtained from peracid oxidation, are generally also those formed metabolically.[38,39]

Stable Phosphorus *S*-Oxide Intermediates. As indicated, *S*-alkyl phosphorothioic *S*-oxides are highly reactive transient intermediates. In contrast, thioperoxoic acids (Figure 11), obtained on MCPBA oxidation of phosphorothioic acids, are sufficiently stable to observe directly, as monitored by ^{31}P NMR. Thus, diethyl phosphorothioic acid (Figure 11, R_1 = R_2 = OEt) (δ ^{31}P 62.07) is oxidized to a new product with

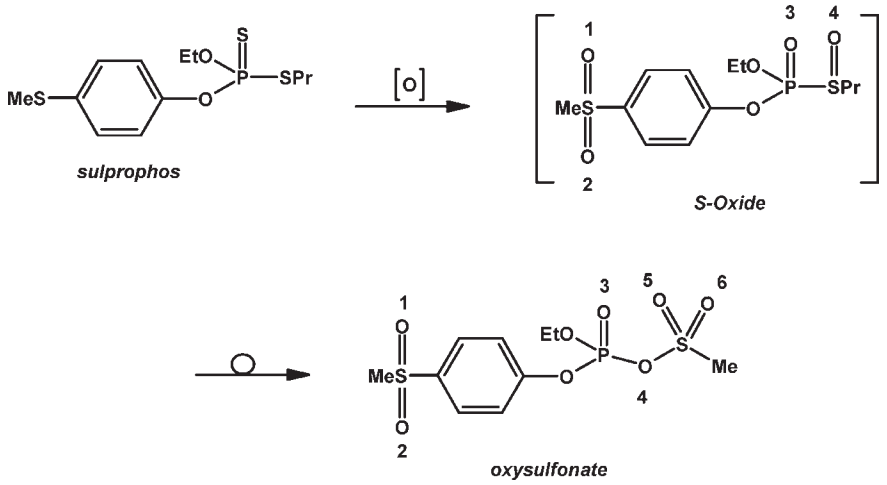

Figure 9. Sequential reaction rate preferences for oxidation of sulprophos insecticide.

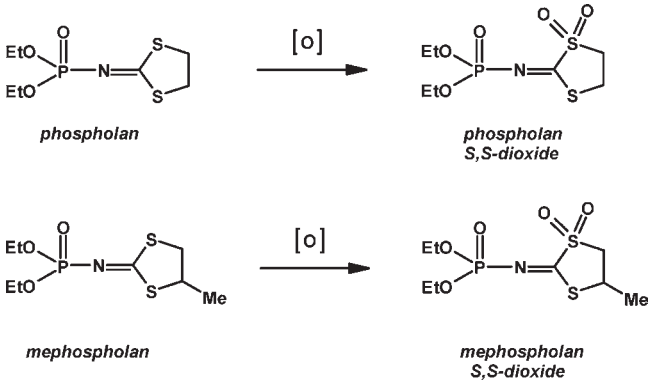

Figure 10. Phospholan and mephospholan pesticides and their *S*,*S*-dioxide analogues.

a ^{31}P NMR signal recorded at 25.53 ppm. This value is intermediate in character between those of the phosphorothioic acid and diethylphosphoric acid. Considering the quintet obtained from the $^{31}P-^{1}H$ coupled spectrum, its structure was tentatively assigned as the appropriate diethylthioperoxoic acid.[40] Further oxidation leads to the analogous *S*,*S*-dioxide, whereas a series of rearrangement reactions leads, via a hydroxyphosphorane intermediate, to oxoperthioic acid (Figure 11). Thioperoxoic acid and its analogous *S*,*S*-dioxide are phosphorylating and sulfonylating agents and, therefore, may inhibit AChE. It is noteworthy that ethanol is sulfurylated by the *S*,*S*-dioxide analogue to diethyl hydrogen phosphonate, a reduced form of phosphorus in a strongly oxidizing environment. This is the first time this type of intermediate could be directly observed. Oxidation of triester phosphorothionates with aqueous solution of magnesium monoperoxyphthalate similarly gave dialkyl hydrogen phosphonates.[41]

The mechanism of phosphorus *S*-oxide conversion to phosphinyloxysulfonate (Figure 1) was reexamined with N,N,N′,N′-tetrasubstituted phospordiamidothioates.[42] The *S*-oxides of this series are expected to be stable due to the electron donation of the nitrogen to the phosphorus center. Indeed, continuous ^{31}P NMR monitoring of the MCPBA oxidation reaction of the phosphordiamidothioates (~36 ppm) revealed two major resonances at ~31 and ~21 ppm that appeared and disappeared with half-lives of 50 and 10 min. They were tentatively assigned as the appropriate sulfoxide and sulfone. In that respect they resemble the *N*,*N*-dialkylcarbamothioate sulfoxide and sulfone, some of which were sufficiently stable to be isolated.[20]

MCPBA Biomimetic Studies with Systems That Were Not Suitable Models: VX and Sulfallate. VX inhibits directly AChE with extremely high reaction rates ($k_i = 1.4 \times 10^8\ M^{-1}\ min^{-1}$ for the P^s enantiomer).[43] Attempted oxidation of VX in pure *tert*-butyl alcohol initially led to a stable *N*-oxide, rather than *S*-oxide, intermediate, which subsequently decomposed to *O*-ethyl *S*-vinyl methylphosphonothioate and diisopropylhydroxylamine via a Cope elimination reaction (Figure 12).[44]

Due to the extensive breakdown of the VX molecule, following N-oxidation, it is concluded that this peracid system is not an appropriate model for its activation.

Treatment of sulfallate (2-chloroallyl *N*,*N*-diethyldithiocarbamate) herbicide, and related alkyl dialkyldithiocarbamates, with MCPBA leads preferentially to the corresponding sulfine (thion *S*-oxide), rather than the sulfoxide (Figure 13). The sulfine derivative of sulfallate is stable for a few hours at 25 °C in methanol but decomposes spontaneously in chloroform or acetone. With excess MCPBA, the major product identified in solution is the corresponding trialkyl iminium salt characterized by its unusual low field resonating iminium proton (10.2 ppm). Therefore, MCPBA is not a suitable biomimetic model for alkyl *N*,*N*-dialkyldithiocarbamates, including mutagenic sulfallate, because the reagent serves probably also as a derivatizing agent during the sequential oxidation reaction.[45]

Conversion of sulfallate promutagen to the ultimate mutagen 2-chloroacrolein was proposed to involve in vivo hydroxylation at α-allylic carbon adjacent to sulfur (proximate mutagen), followed by hydrolysis.[46]

Application to Prodrugs and Highly Toxic Intermediates in OP Synthesis. Biomimetic oxidation studies allow for the design of better prodrugs. MCPBA-mediated oxidation of *S*-alkyl dioxaphosphorinane, with the appropriate stereochemistry at 4-hydroxymethyl (Figure 14), leads to the corresponding bicyclophosphate (BP), a highly potent γ-aminobutyric acid (GABA) antagonist.[47] Due to the internal transesterification reaction, leading to cyclization, the competitive sulfoxide to oxysulfonate rearrangement reaction (Figure 1) is eliminated so that the BP product is obtained almost quantitatively. A similar microsomal oxidase bioactivation mechanism was proposed for the conversion of the dioxaphosphorinane to BP in vivo.

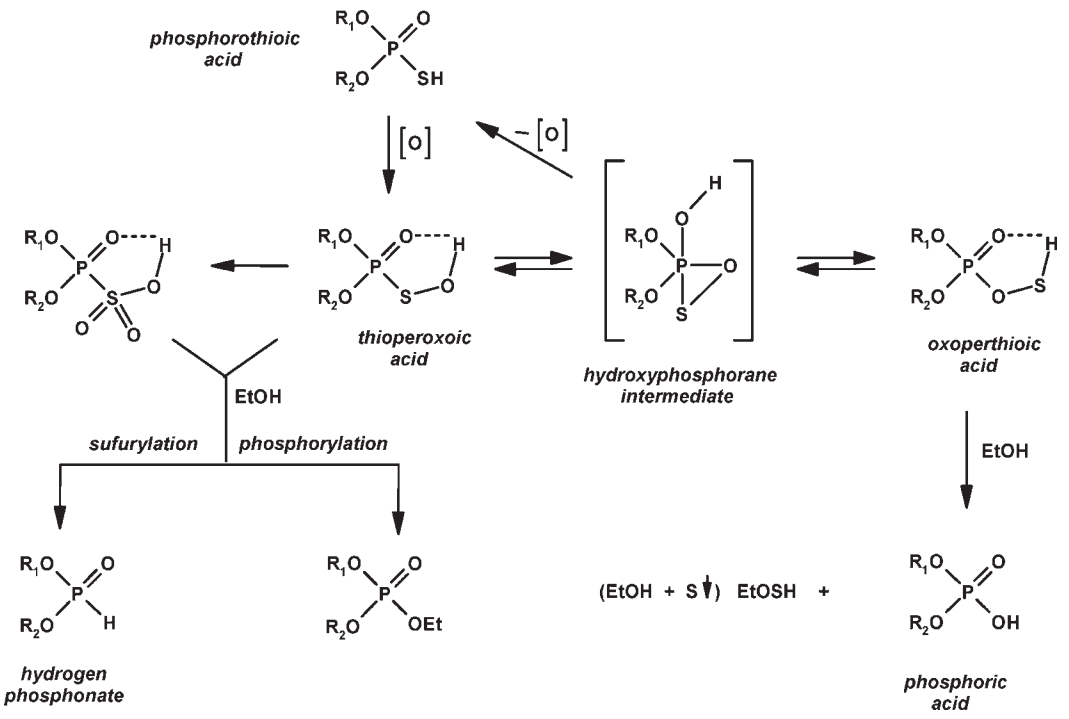

Figure 11. MCPBA oxidation of dialkyl phosphorothioic acids to relatively stable thioperoxoic and oxoperthioic acids.

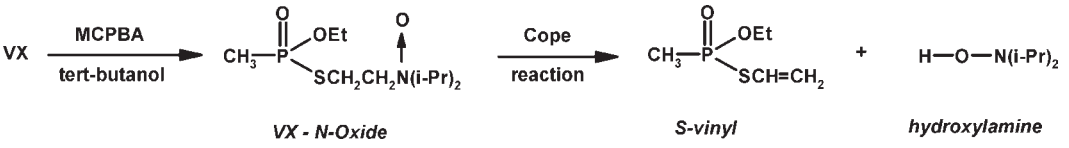

Figure 12. MCPBA oxidation of VX in *tert*-butanol.

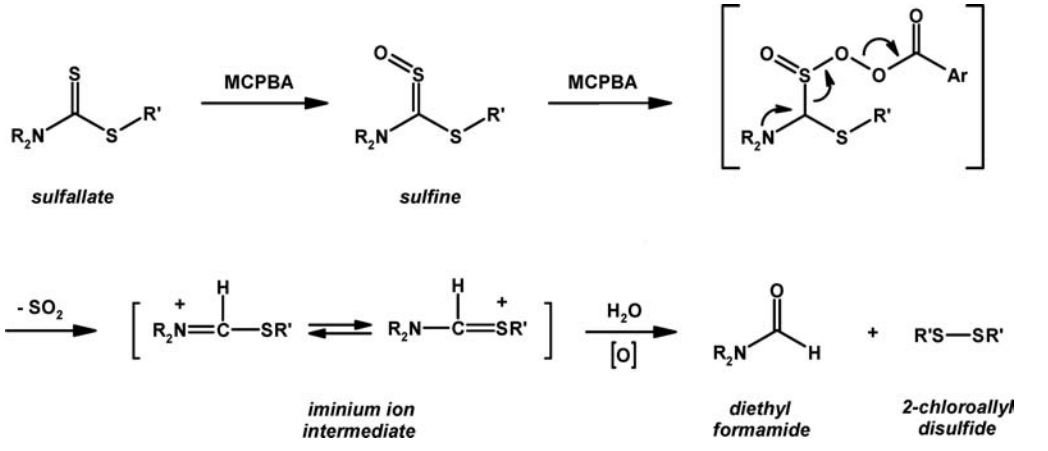

Figure 13. Oxidation of sulfallate (R = C_2H_5, R′ = $CH_2CCl{=}CH_2$) and related alkyl *N*,*N*-dialkyldithiocarbamates to dialkylformamide via sulfine and iminium ion intermediates. Ar = 3-Cl—C_6H_4.

Some major intermediates in OP synthesis are classified as highly toxic to mammals both orally and by inhalation. Early proposed activation of $POCl_3$[48] as anti-AChE was confirmed by preparing pure $Cl_2P(O)OH$, in aprotic solvent, as a primer metabolite (Figure 15).[49]

$Cl_2P(O)OH$ and potassium and dicyclohexylamine (DHA) salts thereof reproduce the action of $POCl_3$ as in vitro equipotent AChE inhibitors and toxicants in mice, indicating that ionized $Cl_2P(O)OH$ is the actual inhibitor. Mortality in mice is consistent with poisoning by hydrolytic products: $Cl_2P(O)OH$ from $POCl_3$ and $Cl_2P(O)SH$ from $PSCl_3$. The latter is resistant to MCPBA oxidation, retaining its thiophosphoryl substituent In Vivo in mice based on the observation, by ^{31}P NMR, of thiophosphoric acid as the primer urinary metabolite.

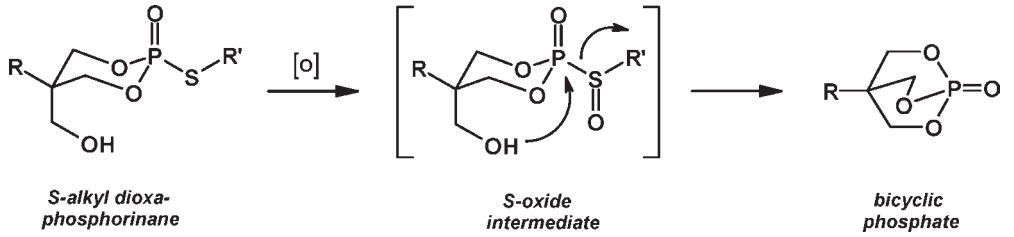

Figure 14. Oxidation of *S*-alkyl dioxaphosphorinane to the corresponding BP, a highly potent GABA antagonist (for R = t-C_4H_9, LD_{50} for mice = 0.036 mg/kg).

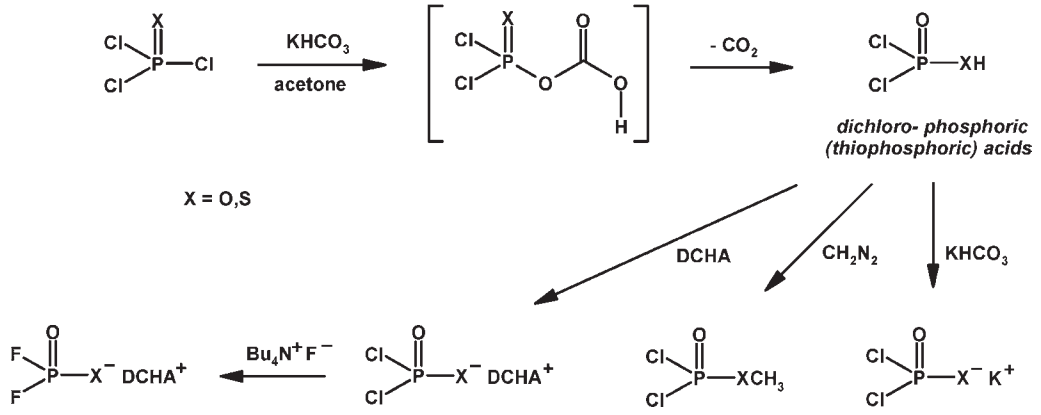

Figure 15. Preparation and reactions of dichlorophosphoric and dichlorothiophosphoric acids and potassium and DHA (dicyclohexylamine) salts starting from $POCl_3$ and $PSCl_3$.

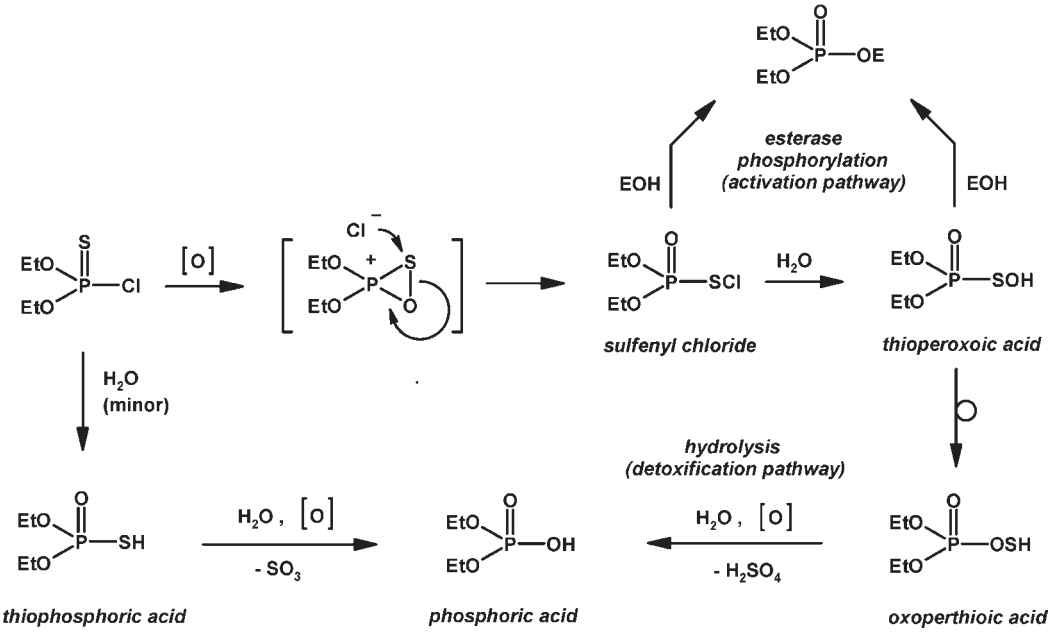

Figure 16. Possible in vivo metabolic bioactivation and detoxification pathways for diethyl thiophosphoryl chloride, $(EtO)_2P(S)Cl$.

Vapors of $(EtO)_2P(S)Cl$ are lethal to houseflies as in vivo AChE inhibitors possibly on oxidative activation by P450. $EtOP(S)Cl_2$ is oxidized by MCPBA to $EtOP(O)Cl_2$ in contrast to $(EtO)_2P(S)Cl$, which converts to $(EtO)_2P(O)SCl$, the structure of which was unequivocally approved by independent synthesis. Its further pathway, involving hydrolysis and rearrangement reactions, may have toxicological relevance (Figure 16).

Initial oxidation takes place at sulfur with release of chloride. In cage back attack of chloride leads to the biologically active sulfenyl chloride. Further hydrolysis gives thioperoxoic acid. Both latter derivatives may phosphorylate the enzyme in an activation pathway. Otherwise, thioperoxoic acid rearranges to the analogous oxoperthioic acid (Figure 16), which lacks phosphorylation properties. Further hydrolysis yields biologically

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inactive diethyl phosphoric acid, an overall detoxification pathway. Thioperoxoic acid, in the absence of enzymes, may partially convert to thiophosphoric acid that on further oxidation, followed by hydrolysis, converts as well to diethyl phosphoric acid.

MCPBA oxidation of $(EtO)_2P(S)Cl$ in ethanol leads quantitatively to $(EtO)_3PO$ and elemental sulfur, emphasizing the sulfenyl chloride and thioperoxoic acid as phosphorylating rather than sulfonylating agents. These biomimetic studies indicate that PCl_3, $POCl_3$, and $PSCl_3$ inhibit serine hydrolases in vitro. Whereas PCl_3 inhibits directly, $POCl_3$ and $PSCl_3$ inhibit by their monohydrolysis acids $Cl_2P(O)OH$ and $Cl_2P(O)SH$. $(EtO)_2P(O)Cl$ acts directly, but $(EtO)_2P(S)Cl$ acts on oxidative bioactivation to $(EtO)_2P(O)SCl$.

Summary and Conclusions. Five types of oxidative/rearrangement model reactions are analyzed for their toxicological relevance in biological systems: (a) *S*-Oxide intermediates from *S*-alkyl phosphorothioate pesticides and their synthetic precursors (Figures 1, 3, 4, 6, 9, 10, 13, and 15) are formed on an activation pathway mechanism that results in direct attack of the enzyme. Otherwise, they undergo rearrangement to the appropriate phosphorus oxysulfonate followed by hydrolysis to biologically inactive metabolites, an overall detoxification pathway. (b) Phosphoramidate pesticides and other extensively used compounds are activated to their *N*-oxide derivatives (Figures 1, 6−8, and 12). Similar activation detoxification rearrangement and cleavage sequences are also noted with these types of compounds. Many of the products obtained on peracid oxidation were in general those formed biologically. (c) Thiocarbamate pesticides and related *S*-alkyl thiocarbamates are converted to their sulfoxides (Figure 5). This type of widely accepted activation mechanism has been established with a variety of herbicides. Depending on the nature of the *S*-alkyl moiety, the *S*-oxide intermediate may hit the target directly or, following a rearrangement/elimination reaction, convert to a highly potent mutagen or detoxify. (d) Dithiocarbamate pesticides and related *S*-alkyl dithiocarbamates are possibly bioactivated (Figure 13). In all cases studied these oxidation reactions failed to activate the dithiocarbamates because the initial oxidation forms sulfine derivatives, which follows extensive breakdown reactions resulting in detoxification of the parent compound. (e) Oxidation of a heteroatom in a remote position from phosphorus may take place (Figure 9). Reactive intermediates, obtained in this category, gave often identical intermediates formed by metabolic activations.

The peracid system is not always a suitable biomimetic model. S-Oxidation and N-oxidation often give the appropriate highly reactive transient oxides that are proposed to be formed metabolically. EPTC, diallate, metribizin, profenophos, methamidophos, glyphosate, and oxime ether pyrethroids are among other pesticides that belong in this category. Thiophosphoryl chloride and its diethyl ester analogue are similarly categorized. However, oxidation of sulfallate herbicide gives products of which none exhibit mutagenic activity in the Ames test, as opposed to its metabolic activation that forms a highly potent mutagen 2-chloroacrolein. VX is primarily oxidized at nitrogen to form the *N*-oxide, which undergoes an elimination reaction leading to products that lack significant biological activity (Figure 12). Therefore, the relevance of the biomimetic model has to be established in each case.

Excluding some thiocarbamate sulfoxides, the *N*- and *S*-oxides studied here are highly reactive transient intermediates that have never been isolated. It appears that the thiophosphorus *S*-oxide moiety is one of the best known leaving groups attached to tetracoordinated phosphorus. All of the *N*-oxides and most of the thiophosphorus *S*-oxides could not be elucidated directly by spectroscopy, and thus their existence as transitory intermediates, as well as their structures, is based on products obtained from their reactions in different media. Some *S*-oxides, with unique structural features, have improved thermal stability and may be observed at room temperature. The *S*-oxides from dialkyl phosphorothioic acids (Figure 11) with internal hydrogen bonding are sufficiently stable to be analyzed by spectroscopy. Phosphoramidothiolate *S*-oxides have enhanced stability due to electron donation by nitrogen to phosphorus. Their structures were elucidated by ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy both as sulfoxides and as sulfones. Accordingly, the *S*-oxides from thiocarbamate pesticides are sufficiently stable to be directly observed.

The major drawbacks of oxidative model systems are the lack of sufficient data related to DME mechanisms and extrapolation from nonprotic solvents to aqueous medium. Despite those disadvantages, the biomimetic model system has major advantages that establish it as a useful tool for studying reactive metabolites: it allows the determination of whether certain biologically active compounds act directly or on preactivation or both, in some cases allowing the study of short-lived intermediates that are formed metabolically; it allows for the design of better biologically active compounds, obtaining intermediates and products in large amount, investigating rapid reactions in organic and protic solvents, and often direct monitoring of intermediates by NMR, monitoring of reactions at low temperature seeking transient intermediates, simplicity for isolation, evaluation of activation and detoxification mechanisms and mode of action based on chiral isomers, and allows for incorporation of ^{18}O, from ^{18}O-enriched MCPBA, for mechanistic studies.

Most of the studies presented here were carried out over the past three decades at ECTL under the supervision of John E. Casida. This concept of biomimetic model systems has been proved to be indispensable to understanding pesticide mode of action, selectivity, and safe use. The idea seems to be intriguing, surprising, fun, and most helpful in obtaining efficient results that otherwise would have been difficult to achieve.

AUTHOR INFORMATION

Corresponding Author
*E-mail: yoffis@iibr.gov.il.

ABBREVIATIONS USED

OP, organophosphorus; DME, drug-metabolizing enzymes; AChE, acetylcholinesterase; ECTL, Environmental Chemistry and Toxicology Laboratory at Univeristy of Calfornia at Berkeley; MCPBA, *meta*-chloroperoxybenzoic acid; MFO, mixed function oxidase; HMPA, hexamethylphosphoramide; DHA, dicyclohexylamine; BP, bicyclophosphate.

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