Bull. Org. mond. Santé }
Bull. Wld Hlth Org. } 1971, **44**, 133-150

Biological and Nonbiological Modifications of Organophosphorus Compounds *

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The general types of biological reaction that are most prominent in the modification of organophosphorus compounds involve the mixed-function oxidases, hydrolases, or transferases. In certain cases, more than one of these reactions may be involved at the same site on the pesticide molecule. Examples of various organophosphorus pesticides that are altered by oxidation, hydrolysis, alkyl- or aryl-group transfer, reduction, and conjugation are discussed. The increase or decrease in toxicity of a pesticide that can result from biological modification is emphasized.

Non-biological transformations of organophosphorus compounds involve the effect ou the compounds of such factors as light, air, temperature, and solvent. These factors are discussed with special emphasis on desulfuration, rearrangement, and oxidation.

Increasing emphasis is being given to research on, and the development of, pesticides that will be effective for the control of insects but that will minimize the " off-target " effects that are characteristic of the chlorinated hydrocarbons. This trend involves both the more effective use of currently available nonpersistent pesticides and the development of new compounds. The organophosphorus compounds are a chemical group of insecticides that are characterized by their nonpersistence in the environment as well as by their wide spectrum of activity. In order to utilize more effectively the inhibitory power of the phosphate moiety in the development of new compounds, a knowledge of the metabolism and fate of organophosphorus esters in various biological systems is necessary. This information is important for an understanding of the processes of intoxication, detoxification, and resistance. Information on the chemical behaviour and reactions of organophosphorus compounds in a nonliving system, the environment, is essential for the assessment of the potential hazards they present to human health, their longevity in the environment, and their efficacy for the control of insects.

The metabolism of organophosphorus compounds by plants and animals, with special emphasis on isolated *in vitro* systems, is discussed in the first section of this paper. The second section is devoted to environmental factors such as light, temperature, air, and solvent and their effects on nonbiological modifications of organophosphorus compounds.

BIOLOGICAL MODIFICATIONS

The metabolism of organophosphorus insecticides in plants and animals has been the subject of several recent reviews (O'Brien, 1967; Hodgson, 1968; Casida & Lykken, 1969; Lykken & Casida, 1969; Fukuto & Metcalf, 1969; Menzie, 1969; Menzer & Dauterman, 1970; Bull, 1970). This portion of the review summarizes and compares the importance of the various biological processes by which plants and animals modify the chemical structure of insecticidally active organophosphorus compounds. Since the literature is so complex, by virtue of the large number of compounds, specific examples have been chosen to illustrate the various types of reaction.

Organophosphorus insecticides are metabolized by four general classes of reaction: (1) reactions

* This study was supported in part by Grant ES-00044 from the US Public Health Service. It is paper No. 3391 of the Journal Series of the North Carolina State University Experiment Station, Raleigh, N.C., USA.

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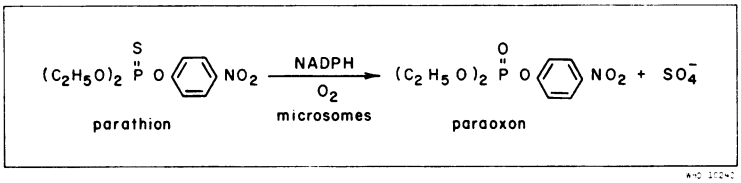
Fig. 1

Oxidative desulfuration of parathion *



* Dahm (1970).

involving the mixed-function oxidases, (2) reactions involving hydrolases, (3) transferase reactions, and (4) miscellaneous reactions. In certain cases, more than one of these reactions may be involved at a common site on the organophosphorus molecule. Therefore the identification of the product is not necessarily indicative of either the type of biological alteration or the route.

MIXED-FUNCTION OXIDASES

The mixed-function oxidases are a group of enzymes that are important in the metabolism of xenobiotics in mammals (Gillette et al., 1969) and insects (Hodgson & Plapp, 1970). The enzyme systems are associated with the post-mitochrondrial supernatant of plant or animal tissue homogenates and are derived from the endoplasmic reticulum. The particulate enzyme system has an essential requirement for NADPH and molecular oxygen in order to modify a xenobiotic.

Oxidative desulfuration

Phosphorothioate and phosphorodithioate esters are poor inhibitors of cholinesterases unless the compounds are oxidatively desulfurated. This generally results in an increase in inhibition of the target enzyme as well as in toxicity to the organism (Heath, 1961; O'Brien, 1960). The *in vivo* activation of parathion to paraoxon has been demonstrated in both insects and mammals (Gage, 1953; Metcalf & March, 1953). Evidence for oxidative desulfuration has been demonstrated in both plants and animals for a wide variety of insecticides: parathion-methyl (Hollingworth et al., 1967), malathion (O'Brien, 1957), dimethoate (Dauterman et al., 1960; Brady & Arthur, 1963), fenitrothion (Hollingworth et al., 1967), and Supracide † (Bull, 1970). *In vitro* studies with microsomal preparations from cockroach fat body (Nakatsugawa & Dahm, 1965), rat liver (Neal, 1967a, 1967b; Nakatsugawa & Dahm, 1967), and housefly abdomen (El Bashir & Oppenoorth, 1969) demonstrated that desulfuration of parathion was accomplished by a mixed-function oxidase system in the presence of NADPH and molecular oxygen (Fig. 1). The sulfur atom is removed during the reaction *in vitro* and bound to the microsomes and the detached sulfur is found *in vivo* as inorganic sulfate (Nakatsugawa & Dahm, 1967; Nakatsugawa et al., 1969b). It seems reasonable to assume that the mixed-function oxidases are responsible for the *in vivo* desulfuration of phosphorothioates in most other biological entities, although this reaction has not been demonstrated *in vitro* in plants.

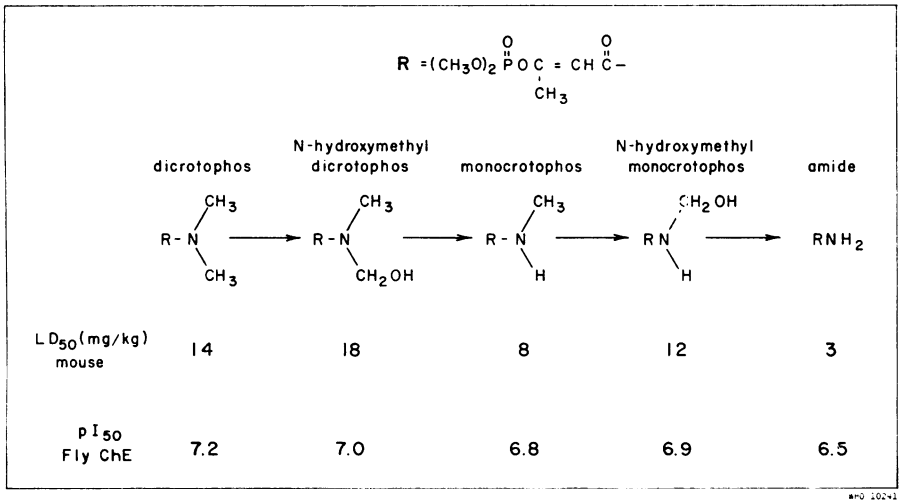
Oxidative N-*dealkylation*

The oxidative *N*-dealkylation by the mixed-function oxidases of many nitrogen-containing xenobiotics in plants and animals is well documented (Frear et al., 1969; Gillette et al., 1969; Casida & Lykken, 1969; Menzer & Dauterman, 1970). A typical example of this reaction is the *N*-demethylation of dicrotophos and moncrotophos (Fig. 2). This reaction has been demonstrated to occur in plants, mammals, and insects (Menzer & Casida, 1965; Bull & Lindquist, 1964, 1966; Lindquist & Bull, 1967). Removal of the *N*-methyl groups proceeds by the formation of relatively stable *N*-hydroxymethyl intermediates followed by the elimination of formaldehyde. The removal or modification of the *N*-substituents can result in an increase, a decrease, or little change in toxicity. *N*-demethylation has been reported to occur with schradan (Spencer et al., 1967), dimethoate (Sanderson & Edson, 1964; Lucier & Menzer, 1968, 1970), and famphur † (O'Brien et al., 1965). *N*-deethylation has been reported to occur with phosphamidon (Bull et al., 1967; Clemmons & Menzer, 1968). With phospha-

† Names against which this symbol appears are identified in the Glossary on pages 445-446.

Fig. 2

Oxidative *N*-dealkylation of dicrotophos *



* Menzer & Casida (1965).

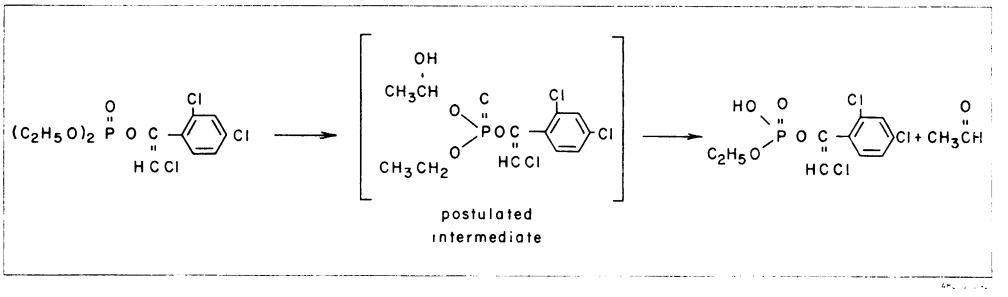
midon the *N*-ethyl groups are sequentially hydroxylated on the α-carbon and then eliminated as acetaldehyde. In all cases studied the relative concentrations of the products formed were usually small but all the metabolites had anticholinesterase properties.

Oxidative O-*dealkylation*

Cleavage of organophosphorus triesters to diesters results in relatively nontoxic metabolites. Since the isolation and identification by Plapp & Casida (1958) of metabolites that were the result of alkyl–phosphate cleavage, this reaction has generally been regarded as being of a hydrolytic nature, catalysed by a phosphatase enzyme. Only recently has *in vitro* evidence been presented to indicate that other mechanisms are also responsible for the formation of dealkylated metabolites (see also the sections on hydrolases and transferases, below). Donninger et al. (1967) demonstrated that chlorfenvinphos was oxidatively *O*-deethylated by liver microsomes in the presence of NADPH and oxygen (Fig. 3). The reaction resulted in the isolation of deethyl chlorfenvinphos and acetaldehyde and it was postulated that an unstable 1-hydroxyethyl intermediate was formed, which subsequently broke down. Oxidative demethylation has also been demonstrated to occur with Gardona,† the dimethyl analogue

Fig. 3

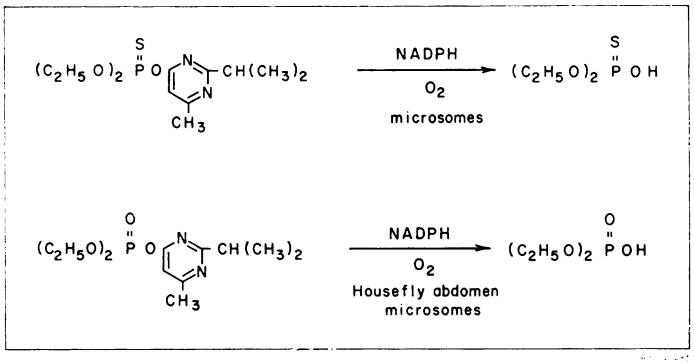
Oxidative *O*-deethylation of chlorfenvinphos *



* Donninger et al. (1967).

Fig. 4

Oxidative dearylation of diazinon *



* Yang et al. (1971).

of chlorfenvinphos (Hutson et al., 1968a). Further work by Hutson et al. (1968b) indicates that oxidative *O*-dealkylation occurs with dimethyl, diethyl, diisopropyl, and di-*n*-butyl analogues of 1-naphthyl phosphate. Lewis (1969) reported that diazoxon (but not diazinon) was deethylated by a microsomal preparation from a resistant strain of the housefly in the presence of NADPH and oxygen.

Other studies have failed to demonstrate a role of the mixed-function oxidases in the *O*-dealkylation of phosphorothioates (Hollingworth, 1969; Nakatsugawa, Tolman & Dahm, 1969a; Yang et al., 1971a, 1971b). If oxidative *O*-dealkylation is an important route of detoxification, it may be primarily involved with the phosphates rather than the phosphorothioates.

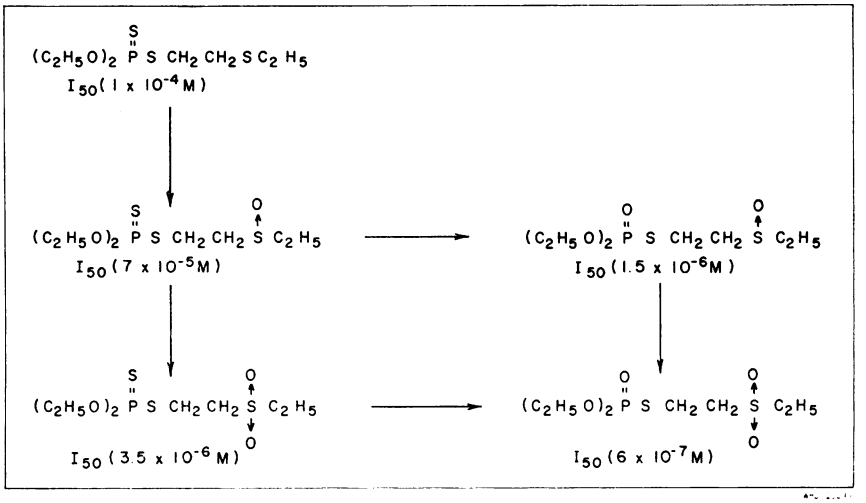
Oxidative dearylation

It is generally agreed that disruption of the acid-anhydride bond of organophosphorus insecticides is the most important mechanism for *in vivo* detoxification. For some time this reaction was regarded as being catalysed exclusively by hydrolases (Aldridge, 1953; Augustinsson & Heimburger, 1954; O'Brien, 1960). While it is true that these enzymes are important for the degradation of some organophosphates, recent *in vitro* studies have also demonstrated that a NADPH-dependent microsomal oxidase system from mammalian liver and housefly abdomens was able to cleave the aryl–phosphate bond (Fukunaga, 1967; Neal, 1967a, 1967b; Nakatsugawa & Dahm, 1967; El Bashir & Oppenoorth, 1969; Lewis, 1969; Yang et al., 1971a, 1971b). Studies with parathion and diazinon resulted in the isolation of diethyl phosphorothioic acid and some diethyl phosphoric acid, which is formed by oxidative desulfuration followed by the breakdown of the oxons (Fig. 4). The mixed-function oxidases from rat liver are able oxidatively to degrade diazinon but not diazoxon (Yang et al., 1971a), whereas the microsomal system from houseflies is able oxidatively to dearylate both diazinon and diazoxon, although diazinon is the preferred substrate (Lewis, 1969; Yang et al., 1971b). In studies by Nakatsugawa et al. (1968), eight substituted and unsubstituted phosphorothioate analogues containing *p*-nitrophenol were oxidatively dearylated to *p*-nitrophenol by microsomes from rat liver, rabbit liver, and housefly abdomens. From the available information it would appear that the cleavage of an aryl–phosphate bond by oxidative dearylation in mammals and insects is an important degradative reaction, especially if the organophosphate contains a thiono sulfur atom. There is no evidence that this reaction occurs in plants.

Thioether oxidation

The thioether oxidation of certain organophosphates has been demonstrated *in vivo* in plants, mammals, and insects. This reaction involves the conversion of the thioether moiety to the respective sulfoxide and sulfone and has been demonstrated with demeton (Fukuto et al., 1955; Fukuto et al., 1956), phorate (Bowman & Casida, 1957, Metcalf et al. 1957), disulfoton (Metcalf et al., 1957; Bull, 1965), and fensulfothion (Benjamini et al., 1959a, 1959b). Thioether oxidation is exemplified by the reactions in Fig. 5, which show the oxidation of

Fig. 5
Thioether oxidation of disulfoton *



* Metcalf et al. (1957).

the thioether moiety of disulfoton to the corresponding sulfoxide and sulfone as well as the desulfuration. The I_{50} values for the various metabolites to fly-head ChE are given. In general the initial oxidation of most thioether-containing organophosphates results in a rapid conversion to the sulfoxide and then a slow conversion to the sulfone. This results in the accumulation of the sulfoxide, which is probably the principal toxicant. With disulfoton, thioether oxidation resulted in an increase in the I_{50} value, but this activation was not as great as that resulting from oxidative desulfuration (Metcalf et al., 1957).

At present, no *in vitro* data are available on the enzymes or enzyme systems responsible for the oxidative reactions with thioether-containing organophosphates. However, *in vitro* studies with carbamates (Tsukamato & Casida, 1967a, 1967b; Andrawes et al., 1967) and sulfur compounds (Parke, 1968; Lee et al., 1970) indicate that the microsomal oxidases are responsible for the conversion of the thioether moiety to the sulfoxide and also the sulfone. It is reasonable to assume that the mixed-function oxidases are also involved in the oxidation of thioether-containing organophosphorus insecticides.

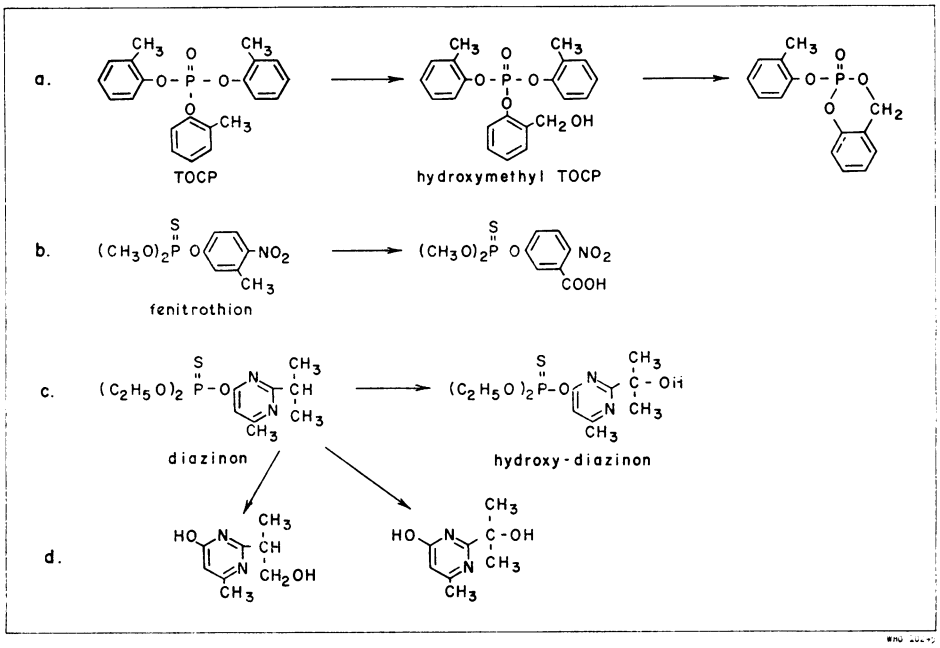
Side-group oxidation

The oxidation of aliphatic ring substituents of certain organophosphorus compounds is probably also the result of microsomal enzymes. Eto et al. (1962), working on the metabolism of tri-*o*-tolyl phosphate (TOCP), obtained evidence that three active metabolites were formed *in vivo* by rats and *in vitro* by rat liver microsomes fortified with NADPH. The structures of the metabolites isolated from rat intestines were characterized by chemical tests, IR spectra, and synthesis. The findings indicated that TOCP was hydroxylated at the *o*-methyl group to form hydroxymethyl TOCP and that this intermediate was then cyclized (Eto et al., 1967) to form cyclic methylene-*o*-phenylene *o*-tolyl phosphate [*o*-tolyl saligenin phosphate] (Fig. 6, reaction a). This reaction resulted in an increase in anticholinesterase activity to a level 12 000 000 times that of the parent TOCP.

Douch et al. (1968) reported that the 3-methyl group of fenitrothion was oxidized to a carboxyl group by mouse liver microsomes fortified with NADP and glucose-6-phosphate (Fig. 6, reaction b). It was suggested that this reaction might be responsible for the low mammalian toxicity of fenitrothion. The reported metabolite was identified by indirect methods and further work is needed to substantiate this identification.

Another example of *in vivo* oxidation of aliphatic ring substituents has recently been reported with the identification of hydroxy diazinon (Pardue et al., 1970). This metabolite was isolated from field-

Fig. 6
Side-group oxidation *



* References: reaction a, Eto et al. (1962); reaction b, Douch et al. (1968); reaction c, Pardue et al. (1970); reaction d, Mücke et al. (1970).

treated kale and is the result of oxidation at the tertiary carbon of the isopropyl group (Fig. 6, reaction c). In another *in vivo* study with diazinon in rats, Mücke et al. (1970) found that the pyrimidine moiety was oxidized at both the primary and the tertiary carbon atoms of the isopropyl group (Fig. 6, reaction d). These metabolites were isolated from rat urine and it was not established whether the pyrimidinol metabolites were formed before or after cleavage of the pyrimidine–phosphate linkage.

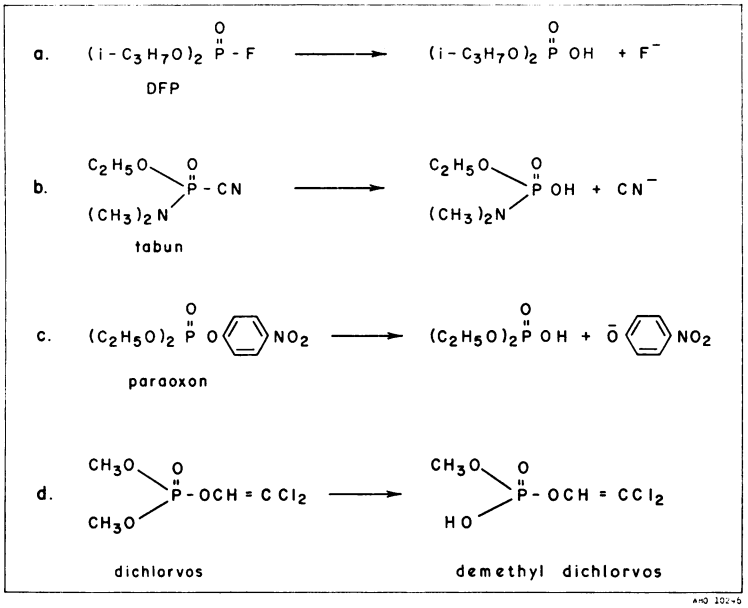
HYDROLASES

Most organophosphorus compounds are degraded to some extent by various hydrolases (Heath, 1961; O'Brien, 1960, 1967). These enzymes may hydrolytically attack the phosphorus ester bond or the anhydride bond as well as an ester or amide bond in the leaving group. The disruption of any of these bonds to form a diester or a monocarboxylic acid is probably one of the most important mechanisms of inactivation and detoxification. Hydrolases responsible for organophosphate hydrolysis are present in many biological systems and are widely distributed in different organs and tissues, as well as being found in both soluble and subcellular fractions.

Triester hydrolysis

The first enzymatic hydrolysis of an organophosphorus compound to be reported was that of DFP † (Mazur, 1946). The findings indicated that DFP and various analogues were hydrolysed at the P–F bond by various rabbit tissue homogenates. Evidence for the hydrolysis of the acid anhydride bond by arylesterases has subsequently been demonstrated both with mammalian and insect tissue homogenates and with partially purified enzymes with DFP (Mounter, 1956; Cohen & Warringa, 1957), tabun † (Augustinsson & Heimburger, 1954), sarin † (Adie, 1956), paraoxon and analogues (Aldridge, 1953; Main, 1960a, 1960b; Jarczyk, 1966), and diazinon (Matsumura & Hogendijk, 1964a). With all the compounds mentioned above the hydrolysis occurred at P–O–C, P–CN, or P–F bonds and resulted in relatively nontoxic products (Fig. 7). At present most of the available evidence indicates

Fig. 7
Triester hydrolysis



that the phosphates are probably the preferred substrates for enzymatic hydrolysis rather than the phosphorothioate analogues.

O-dealkylation is another possible mechanism by which organophosphorus triesters may be hydrolysed. Hodgson & Casida (1962) reported that dichlorvos was *O*-demethylated by a soluble enzyme fraction from rat liver (Fig. 7, reaction d). Their findings indicated that this reaction was not associated with the microsomal fraction and that NADPH was not required; therefore, it was not the result of oxidative *O*-dealkylation. However, endogenous glutathione might have been present in the soluble fraction in sufficient quantities to mediate the *O*-dealkylation (see glutathione *S*-alkyl transferase, below). Recently Nolan & O'Brien (1970) reported that ^{3}H-paraoxon was *O*-dealkylated in houseflies, resulting in the formation of labelled ethanol and derivatives. Since ethanol was found rather than the aldehyde or *S*-ethyl glutathione, one may conclude that this reaction may have been mediated by a hydrolase. In general very little information is available on this reaction and further work is needed to clarify its importance *in vivo*.

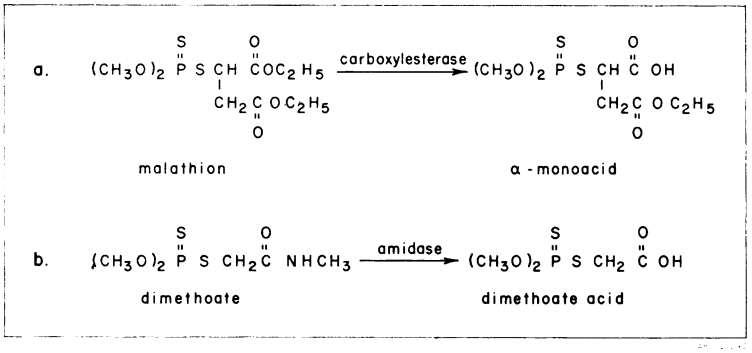
Carboxylesterases

Carboxylesterases, or carboxylic-ester hydrolases, have been shown to be important in the detoxification of organophosphorus compounds such as malathion and acethion † (O'Brien, 1960). This detoxification reaction involves the hydrolysis of a carboxyester linkage, resulting in a nontoxic ionic product, the mono-acid of malathion (Cook & Yip, 1958) (Fig. 8, reaction a). The enzyme is widely distributed in mammals and has been found in the liver, kidney, serum, lung, spleen, and ileum of the rat (Seume & O'Brien, 1960), the mouse, the guinea-pig, and the dog (Murphy & DuBois, 1957). In each of the insect species studied the activity of the enzyme was low in (or the enzyme was absent from) susceptible insects (Kojima, 1961), partly explaining the selective toxicity of the compound. This hydrolase is present in certain malathion-resistant insects and it is reasonable to assume that resistance to malathion is at least partly due to carboxylesterase activity (Dauterman & Matsumura, 1962; Matsumura & Brown, 1963; Matsumura & Hogendijk, 1964b).

Main & Braid (1962) partially purified from rat liver an enzyme that hydrolysed malathion. This

Fig. 8

Hydrolases of functional groups



enzyme cleaved only one of the carbethoxy groups and was characterized as a carboxylesterase.

Summarizing some of the findings on this enzyme from our laboratory, only one of the carbethoxy groups is hydrolysed *in vivo* by the rat and by the purified enzyme and the resulting metabolite, as identified by NMR spectroscopy, is the α-monoacid (Chen et al., 1969) (Fig. 8a). This enzyme is unable to hydrolyse the second carbethoxy group and can attack only un-ionized substrates. Long-chained carbalkoxy compounds are better substrates than carbmethoxy compounds (Dauterman & Main, 1966). Malaoxon is both a substrate and an inhibitor of the carboxylesterase (Main & Dauterman, 1967). The enzyme is unable to hydrolyse the carboxyamide bond of compounds like dimethoate.

Amidases

Carboxyamidases have been implicated in the metabolism in plants and animals of a number of organophosphorus insecticides containing carboxyamide groups—e.g., dimethoate, dicrotophos, monocrotophos, and phosphamidon (Menzie, 1969). In each case a carboxylic acid metabolite was identified as an alteration product (Fig. 8b). Since these compounds were either substituted *N.N*-dimethyl, *N*-methyl, or *N,N*-diethyl amides, one must assume that carboxyamidases are able to hydrolyse various substituted *N*-alkyl groups.

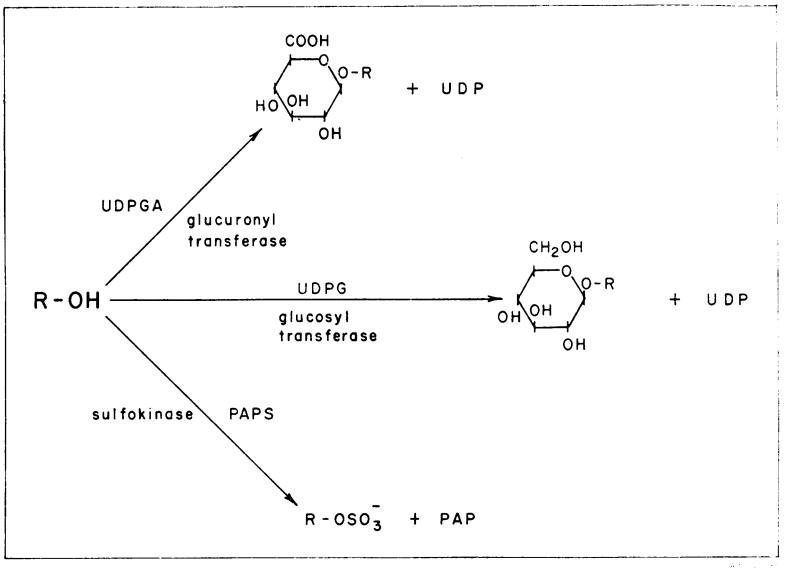
Most of the *in vitro* studies on the amidases have been conducted with mammalian tissues being the source of the enzyme. In mammals the enzyme appears to be concentrated in the liver (Uchida et al., 1964) and is primarily associated with the microsomal fraction (Uchida & O'Brien, 1967). The latter workers found a correlation between the *in vitro* degradation of dimethoate by liver homogenates from six vertebrates with the *in vivo* toxicity for each species.

With this in mind we undertook an investigation of some of the properties of the mammalian amidase. Preliminary findings indicated that the enzyme could be rendered soluble by repeated freezing and thawing of sheep liver microsomes. Utilizing rapid-flow hydroxylapatite gel chromatography followed by benzyl-DEAE column chromatography it was possible to obtain a 50-fold purification based on protein. Disc electrophoresis indicated that the enzyme was a single protein band (Chen & Dauterman, unpublished results). Our results showed that the enzyme has an optimum pH of 9 and a molecular weight of approximately 230 000–250 000. Divalent cations did not stimulate the enzyme at a 10^{-6}M concentration, indicating that it is not an arylamidase since these ions activate arylamidase activity. The various nucleotides, in either the oxidized or the reduced form, did not enhance the activity. Whereas malaoxon is both a substrate and an inhibitor of carboxylesterase, the oxygen analogue of dimethoate is only an inhibitor of the amidase and is not hydrolysed by it.

TRANSFERASES

Studies on transferases or conjugation reactions involving organophosphorus insecticides have generally been neglected. Most of the emphasis in organophosphorus metabolism has been on the identification of the hydrolysis products containing the P atom rather than on other portions of the molecule that could be conjugated. With the carbamates much more information is available on

Fig. 9
Transferase reactions



conjugates, as has been described by Knaak (1971). However, since organophosphorus compounds can undergo a wide variety of biological modifications that may result in the introduction of sites for conjugation, one must assume that a portion of these metabolites are conjugated by the general transferase reactions.

Some important conjugation reactions that may be involved in the *in vivo* metabolism of organophosphates are summarized in Fig. 9. Of those listed, glucuronides are reported to be formed in vertebrates, glucosides in plants and insects, and etheral sulfates in vertebrates. These reactions are some of those most frequently described in the literature. All of these transferase reactions required a high energy donor as well as a suitable enzyme. With glucuronide formation the reaction requires uridine diphosphate glucuronic acid and the glucuronyl transferase enzyme, which is associated with the microsomal fraction of mammalian livers or kidneys. Glucuronic acid may be transferred to phenolic, hydroxylamino, and alcoholic hydroxyl groups; carboxyl groups; amino and imino groups; and sulfhydryl groups (Smith & Williams, 1966).

The formation of glycosidic conjugates requires a uridine diphosphate glucose donor, a glucosyl transferase, and acceptor groups similar to those required for glucuronide formation. Under certain conditions sugars other than glucose may be involved as a part of the uridine donor.

Sulfate conjugation primarily occurs with aromatic amino groups, phenolic hydroxyl groups and aliphatic alcoholic hydroxyl groups (Parke, 1968). Sulfate esters are synthesized biochemically by the transfer of sulfate from adenosine-3′-phosphate-5′-phosphosulfate to the phenol, alcohol, or amine by the sulfate-transferring enzyme aryl sulfotransferase.

The accompanying table lists some of the conjugation products that have been identified for a number of organophosphorus insecticides. All of the conjugated products reported are secondary metabolites in which the phosphorus moiety is lacking. From these data it would appear that conjugates of the leaving group are synthesized after the cleavage of the acid anhydride bond.

None of the other conjugation reactions—such as methylation, peptide conjugation, acetylation, or phosphate conjugation—has been reported to occur with organophosphorus insecticides.

Alkyl transferases

Glutathione conjugations have been found to be extremely important in the metabolism of organo-

Conjugation products of metabolites of organophosphorus insecticides

Compound	Organism	Product	Reference
Abate †	plant	4,4'-thiodiphenol, 4,4'-sulfinyldiphenol, and 4,4'-sulfonyldiphenol glucosides	Blinn (1968)
chlorfenvinphos	rat, dog	2,4-dichlorophenylethyl and ethanediol glucuronides	Hutson et al. (1967)
Colep †	rat plant	phenylsulfuric acid phenyl α and β glucosides	Marco & Jaworski (1964)
famphur †	calf	methyl and dimethyl sulfamoylphenyl glucuronides and sulfates	Gatterdam et al. (1967)
Gardona †	rat, dog	2,4,5-trichlorophenylethyl and ethanediol glucuronides	Whetstone et al. (1966)
parathion	cow	aminophenol glucuronide	Pankaskie et al. (1952)
phosalone	plant	benzoxazolone glycoside	Colinese & Terry (1968)
trichlorfon	insect	trichloroethanol glucuronide	Hassan et al. (1965)
dichlorvos	rat (*in vitro*)	dichloroethanol glucuronide	Hodgson & Casida (1962)

phosphates because of the formation of primary metabolites (Fig. 10, reaction a). Studies on the detoxification of parathion-methyl and the dimethyl homologue of paraoxon by mammals and insects demonstrated that *O*-demethylation occurred in the presence of a soluble enzyme preparation and reduced glutathione (Fukami & Shishido, 1963, 1966; Shishido & Fukami, 1963). Subsequent *in vitro* studies on the dimethyl analogue of chlorfenvinphos (Hutson et al., 1968a), mevinphos (Morello et al., 1968), fenitrothion and methyl paraoxon analogues (Hollingworth, 1969), bromophos (Stenersen, 1969), and diazinon (Lewis, 1969) have demonstrated that *O*-dealkylation also occurred with other organophosphorus compounds. The available information indicates that the alkyl transferase reaction is dependent on the presence of reduced glutathione, that the enzyme is in the soluble fraction and is probably identical to glutathione *S*-alkyl transferase, and that the substrate specificity favours the dimethyl esters and will *O*-dealkylate both phosphorothioates and phosphates, resulting in *S*-alkyl glutathione and *O*-dealkyl derivatives. The transferase activity is greatest in soluble fractions of mammalian liver and in the midgut and fat body of insects. All available information indicates that only one alkyl group is *O*-dealkylated. Stenersen (1969) reported that both *O*-methyl groups

Fig. 10

S-alkyl and *S*-aryl transferases

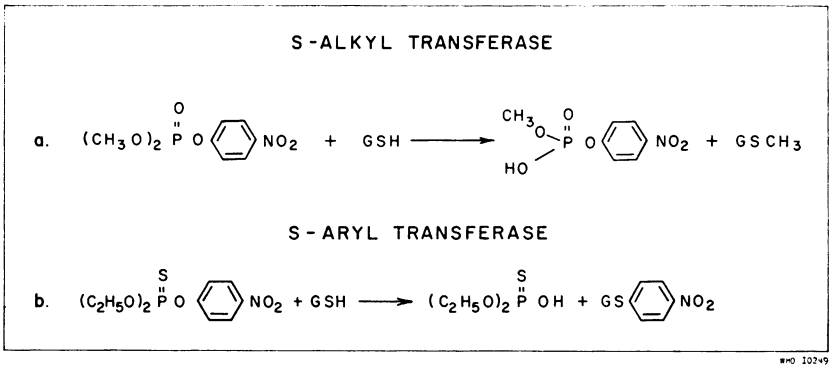
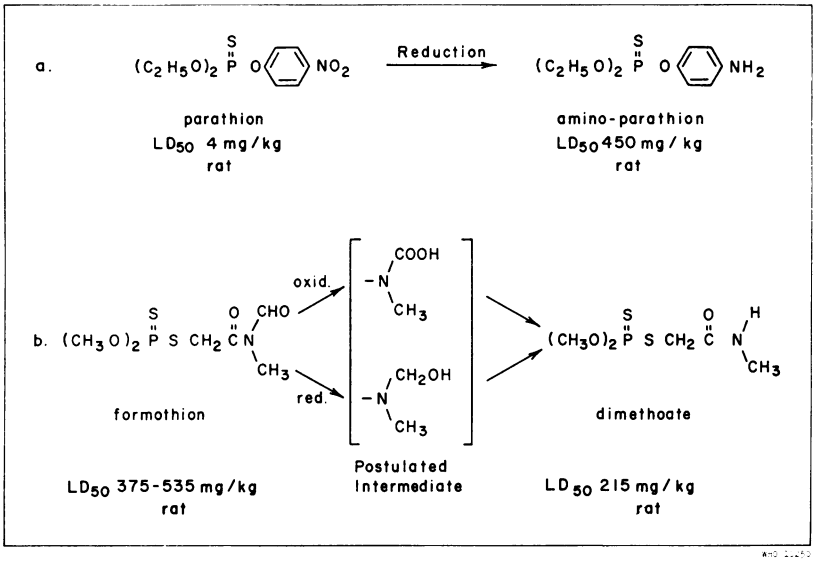


Fig. 11

Miscellaneous reactions *



* References: reaction a, Ahmed et al. (1958); reaction b, Laroche et al. (1970).

of bromophos were removed; however, he subsequently indicated that bromophos is only monodemethylated.

Aryl transferases

Preliminary data also indicate that a glutathione transferase reaction might be responsible for the transfer of aryl groups (Fig. 10, reaction b). Dahm (1970) reported some evidence that the P-O–aryl bond of parathion was cleaved by a non-oxidative soluble enzyme requiring glutathione. Studying the *in vitro* metabolism of diazinon in the housefly, we found that diethyl phosphoric acid and diethyl phosphorothioic acid were produced in the presence of GSH and the soluble enzyme fraction, thus indicating a possible aryl transfer (Yang et al., 1971b). However, we have not actually identified an *S*-aryl glutathione metabolite.

MISCELLANEOUS REACTIONS

There are reports of reductive reactions involved in the metabolism of organophosphates. The *in vivo* reduction of parathion and paraoxon to the amino derivatives is an important detoxification mechanism in ruminants, but is of minor importance in other animals (Ahmed et al., 1958). The toxicity of the amino derivatives is much lower than that of the parent compound (Fig. 11, reaction a). Recent *in vitro* studies (Hitchcock & Murphy, 1967) on the reduction of nitrophenyl-containing insecticides indicated that reductase activity was uniformly distributed between various cell fractions and that the enzyme required NADPH for activity. Reductase activity was greatest in liver and kidney but was also found in several other tissues.

Evidence that formothion is metabolized *in vivo* to dimethoate was recently obtained by Laroche et al. (1970). It is possible to postulate two routes of metabolism (Fig. 11, reaction b), one involving the oxidation of the aldehyde to the acid followed by decarboxylation and the other involving the reduction of the aldehyde to the *N*-hydroxymethyl group. This reaction can be considered an intoxication reaction since dimethoate is more toxic than formothion.

NONBIOLOGICAL MODIFICATIONS

Considerable research has been conducted on the "biological modifications" of organophosphorus compounds, but less attention has been devoted to the physical factors that may modify these compounds (Crosby, 1969). Some of the physical factors that may alter or modify the structure of organophosphorus compounds are light, temperature, air, and solvent. When a pesticide is used for insect control it is exposed to various environmental factors, which may alter it in the absence of a living organism. Since many of the alteration products that have been isolated and identified are quite similar to those obtained from biological systems, these reactions may also be important for the intoxication and detoxification of organophosphorus compounds, and may present a health hazard in the environment.

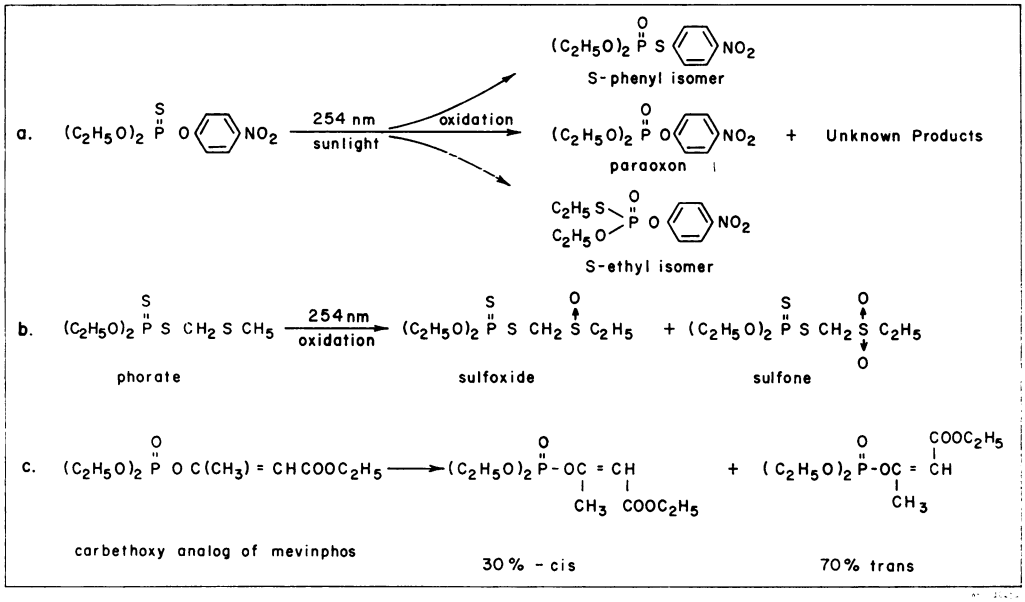
Light

Of all the physical factors that are responsible for chemical change, light is probably one of the most important. Most photochemical reactions appear to be mediated by the short-wavelength or the ultraviolet component of sunlight.

Parathion was one of the first organophosphorus compounds whose anticholinesterase activity was shown experimentally to increase during exposure to UV light and sunlight (Payton, 1953). Subsequent work by Cook (1955) and Cook & Pugh (1957) indicated that the toxicity of parathion decreased under UV light, but the *in vitro* anticholinesterase activity increased as the result of the formation of more polar products. Frawley et al. (1958) found that the exposure of parathion to UV light resulted in a mixture of compounds with greater *in vitro* anticholinesterase activity than parathion. A study by Mitchell (1961) indicated that most organophosphorus compounds break down to form a wide variety of new compounds on irradiation, but no attempt was made to characterize them. Studies by Koivistoinen & Meriläinen (1963) demonstrated that both UV light and sunlight changed parathion to several cholinesterase inhibitors. On the basis of chromatographic behaviour, the metabolites were identified as paraoxon and the *S*-ethyl and *S*-phenyl isomers of parathion, together with unknown products (Fig. 12). This study showed that UV light is able to oxidize as well as isomerize parathion. When

Fig. 12

Effect of light on some organophosphorus insecticides



parathion-methyl was given the same UV treatment only the methyl homologue of paraoxon was found. A similar study in which EPN † was exposed to UV light resulted in the identification of the oxygen analogue of EPN and *p*-nitrophenol, together with unidentified resins (Okada & Uchida, 1964), also indicating cleavage of the P-O–aryl bond.

Studies with seven organophosphorus pesticides containing sulfur in a thioether group indicated that exposure to UV light (254 nm) resulted in a variety of oxidation products (Mitchell et al., 1968). With phorate, disulfoton, and thiometon the corresponding sulfoxides and sulfones were identified as products of UV irradiation. With thiometon evidence of oxidation of the thiono sulfur was also obtained. In all cases the oxidation products were more toxic than the parent compound (see *Thioether oxidation*, above).

Ultraviolet irradiation of a carbethoxy analogue of mevinphos results in another type of photoisomerization (Fig. 12) (Casida, 1955). Starting with either the *cis* or the *trans* isomer or a mixture of the isomers and exposing the compounds to UV light resulted in a mixture of approximately 30% of the *cis* and 70% of the *trans* isomer. In all cases the *trans* isomer was predominant.

When Dursban † is exposed to UV light or sunlight it undergoes hydrolysis in the presence of water to liberate 3,5,6-trichloro-2-pyridinol (Smith, 1968), which then undergoes complete photodechlorination with the formation of diols, triols, and tetraols.

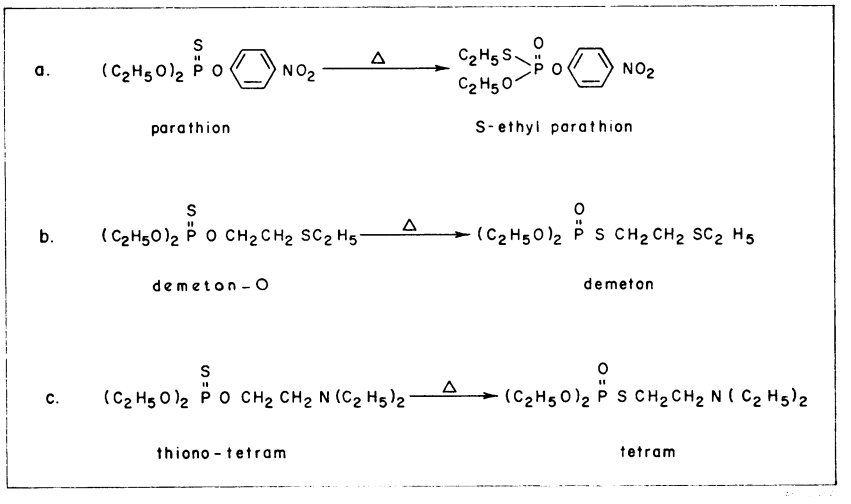
The effect of light, therefore, can be responsible for oxidation of thiono sulfur as well as thioether groups, isomerization of the thiono sulfur and isomerization across double bonds, as well as hydrolysis and dehalogenation.

Temperature

The first report of thermal isomerization of an alkyl phosphorothioate was published by Emmett & Jones (1911), who reported that *O,O,O*-trimethyl phosphorothioate, when heated in a sealed tube at 150°C, isomerized to *O,O,S*-trimethyl phosphorothioate. Subsequent studies with insecticidal organophosphates also indicated that heat modifies the biological activity.

Metcalf & March (1953) found that when parathion was heated at 150°C for 24 hours, 8 breakdown products were formed. Five of the compounds were identified: parathion, paraoxon, *p*-nitrophenol, bis(*p*-nitrophenyl) thionophosphate, and—the principal product—*S*-ethyl parathion (Fig. 13, reaction a). Thermal isomerization has also been demonstrated for malathion, parathion-methyl, the diisopropyl homologue of parathion, EPN † (Metcalf & March 1953); Chlorthion,† diazinon (Augustinsson & Jonsson, 1957); and fensulfothion (Benjamini et al., 1959a). In each case the *S*-alkyl isomer showed greater *in vitro* anticholinesterase activity than the

Fig. 13
Thermal isomerization



parent compound. Heating insecticidal organophosphates above 200°C also results in decomposition. With parathion-methyl, isomerization to the *S*-methyl isomer is followed by the generation of dimethyl sulfide and sulfur dioxide and a mixture of poly(aryl metaphosphates), which decompose to a carbonaceous residue that is explosive (McPherson & Johnson, 1956). Parathion-methyl, malathion, Chlorthion,† and dicapthon † may also decompose at temperatures between 65°C and 115°C. However, the time for decomposition is measured in days rather than minutes.

A second type of thermal isomerization involves compounds containing sulfur or nitrogen in the side chain. *O*-dialkylaminoethyl and *O*-alkylthioethyl dialkyl phosphorothioates isomerize readily to yield the *S*-2-ethylthioethyl and *S*-2-dialkylaminoethyl isomers (Fukuto & Metcalf, 1954; Fukuto & Stafford, 1957; Tammelin, 1957) (Fig. 13, reactions b and c). No evidence was found for *S*-ethyl or *S*-methyl isomerization. It is generally accepted that this type of isomerization probably proceeds via a cyclic intermediate and results in an increase in toxicity as well as an increase in the anticholinesterase properties.

Simple alkyl and aryl phosphates also decompose under the influence of heat. A temperature of 100–200°C is required before the extent of decomposition becomes significant. Two types of reaction are shown in Fig. 14. In the first reaction, heating a diaryl alkyl phosphate results in the formation of an unsaturated aliphatic hydrocarbon and a diaryl phosphate. For this type of thermal decomposition, it is postulated that an available hydrogen is necessary on carbon number 2 for the formation of a 1-alkene (Gamrath et al., 1954). For the second type of reaction, compounds containing a pyrophosphate bond tend to undergo dissociation and rearrangement on heating. This reaction is catalysed by acids, which are readily formed by hydrolysis if a trace of water is present, and is known to occur with TEPP.

Heat may be extremely important in the manufacture, purification, and storage of organophosphorus insecticides. This effect of temperature may increase—or, with some compounds, decrease—the toxicity. From this limited discussion it is obvious that the effect of temperature on organophosphorus compounds is extremely complex—both chemically and biologically.

Air

It is generally recognized that a component of air, oxygen, is required for many biological reactions catalysed by the mixed-function oxidases. Generally, it has been assumed that the conversion of P=S to P=O compounds was strictly a biological reaction. However, when dimethoate was exposed to air in the absence of UV light, dimethoxon was rapidly formed. This reaction occurred on leaves of cotton, potato, and maize (corn) and on glass plates (Dauterman et al., 1960). Koivistoinen & Meriläinen (1963) also showed that when parathion was exposed as a thin film, trace amounts of paraoxon were formed in the absence of light.

Solvents

Various types of solvent may have a marked effect on the stability as well as the toxicity of organophosphorus compounds. It is generally recognized that in aqueous solution most organophosphorus esters are readily hydrolysed. Under acid or neutral conditions the alkyl–oxygen bond is attacked, while in alkaline conditions the phosphorus–oxygen bond is ruptured. The hydrolysis products are generally less susceptible to further

Fig. 14

Effect of heat on organophosphorus compounds

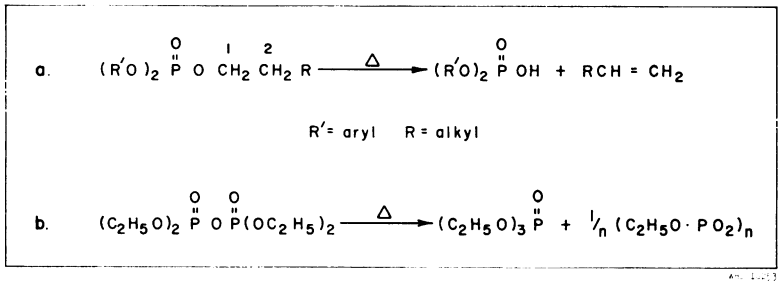
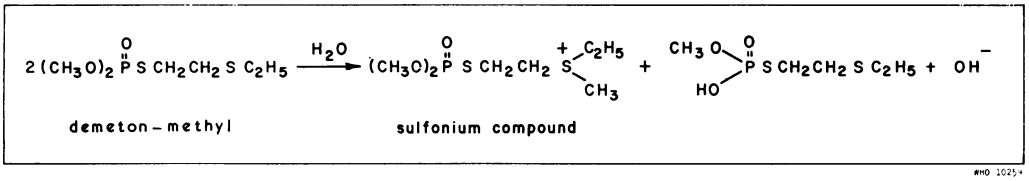


Fig. 15

Transalkylation *



* Heath & Vandekar (1957). The intravenous LD_{50} for rats is 65 mg/kg of demeton-methyl and 0.06 mg/kg of the sulfonium compound.

degradation and are markedly less toxic to insects and mammals (O'Brien, 1960). A number of agents such as amino acids, hydroxylammonium derivatives, and metal ions such as Cu^{++} catalyse the hydrolysis of the phosphorus esters.

Solvents are also used in formulating organophosphates to obtain properties that will increase the chances of contact between the insecticide and the pest. Casida & Sanderson (1963) found that dimethoate increases in toxicity on storage in certain hydroxylic solvents, particularly 2-alkoxyethanols. The oral LD_{50} for the rat decreased on storage from 150–250 mg/kg to 30–40 mg/kg after 7 months at normal temperatures. Studies indicated that 14 phosphorus-containing metabolites were formed in the presence of methyl Cellosolve. The degradation involved hydrolysis of the amide bond, hydrolysis of all the ester groups, and loss of the thiono sulfur. The most toxic fraction was identified as an *O,O*-dialkyl *S*-(*N*-methylcarbamoylmethyl) phosphorothioate with probably one, but possibly both, of the methyl groups replaced by 2-methoxyethyl groups. No evidence was obtained for the formation of pyrophosphates. The toxicity of a few other phosphorothioate insecticides also increased in the presence of 2-methoxyethanol.

Another type of reaction occurs when organophosphorus compounds containing a secondary sulfur are stored undiluted or in an aqueous solution. This reaction involves one molecule of the compound alkylating another (Fig. 15). Heath & Vandekar (1957) observed that a 1% solution of demeton-methyl increased in toxicity spontaneously at 35°C during the course of one day. This increase was due to the formation of the sulfonium ion, whose toxicity is more than 1 000 times that of the parent compound. A similar reaction has also been shown to take place with demeton-O. Samples of demeton-methyl that have been stored for a few months may contain up to 4% of the sulfonium compound. The transalkylation is extremely rapid with demeton-methyl and slower with demeton.

CONCLUSIONS

From this brief review it is obvious that our knowledge of the different reactions involved in the biological and nonbiological modification of organophosphorus compounds is advancing but is far from complete. Recent *in vitro* studies, especially those involving the mixed-function oxidases, have helped to elucidate some of these reactions. However, a number of different enzymatic reactions can produce the same metabolites *in vitro* (i.e., oxidative dearylation, hydrolysis, and aryl transfer). Therefore, it is difficult to assess the importance of the reaction *in vivo*. Further work is needed to evaluate these reactions and determine their relative importance *in vivo* in both target and nontarget organisms.

The metabolic fate of many of the leaving groups of organophosphorus compounds has only been partially investigated. Since in many instances the basic anticholinesterase activity has been destroyed by both environmental and biological reactions, it has generally been assumed that the heterocyclic, aryl, and alkyl leaving groups are nontoxic in the environment. Further research is needed to clarify the importance of these chemical breakdown products as possible hazards in the environment and the extent to which they may accumulate therein.

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