an amino group. Data also indicated that methyl parathion, a phosphorothioate, was not only a poor ChE inhibitor but also almost nontoxic to houseflies at the test concentration without proper biological oxidation (Table VII).

Other pyrethrin synergists, such as piperonyl butoxide, sulfoxide, and propyl isome, also synergized the toxicity to houseflies of pyrethrins, SD 2966, SD 5656, and SD 3562, and reduced the toxicity of methyl parathion (Table III). The same mode of action on certain organophosphorus and chlorinated insecticides may occur to all four synergists, each of which has an active methylenedioxyphenyl group.

Some of the exceptional cases may be explained by the stabilizing effect of synergists on some insecticides and their joint action. For example, Phosdrin, methyl paraoxon, dieldrin, and DDT (Table IV), which are probably not affected by the mode of action of sesamex under discussion, have shown low increases in toxicity when mixed with sesamex. In other cases, Guthion, isodrin, and SD 2642 (Table IV) did not show decreases in toxicity in their sesamex mixtures. This may be explained by the degree of changes, the toxicity of oxidized products, and the effects of sesamex on the stabilization and penetration of toxicants and their oxidized products. In order to explain these factors more fully it is necessary to study each case individually.

Metcalf (*6*) made an excellent review on the synergism between pyrethrins and pyrethrin synergists. The general opinion was that synergists prevented the detoxification of pyrethrins in insects. Although the toxicity of pyrethrum would be greatly reduced under light and in air, and antioxidants have been widely used to prevent the deterioration of pyrethrum and its extracts, enzymic hydrolysis, rather than enzymic oxidation, was considered by most authors as a possible cause for detoxification. On the basis of the present study on synergistic and antagonistic action of pyrethrin synergists, a similar degree of synergistic action of four synergists to pyrethrins and three organophosphorus insecticides (Table III), their high synergistic action against houseflies, the inhibition of biological oxidation of methyl parathion, a phosphorothioate (Table VII), and of aldrin to dieldrin (Table VIII) lead to the speculation that pyrethrins may also be detoxified by biological oxidations and that the synergism produced by pyrethrins and synergists may be due to the inhibition of such oxidation.

Results on pea aphids and two-spotted spider mites were somewhat different from those on houseflies. Mixtures containing SD 2966–sesamex and SD 3562–sesamex gave only low increases in toxicity (Table VI). This relatively low order of increase was also reported in the literature on pyrethrins-synergist combinations against many species other than houseflies. However, the reduction in toxicity of methyl parathion or parathion was of a similar order for houseflies (Table IV) and pea aphids (Table VI). These indicated that aphids and/or mites, as compared to houseflies, reacted differently to SD 2966–sesamex or SD 3562-sesamex combinations but reacted similarly to parathion-sesamex mixtures. In other words, synergists may affect two or more biological oxidation systems which may be associated with oxidative enzymes. One may be associated with the oxidation of organic thionophosphorus compounds and possibly certain cyclodiene compounds, and others may act more specifically on compounds containing an amino or an amido group. The exceptions to this generalization are SD 2438 and schradan (Table IV), the amido group of which is attached to a phosphorus rather than a carbon atom. This difference may be related to the fact that contrary to the oxidation of schradan into a more active compound (*2*), other organophosphorus compounds containing an amino or an amido group are probably detoxified by certain biological oxidations.

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Metabolism of Insecticides by Various Insect Species

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A NUMBER of reviews on insect resistance to chemicals have appeared in the last decade (*4, 13, 14, 17, 27, 29, 40, 54, 73*). Several reviews have covered in detail the metabolic fate of insecticides in various insect species. This paper discusses only the highlights of this problem—i.e., what happens to the insecticide after it has penetrated the insect's tissues.

Like most other animals, insects must degrade or chemically alter a large variety of compounds to maintain their normal body functions. It is not surprising, therefore, that many foreign compounds, including poisons, are attacked in the metabolic process.

The fact that insects differ in their response to a chemical indicates the presence of inheritable variations arising from differences in the genetic constitution of individuals within a population or between populations of different origins. Our modern genetic theories postulate that genes, or units of inheritance, function in directing enzyme specificities, which, in turn, catalyze the innumerable biochemical reactions in the body. From a biochemical standpoint, it appears that resistance to insecticides results from the selection of those variants that can cope with the chemical more efficiently. The mechanisms by which insects accomplish these protective feats are discussed below.

Detoxication mechanisms involving

Most chlorinated hydrocarbon insecticides and many organophosphorus compounds are metabolized by insect species. The metabolic processes that bring about these chemical changes may be classified as "activating" and "detoxifying." Activating mechanisms usually involve epoxidation reactions, such as conversion of heptachlor to heptachlor epoxide and aldrin to dieldrin; or oxidation reactions, such as conversion of thionophosphates to phosphates, oxidation of thiol ethers to sulfoxides and sulfones, and oxidation of phosphoramides to more potent cholinesterase inhibitors. Detoxifying processes may convert insecticide to nontoxic metabolities, which are retained in the tissues or rapidly excreted. Detoxication of organophosphorus compounds in most cases involves hydrolytic reactions. The type of change is dependent on the chemical structure of the compound and the insect species—DDT is metabolized by the housefly, body louse, certain mosquitoes, American roach, Mexican bean beetle, boll weevil, milkweed bug, fruit fly, etc., but this process follows four or five metabolic pathways. Many of these reactions are enzymatically catalyzed.

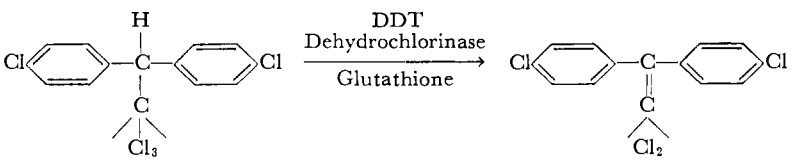
some insecticides and noninsecticidal compounds have been reviewed in detail by Smith (*60*). Prominent among them are (1) conjugation processes, such as the formation of hippuric acid from benzoic acid, formation of etheral sulfates and β-glucosides, acetylation, and methylation; (2) oxidation reactions, such as aliphatic oxidation, aromatic oxidation and epoxidation, hydroxylation of aromatic rings, and oxidation of thiophosphate and pyrophosphoramide insecticides; (3) reduction; (4) dehydrochlorination of insecticides; and (5) detoxication of heavy metal poisons by reaction with sulfur compounds. The present discussion is concerned primarily with the metabolism of chlorinated hydrocarbon insecticides and organophosphorus compounds.

Halogenated Hydrocarbon Insecticides

DDT. Since the appearance of DDT resistance in the housefly in 1946, numerous investigations have been undertaken to determine its cause. Among the many theories advanced, the most popular explanation was the finding that houseflies were able to convert DDT [1,1,1-trichloro - 2,2 - bis(*p* - chlorophenyl)-ethane] to the nontoxic derivative 1,1 - dichloro - 2,2 - bis - (*p* - chlorophenyl)-ethylene (DDE) (*34, 57, 63, 67, 76*).

Many investigators have confirmed this finding and have variously demonstrated that both susceptible and DDT-resistant houseflies are able to convert DDT to DDE, or that only the resistant strain is capable of accomplishing this conversion (*21, 57*).

Detoxication of DDT by houseflies has long been considered a major factor in the defense against the lethal action of this insecticide. This hypothesis was greatly strengthened by the isolation of the enzyme DDT-dehydrochlorinase (*64, 65*), which in the presence of glutathione catalyzes the dehydrochlorination of DDT in resistant houseflies according to the following reaction:



Recently, DDT-dehydrochlorinase has been isolated from susceptible houseflies also, but in much smaller titer (*33*). The enzyme has a pH optimum of 7.4 and a temperature optimum of 37° C. and requires glutathione for activation. It also catalyzes the dehydrochlorination of 1,1 - dichloro - 2,2 - bis - (*p* - chlorophenyl)-ethane (DDD) to 2,2-bis-(*p*-chlorophenyl)-chloroethylene. It does not attack *o,p'*-DDT, indicating some degree of specificity with regard to position and orientation of certain halogen groups in the molecule. DDT-dehydrochlorinase also has been isolated from Mexican bean beetle larvae and pupae (*19, 66*).

Enzymic breakdown of DDT has been demonstrated in the human body louse (*55*). The crude enzyme which has been isolated from susceptible and DDT-resistant body lice has a pH optimum of 9 to 9.5, is stable at high temperature, can withstand digestion by proteolytic enzymes without loss of activity, and may be activated with glutathione, cysteine, thioglycollic acid, ascorbic acid, or coenzyme A. The metabolite resulting from DDT breakdown in the body louse is a water-soluble acidic conjugate, which yields a pink complex when analyzed by the method of Schechter *et al.* (*59*).

Many other insects are capable of converting DDT to one or more metabolites. A fairly complete list showing quantitative data on the metabolism of DDT by various insects has been compiled (*53*). DDT metabolism follows at least five different metabolic pathways, as shown in Figure 1.

It is assumed that catalytic reactions involving the same substrate and yielding similar metabolic end products should be mediated by the same enzyme system. Several species of DDT-resistant mosquitoes, such as *Aedes aegypti*, *Aedes taeniorhynchus*, *Anopheles sacharovi*, and *Culex fatigans* have been shown to convert large amounts of DDT to DDE in vivo (*5, 15, 52*) but so far all attempts to isolate the mechanism in vitro have failed (*15, 52*). This indicates species specificity with regard to the enzymatic system involved.

Figure 1 shows that many intermediate or end products of DDT metabolism have not been identified yet. Judging

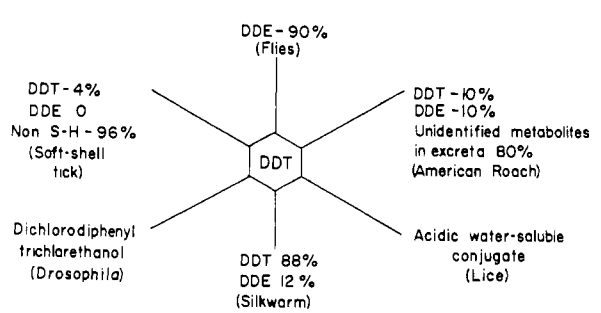


Figure 1. Pathways of DDT metabolism as illustrated in various insect species

from extraction procedures used, it would appear that many of the polar substances isolated are metabolite conjugates. The nature of the conjugation, however, has not been determined. The metabolites that have been identified include DDE in the housefly, certain mosquito species, the tent caterpillar, the European corn borer, the cabbage worm, the Mexican bean beetle, the red banded leafroller, and two grasshopper species (*53*). The metabolite *p,p'*-dichlorobenzophenone was detected in the excreta of DDT-treated American roaches (*28*), and 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)-ethanol was found in two species of *Drosophila* after they were exposed to DDT (*68*).

Benzene Hexachloride (BHC)

The weight of evidence and agreement that is nearly general among experimenters who have worked with this compound is that gamma-BHC (lindane) is metabolized by the housefly into polar metabolites, which are rapidly excreted.

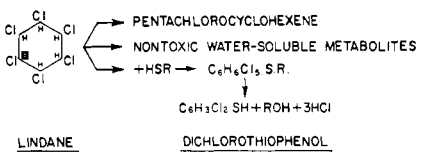
An early work (*7*) showed that pupae and adult houseflies originating from larvae reared in a gamma-BHC medium contained significant amounts of unchanged insecticide, which indicated the absence of an efficient detoxication mechanism. On the other hand, injection of gamma-BHC into resistant and susceptible houseflies (*49*) resulted in rapid breakdown of the chemical in the resistant strain and slower metabolism in the susceptible strain.

It has been demonstrated that metabolism of the alpha-, beta-, gamma-, and delta- isomers of BHC is faster in resistant than in susceptible houseflies (*8, 50*).

Using spectrophotometric and chromatographic methods, it was shown (*62*) that the first product of gamma-BHC metabolism was pentachlorocyclohexene. It was considered to be an intermediate metabolite, because the amount of gamma-BHC in the housefly decreased with an increased time interval but the metabolite did not increase in proportion. Pentachlorocyclohexene was shown to be metabolized further to unidentified products by resistant and susceptible houseflies. The enzyme DDT-dehydrochlorinase was not involved in lindane metabolism. Other studies using C^{14}-labeled alpha-, gamma-, and delta-BHC (*11*) showed that similar amounts of 1-chloro-2,4-dinitrobenzene occurred in houseflies treated with any of the three isomers, amounting to about 17% of the absorbed dose.

However, when a more specific isotope dilution technique was used, approximately 1.5% of the gamma-BHC absorbed by resistant and susceptible houseflies was detected as γ-pentachlorocyclohexene (*9*). The larger amount of 1-chloro-2,4-dinitrobenzene present was attributed to other possible metabolites giving rise to chlorobenzene on reduction. These and other experiments (*11*) suggested that chlorodinitrobenzene arises from three sources: chlorobenzene, occurring as a trace metabolite; some water-soluble metabolite which under reducing conditions gives rise to chlorobenzene; and pentachlorocyclohexene. The absence of larger amounts of pentachlorocyclohexene led to the conclusion that monodehydrochlorination is not the first step in a major pathway for the metabolism of gamma-BHC in houseflies. Quantitative data on the metabolism of gamma-BHC in several insect species (*6*) indicated that the housefly is unique in that respect, in that it possesses an efficient detoxifying mechanism before any selection pressure is applied.

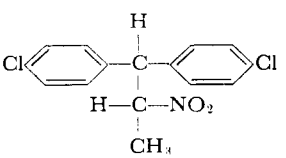
The latest experiments on the metabolism of gamma-BHC (*10*) have shown that alkaline hydrolysis of the metabolic products of alpha- or gamma-BHC yields dichlorothiophenols. It was inferred that the first step in the metabolism of BHC involves the removal of one chlorine atom and the formation of a C—S bond, followed by further dehydrochlorination and the subsequent formation of dichlorothiophenol according to the following scheme:



The nature of the RSH group has not been determined. However, when the procedure used for measurement of DDT-dehydrochlorinase in houseflies was followed in detail (*65*), it was shown that in vitro conversion of alpha- or gamma-BHC into water-soluble metabolites requires reduced glutathione for activation of the enzyme. Thus, gluthathione might be the source of sulfur for the C—S bond.

Prolan

Prolan [1,1-bis-(*p*-chlorophenyl)-2-nitropropane] is one of the two constituents of the insecticide Dilan, the other constituent being Bulan [1,1-bis-(*p*-chlorophenyl)-2-nitrobutane]. Although Prolan contains no chlorine atoms in the propane moiety, Prolan-resistant houseflies were shown to metabolize this insecticide readily into a neutral compound and an acidic derivative, both of which were excreted (*56*).



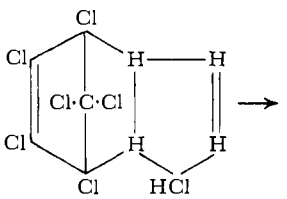
→ Neutral compound similar to Prolan

→ Acidic metabolite, possibly bis-(*p*-chlorophenyl) pyruvic acid

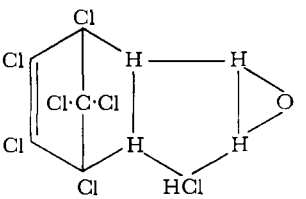
The neutral metabolite was soluble in common organic solvents, was similar to Prolan in infrared, ultraviolet, and colorimetric absorption spectra, and was almost as toxic to mosquito larvae as the parent compound. The acidic metabolite was extractable with dilute alkali, was different from Prolan in photometric absorption spectra and in chemical properties (showing loss of the NO_2 group in the propane moiety), and was much less toxic to mosquito larvae. Assessment of the relative rate of formation of the two metabolic products indicated that excretion of the toxic material was more important to the fly's survival in the initial stages of poisoning.

Cyclodiene Insecticides

Heptachlor. Topically applied, heptachlor was rapidly absorbed by resistant houseflies and converted to heptachlor epoxide (*58*).



Heptachlor

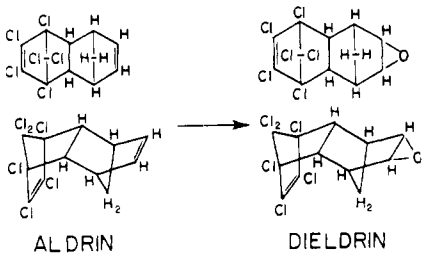


Heptachlor epoxide

Total recovery at the 24-hour interval after application, or beyond this period, did not exceed 53% of the amount applied. More recent information indicates that the missing portion is volatilized from the surface of the fly. The heptachlor epoxide extracted from houseflies was found to be identical in physical and chemical properties with an authentic sample of heptachlor epoxide melting at 159–60.5° C. It also was as toxic as heptachlor to susceptible houseflies and to mosquito larvae. Other experiments showed that the onset of symptoms of poisoning, following application of heptachlor to susceptible houseflies, coincides with the appearance of

heptachlor epoxide in the tissues. From these and other results, it was inferred that poisoning resulted from this conversion—a process which might be called "autointoxication." Neither the synthesized epoxide nor that recovered from houseflies was further metabolized.

Aldrin and Dieldrin. The metab-



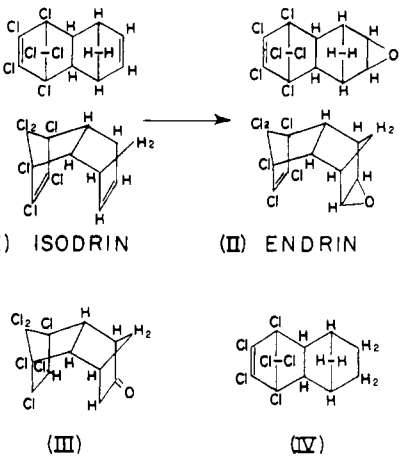
olism of aldrin in insects follows much the same pattern as that of heptachlor. Topical application or injection of aldrin into the American cockroach resulted in partial conversion of the aldrin to the corresponding epoxide, dieldrin (*24*). Tests with dieldrin-resistant houseflies (*70*) showed that more than 80% of the Cl^{38}- or C^{14}-labeled aldrin absorbed was converted to dieldrin within 24 hours following topical application of 5 to 10 γ of aldrin per fly. Conversion to dieldrin was virtually complete at the end of 96 hours. From 30 to 40% of the total radioactivity was lost by volatilization of the aldrin. This radioactivity was recovered by trapping the air from the holding container and assaying in a liquid scintillation counter.

Topical application of dieldrin to resistant or susceptible houseflies resulted in almost quantitative recovery of the unchanged chemical as long as 4 days after application. Eight to 12% of the dosage applied was found in the container, and this might be due to rub-off, egestion, and excretion.

Susceptible houseflies also convert aldrin to dieldrin, and the onset of symptoms of poisoning after a latent period of 2 to 4 hours coincides with the appearance of dieldrin in the tissues. From this observation, it might be inferred that toxicity is due to this conversion. On the other hand, it has been shown (*72*) that the reduction product (IV) of aldrin, which cannot be oxidized, is still toxic to houseflies. This finding suggests that the aldrin type of molecule has an intrinsic toxicity independent of its conversion to the epoxide.

The sulfur analog of dieldrin (S^{35}-labeled) was shown to be partly metabolized by resistant and susceptible houseflies (*74*). In addition, approximately 3% of the dosage applied was excreted unchanged, and 1 to 2% was excreted as water-soluble and insoluble metabolites. Twenty-seven to 34% of the radioactivity was lost by volatilization of the compound. A bromine analog of dieldrin (Br^{82}-labeled) was shown to be excreted unchanged in equal proportion by both resistant and susceptible houseflies (*74*). Small amounts of water-soluble metabolites were also produced.

Isodrin and Endrin. Topically



applied isodrin (I) and endrin (II) (C^{14}-labeled in the terminal unchlorinated ring) were found to be less toxic than aldrin and dieldrin to susceptible houseflies but more toxic to dieldrin-resistant houseflies. Both strains of houseflies converted isodrin to the corresponding epoxide endrin. Small amounts of endrin also were recovered in the external rinse. Endrin was not formed in the tissues of heat-killed insects, suggesting an enzymic epoxidation (*72*). Acetone extracts of live houseflies treated with isodrin or endrin contained small amounts of a nontoxic water-insoluble product, which behaved as a ketone (III) derivative of endrin. There was no evidence that radioactive material was excreted.

Chlordan and Toxaphene. Very little is known about the metabolic fate of chlordan and toxaphene in insects. The paucity of data might be due to lack of sensitive analytical methods (on a microgram scale) for toxaphene, and the fact that chlordan is not a pure chemical but is composed of at least five constituents, three of which are toxic to houseflies and other insects (*20, 35*).

Bioassay techniques for the estimation of chlordan and toxaphene have shown (*25*) that 74% of the absorbed toxaphene and 86% of the absorbed chlordan were metabolized to nontoxic derivatives within 24 hours following topical application of the chemicals.

Using Davidow's colorimetric procedure for chlordan (*20*), it was found (*69*) that 72 hours following topical application of chlordan to chlordan-resistant German roaches, 31% of the 100-γ dose per individual roach was unabsorbed, and 20% was recovered unchanged in the tissues. Forty-nine per cent remained unaccounted for and, hence, might be presumed to have been metabolized. No chlordan was found in the excreta.

Pyrethrins

The reversal of paralytic symptoms and the knockdown induced by pyrethrum suggest that insects may possess a detoxifying mechanism capable of attacking the compound at certain reactive sites. It was first suggested (*1*) that hydrolytic enzymes such as esterases might be involved in decomposing pyrethrins to nontoxic products.

Detoxication of pyrethrins was demonstrated first in the southern army worm by bioassaying tissue extracts of the treated insects against mosquito larvae (*77*). Greatest detoxication in vitro was brought about by fat body, followed by skin and muscle, digestive tract, and blood. Lipase extracts of roaches and houseflies readily hydrolyzed pyrethrin esters to nontoxic derivatives (*18*). Piperonyl butoxide (a pyrethrum synergist) inhibited lipase activity to some extent, and, consequently, detoxication of pyrethrins was diminished.

The American roach has been shown to hydrolyze C^{14}-labeled pyrethrins and cinerins to the corresponding keto alcohols and chrysanthemum monocarboxylic acids, plus unchanged esters, and several unidentified metabolites (*78*). Eight to 12% of the radioactivity was excreted as $C^{14}O_2$.

Houseflies were shown to metabolize significant amounts of C^{14}-pyrethrins and allethrins to nontoxic substances (nonpyrethroid derivatives) within 24 hours after application (*75*). Characterization of metabolites was shown by reversed phase paper chromatography (*72*). The synergist piperonyl cyclonene inhibited the detoxication of pyrethrins more than that of allethrin (*75*). This finding led to the conclusion that the mechanism of detoxication of pyrethrins and allethrins might differ in some respects. No C^{14} was lost by excretion or by expiration of $C^{14}O_2$.

An investigation of the metabolic fate of C^{14}-labeled allethrin (C^{14} on the chrysanthemum monocarboxylic acid moiety) in the housefly (*26*) showed that allethrin was rapidly absorbed, metabolized, and excreted. Twenty-four hours after treatment approximately 44% of the absorbed dose was excreted. Piperonyl butoxide depressed the rate of excretion, and this might account for the increase in mortality. Analysis of fecal and tissue extracts by paper chromatography demonstrated the presence of large amounts of a polar compound with the same R_f value as that of allethrolone. This indicates changes in the molecule other than those caused by hydrolysis of the ester linkage. If hydrolysis occurs, it indicates further degradation or conjugation of the free acids.

The above studies suggest somewhat differing metabolic pathways for pyrethrins and allethrins and differing detoxication mechanisms in houseflies and roaches.

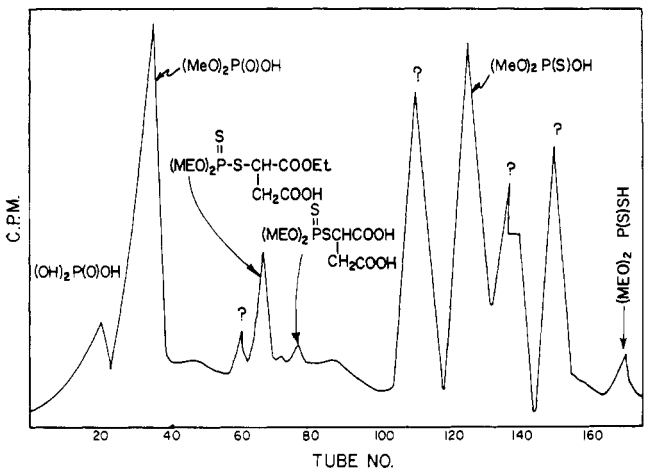


Figure 2. Ion exchange chromatography of metabolites 4 hours after application of P^{32}-malathion to housefly

Organophosphorus Insecticides

Several excellent reviews on the metabolism of organophosphorus compounds in insects, mammals, and plants have appeared in the last decade (*16, 22, 38, 39, 61*).

To be effective as an insecticide, an organophosphorus (OP) compound must first possess sufficient stability and suitable physiochemical properties to be absorbed and transported to the site of action. A second prerequisite is that the compound must be sufficiently unstable to act as a reactive phosphorylating agent. When these conditions are met, the OP compound will, at the site of action, combine with and inhibit a vital enzyme, cholinesterase, although other esterases have been implicated in the poisoning process and other modes of action have been suggested.

The metabolic fate of many of the OP compounds follow two general types of reactivity: metabolic intoxication of nontoxic or moderately toxic compounds to more active derivatives, and metabolic detoxication leading to destructive hydrolysis.

Metabolic intoxication may be brought about by oxidation of esters containing P=S groupings to P=O derivatives, such as occur in parathion, malathion, EPN, Thimet, diazinon; oxidation of thiol ether —S— to sulfoxides —S(=O)— and sulfones —S(=O)(=O)—, as in Systox, Di-Syston, and Trithion; and oxidation of —N—CH_3 to —N(=O)—CH_3 groups followed by rapid rearrangement to the methylol derivative —N—CH_2OH, as in the weakly cholinergic phosphoramides schradan and dimefox. These enzymatic oxidations result in the establishment in the molecule of an electrophilic center, which increases the instability and the consequent phosphorylating ability of the compound.

Other types of activation also occur. For example, *O,O*-dimethyl-2,2,2-trichloro - 1 - hydroxyethyl phosphonate (Dipterex) is dehydrochlorinated to the more active anticholinesterase, *O,O*-dimethyl-2,2-dichlorovinyl phosphate (DDVP). In fact, it has been demonstrated that the toxicity of Dipterex is due to its conversion to DDVP (*41*). This dehydrochlorination process does not involve the enzyme DDT-dehydrochlorinase (*61*).

It is evident that the toxicity of an OP compound to an organism will be markedly affected by the presence of enzymes capable of metabolizing the compound to inactive products. Metabolic detoxication is largely accomplished by base-catalyzed hydrolysis, which generally affects the labile bond broken during phosphorylation of the enzyme, leaving a dialkyl phosphoric acid ester and an alcohol or a phenol. In mammals, this hydrolytic attack is mediated by specific enzymes such as DFP-ase, which hydrolyzes diisopropyl fluorophosphate; tabunase, which splits CN^- from tabun; A-esterase, which hydrolyzes paraoxon; TEPP-esterase, etc. In insects, an aromatic esterase has been shown to hydrolyze parathion and paraoxon to liberate *p*-nitrophenate ion (*43*).

Malathion, *S*-[1,2-bis(ethoxycarbonyl)ethyl]*O,O*-dimethyl phosphorodithioate. Detoxication of OP compounds also may involve enzymatic hydrolysis of the carboxylic ester groups, either before or after oxidation. For instance, malathion appears to be degraded and rapidly excreted by the housefly through intermediates involving hydrolysis of the diethyl succinate nucleus and hydrolysis of the P—S and S—C bonds, as shown in Figure 2. In the American roach, this type of metabolism is less extensive, as judged by the limited number of metabolites resolved by paper chromatography (*36*).

This indicates that in the cockroach, activation (oxidation) is more rapid than hydrolysis, and the oxidation product malaoxon presumably accumulates to a lethal level (*44*). Later studies (*31*) have shown extensive degradation of malathion in the American roach, German roach, and housefly. This process involves at least two major degradative pathways: that due to phosphatases attacking P—S—C bonds, and that owing to carboxyesterases attacking the $COOC_2H_5$ moiety (cf. Figure 2). In the cockroach, both processes are about equal, but phosphatase attack predominates in the housefly.

With comparable doses of P^{32}-malathion, there seems to be little difference between susceptible and malathion-resistant houseflies in rate of excretion of nontoxic metabolites, but the tissues of susceptible houseflies contain a higher titer of a toxic compound that is not malathion (it is presumably malaoxon) (*71*).

Parathion, *O,O*-diethyl *O*-*p*-nitrophenylphosphorothioate. The toxicity of parathion to the American roach has been shown to be due to the conversion of parathion to paraoxon (*42*). The accumulation of paraoxon in susceptible houseflies but to a much lesser extent in parathion-resistant houseflies has been demonstrated in three strains of varying susceptibility (*51*). The failure of resistant houseflies to accumulate paraoxon might be attributable to slow rate of activation of parathion, rapid rate of degradation of parathion, and rapid rate of detoxication of malaoxon. As resistance to parathion extended also to paraoxon, it must be assumed that protection was afforded by the more rapid elimination of paraoxon.

Diazinon, *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate. Diazinon-resistant houseflies were shown to display a 40-fold resistance, as compared with a normal strain. Practically no difference was found in cuticle permeability from topical application of Diazinon. The level of Diazoxon (the oxidation product of Diazinon) was 2.5-fold higher, and the chloroform-extractable material 3-fold higher in normal than in resistant houseflies (*32*). These differences appear to be small in comparison with the magnitude of resistance demonstrated, and other factors than metabolism of the compound may play a vital part in the final toxicity.

After injection of Diazinon into the American roach at a rate of 30 γ per gram, the chloroform-extractables, which contain the toxic compounds, decreased slowly, indicating a slow degradative capacity. The P=O analog constituted

3 to 7% of the chloroform-extractables (*32*). In all probability the slow degradative process was due to the high dosage administered, as compared with the LD_{50} of only 0.75 γ per gram, for at a lower dosage, the degradative capacity was much more pronounced.

Dipterex, *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate. The metabolism of P^{32}-labeled Dipterex, its acetyl derivative (*O,O*-dimethyl 2,2,2-trichloro-1-acetoxyethyl phosphonate), and its vinyl derivative formed on dehydrochlorination (*O,O*-dimethyl 2,2-dichlorovinyl phosphate, DDVP) has been investigated in plants; in the dog and rat; and in the housefly, American roach, and several other insects (*2*). Of the three compounds, the vinyl derivative (DDVP) was generally the most toxic, but it was less selectively toxic than the two phosphonates. A large proportion of the Dipterex applied to the housefly was hydrolyzed, but no other P^{32}-containing metabolites could be detected by solubility characteristics, change in anticholinesterase activity, or permanganate-reducing characteristics. Rate of detoxication of DDVP by the housefly was slower than that of Dipterex. In vivo studies with two species of roaches failed again to demonstrate the presence of a vinyl derivative resulting from Dipterex metabolism. Furthermore, the anticholinesterase activity of Dipterex was not affected by incubation with whole roach intestines. Thus, no evidence was obtained that the toxicity of Dipterex was due to dehydrochlorination and rearrangement to the more active anticholinesterase DDVP, or that the in vivo detoxication rate can explain the relative susceptibility of houseflies to the two compounds.

Homogenates of housefly heads, imported cabbageworm heads, rat serum, rat liver, and whole pea aphids rapidly hydrolyzed the acetyl derivative of Dipterex, but failed to hydrolyze Dipterex or DDVP.

The low mammalian toxicity of Dipterex appears to be due to phosphonate hydrolysis by serum esterases and elimination in the urine of the trichloro portion of the molecule as trichloroethyl glucuronide (*2*).

More recent studies (*41*) using P^{32}-labeled Dipterex and DDVP showed that Dipterex is rapidly converted to DDVP under mild alkaline conditions. The reaction showed marked pH dependency ranging from 11% at pH 5.4 to 100% at pH 8.0. In vivo studies of the toxic action of the two compounds to the housefly showed that the rate of knockdown of houseflies feeding on Dipterex-sugar bait was much higher at pH 7.0 than at pH 5.4. This information together with the 4- to 7-fold greater toxicity of DDVP and the isolation by chromatography, anticholinesterase activity, and other techniques, of about 5% of P^{32}-DDVP from Dipterex-poisoned houseflies, strongly indicates that DDVP is responsible for the toxic action of Dipterex.

Delnav, 2,3-*p*-dioxanedithiol *S,S*-bis-(*O,O* - diethyl phosphorodithioate). P^{32}-labeled technical Delnav was separated by partition chromatography into eight fractions. The major components of interest were the cis- and trans-isomers and two minor components, 2-*p*-dioxenethiol *S*-(*O,O*-diethyl phosphorodithioate) and bis-(diethoxyphosphinothioyl) disulfide. The cis-isomer was the most toxic to houseflies and rats in vivo; but the in vitro anticholinesterase activity was least with the cis, trans, and dioxene derivatives, and greatest with the less toxic components. Two hours following topical application of the various fractions to the American roach at a rate of 200 γ per gram, the insects were sacrificed and the unchanged and hydrolyzed portions of the chemical were measured. The hydrolysis products accounted for 47.7% of the total radioactivity in fraction I, 26.2% in the trans- isomer, 17.6% in the cis- isomer, and 38.4% in the dioxene fraction. Thus, the greater toxicity of the cis- and trans- isomers correlates favorably with their greater stability to hydrolysis. Rats treated orally with technical Delnav excreted considerable amounts of hydrolytic metabolites in the urine and smaller amounts in the feces. The hydrolytic products recovered were identified as *O,O*-diethyl phosphoric, phosphorothioic, and phosphorodithioic acids (*3*).

Co-Ral, *O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate. Recently, the metabolism of Co-Ral has been investigated in cattle grubs and houseflies (*48*). Activation of Co-Ral to the oxygen analog, Coroxon, was pronounced in the housefly and less extensive but well evident in the cattle grub. Degradation to nontoxic derivatives was minimal for the insect species mentioned and for the mouse, but was fairly rapid for the ox and rat. Co-Ral is degraded primarily at the P—O—coumarinyl link and to a lesser extent at the ethoxy link (*30*).

Acethion, *O,O*-diethyl *S*-carboethoxymethyl phosphorodithioate. Compared with mouse liver slices, which degrade acethion extremely rapidly, minced housefly preparations showed some activating capacity. Minced cockroach preparations were effective in degrading acethion but were far less effective than mouse liver (*47*). Chromatographic separation of acethion and its metabolic products indicated that the principal metabolite was acethion acid. The degradative activity is reflected in the comparative toxicity of acethion to the three species: $LD_{50's}$ of 15, 375, and 1280 γ per gram for the housefly, the American roach, and the mouse, respectively (*32*). The levesl of the P=O analog of acethion, acetoxon, and the chloroform-extractable material were much higher in the housefly than in the cockroach 0.5 hour after treatment, but these levels reached almost the same value 2 hours after treatment. Throughout the experiment, these levels were lowest in the mouse. Thus, selective toxicity is apparent between insect and mammal but is not too convincing between insect species.

Dimethoate, *O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate. Injection of 0.5 γ per gram of dimethoate in the housefly, cockroach, and mouse resulted in very rapid detoxication in the mouse (89% in 0.5 hour) and much slower degradation in the housefly and cockroach (32 and 16%, respectively). The P=O analog of dimethoate was approximately three times greater in the cockroach than in the mouse. A high degree of selectivity is indicated by the following $LD_{50's}$: 1.0, 1.0, and 140.0 γ per gram by injection for the housefly, the cockroach, and the mouse, respectively (*32*).

Schradan, octamethyl pyrophosphoramide. Scradan is a very weak anticholinesterase in vitro. It is practically nontoxic to certain insects but highly toxic to others (*39*). Schradan is converted by mammalian liver to a potent anticholinesterase. In susceptible insects, it completely inhibits cholinesterase. Tissues of both susceptible and nonsusceptible insect species contain an enzyme system capable of activating schradan. In the American roach—a nonsusceptible species—the fat body, alimentary canal, cuticle, and nerve cord are active in converting schradan to an anticholinesterase (*45*). It has been postulated that susceptibility of insects to schradan depends on activation of the compound within nerve tissue and that in nonsusceptible species the rate of conversion in the fat body is so rapid that little or no unconverted compound reaches the nerve tissue. Furthermore, the converted schradan is so unstable that it fails to reach and penetrate the lipoid sheath (Hoyle sheath) of insect nerve (*45*).

The active product of schradan metabolism in the American roach was found to be similar to one of the permanganate-oxidation products of schradan (*46*). Possible pathways for the hydrolytic breakdown of schradan that could account for its ineffectiveness against many insect species have not been found.

It was previously suggested (*45*) that the enzyme responsible for the conversion of schradan to its oxide might be trimethylamine oxidase. However, further studies showed evidence against the presence of this enzyme in insects (*46*).

Systox (Demeton), *O,O*-diethyl *O*-

(and *S*)-ethyl-2-thioethyl phosphorothioates. The sytemic insecticide Systox consists of two isomers, the thiono isomer (*O*-ethyl-2-mercaptoethyl moiety) and the thiol isomer (*S*-ethyl-2-mercaptoethyl moiety). Both isomers are rapidly metabolized, degraded, and eliminated in the mouse and cockroach by three different biochemical mechanisms (*37*). The biochemical system of primary importance from the toxicological standpoint involves the oxidation of the mercapto sulfur of the ethylthioethyl moiety of both isomers first to the sulfoxide and then to the sulfone. A second important mechanism, which is concerned only with the thiono isomer and its metabolites, involves the oxidation of the thiono sulfur to produce the respective phosphate and its sulfoxide and sulfone. Unlike the more toxic phosphate analogs produced by other OP compounds, such as parathion and malathion, the phosphate metabolites produced by thiono-Systox are of short duration, due perhaps to their rapid hydrolysis (*23*). They are, therefore, of no toxicological importance. The third biochemical mechanism is a degradative process and involves the hydrolysis of the P—O or P—S bonds of the ethylmercaptoethyl moiety to form the nontoxic ionic derivatives diethyl phosphoric acid or diethyl thiophosphoric acid and the respective alcohols.

These mechanisms follow the same pattern in mammals, insects, and plants; but the oxidation and hydrolytic rates are slower in insects than in mammals.

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