It is not known whether this protection is due to the accumulation of a substance that competes with TMPP for active sites on cholinesterase, to a change in membrane permeability barring the entrance of TMPP into esteratic sites, or to the failure of the circulatory system to transport TMPP through the body.

The in vivo protection against TMPP may also be cited as an example of a reversible phenomenon. DDT-prostrate cockroaches tolerated an injected dosage of TMPP that inhibited 90% of the cholinesterase of a normal cockroach, but if the DDT-prostrate cockroaches were allowed to recover even briefly by raising the temperature before injection of TMPP, no protection occurred.

The exact role in the mode of action of DDT for the events discussed is not known. Perhaps some of them are merely secondary, and are a result rather than a cause of poisoning. The primary action of DDT on sensory nerves still remains unexplained. Before there is a complete understanding of the mode of action of compounds affecting neurotransmission in insects, the basic biochemistry and physiology of insect excitatory tissue must be more thoroughly investigated. The chemical structures of compounds apparently involved with either cardiac or neuroactivity must be determined before their role under normal conditions can be understood. Only then can their part, if any, in the mode of action of pesticides be determined.


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# Synergistic and Antagonistic Actions of Insecticide-Synergist Combinations and Their Mode of Action


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Synergistic and antagonistic actions of pyrethrin synergists in combination with many organophosphorus and chlorinated insecticides have been studied on several species of insects and mites. The highest increase in toxicity to houseflies was 38.7 times for a mixture containing 1% sesamex and methyl 3-[ethoxy(p-dimethylaminophenyl)phosphinyloxy]crotonate. Pyrethrin synergists also reduced the toxicity of some organic thionophosphorus and cyclodiene insecticides. Toxicity results as well as colorimetric and enzymic analyses indicate that synergistic or antagonistic action caused by a pyrethrin synergist appears to be mainly due to the inhibition of certain biological oxidations which either activate or detoxify the compounds.


In the study of joint action of insecticides, a true synergistic action resulting from a mixture of two or more insecticides is rarely found. Although high increases in toxicity to houseflies of pyrethrins, allethrin, etc., in combination with pyrethrin synergists (or activators) are well known, only small increases in toxicity have been reported on other species of insects.

Many pyrethrin synergists have been discovered, but little progress has been made on the study of their mode of action. This report includes not only the studies on the synergistic and antagonistic actions of pyrethrin synergists to vinyl phosphates, phosphonates, and other insecticides, but also the elucidation of a possible mode of action.

### Materials and Methods

With the exception of common insecticides and synergists, chemical names

of newer compounds are given in Table I. Purified samples of aldrin (m.p. 103–04° C.), dieldrin (m.p. 175–76° C.), endrin (m.p. 229–30° C.), isodrin (m.p. 234–37° C.), lindane (m.p. 110° C.), DDT (m.p. 107° C.), heptachlor (m.p. 95° C.), heptachlor epoxide (m.p. 163° C.), SD 3110 (m.p. 163–64° C.), SD 3450 (m.p. 256° C.), SD 2642 (m.p. 142.5–44° C.), SD 2648 (m.p. 221–22° C.), methyl parathion and methyl paraoxon, technical grades of DDVP, Amiton-oxalate, phosphamidon, Phosdrin, Diazinon, Guthion, malathion, EPN, parathion, Chlorthion, schradan, pyrethrins (20% concentrate), piperonyl butoxide, sulfoxide, propyl isome, sesamex, and distilled samples of other SD compounds were used for tests. In spray tests the percentage concentration of each toxicant and each synergist was expressed as grams of active ingredient per 100 ml. of solution.

**Table I. Chemical Names of Newer Compounds**

| Compound | Chemical Name |
|---|---|
| SD 2244 | 3-(Diethoxyphosphinyloxy)-*N,N*-dimethylcrotonamide |
| SD 2409 | Ethyl 3-[ethoxy(*p*-nitrophenoxy)phosphinyloxy]-crotonate |
| SD 2438 | Ethyl 3-(dimethylamino)-phenylphosphinyloxy)-crotonate |
| SD 2642 | 5,6,7,8,9,9-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-epoxy-*exo,endo*-5,8-methanonaphthalene |
| SD 2648 | 5,6,7,8,9,9-Hexachloro-1,2,3,4,4a,5,8,8a-octahydro-1,2,3,4-diepoxy-*exo,endo*-5,8-methanonaphthalene |
| SD 2966 | Methyl 3-[ethoxy(*p*-dimethylaminophenyl)phosphinyloxy]-crotonate |
| SD 3110 | 5,6,7,8,9,9-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanophthalazine |
| SD 3450 | 5,6,7,8,9,9-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanophthalazine 2-oxide |
| SD 3562 | 3-(Dimethoxyphosphinyloxy)-*N,N*-dimethylcrotonamide |
| SD 4092 | Benzyl 3-(dimethoxyphosphinyloxy)crotonate |
| SD 4239 | *p*-Chlorobenzyl 3-(dimethoxyphosphinyloxy)crotonate |
| SD 5567 | 4′-Chloro-3-(dimethoxyphosphinyloxy)crotonanilide |
| SD 5656 | *N,N*-Diallyl-3-(dimethoxyphosphinyloxy)crotonamide |
| SD 5742 | *N,N*-Diallyl-2-chloro-3-(dimethoxyphosphinyloxy)-crotonamide |
| Amiton-oxalate | *S*-(2-Diethylamino)ethyl *O,O*-diethyl phosphorothioate hydrogen oxalate |

**Table II. Toxicity to Houseflies of SD 3562, Methyl Parathion, and Their Mixtures**

| Material | $LC_{50}$, % | Cotoxicity Coefficient of Mixture |
|---|---|---|
| SD 3562 | 0.059 | |
| SD 3562 + 1% sesamex[a] | 0.0030[b] | 19.7 |
| Methyl parathion | 0.0055 | |
| Methyl parathion + 1% sesamex | 0.015[c] | 0.37 |

[a] 1% sesamex solution is not more toxic than kerosine alone.
[b] $LC_{50}$ of SD 3562 in mixture.
[c] $LC_{50}$ of methyl parathion in mixture.

In order to find the synergistic action of a mixture, the concentration of synergist should be so low that it would give little or no toxicity when applied alone. A 1% concentration was selected for most tests on the housefly because it is not more toxic than kerosine alone, because it is the concentration of pyrethrin synergists used in many commercial formulations, and because insecticides having a wide range of toxicity can be compared on equal weight of synergists by using a constant concentration of synergist instead of a definite ratio of insecticide and synergist. Other concentrations were selected for other tests because they were not more toxic than the control to the species tested.

The spray method used for testing houseflies, *Musca domestica* L. (National Association of Insecticide and Disinfectant Manufacturers 1948 strain), was essentially the same as that described by Sun (*10*). Houseflies were reared and tested at 80° ± 2° F. and at 40 to 50% relative humidity. Four-day-old flies of both sexes were thoroughly mixed and properly sampled before 100 individuals were counted into each cage. Three cages of flies were each sprayed with 0.6 ml. of refined kerosine (No. 10 oil) solutions. The average deposit was 41 μl. per 100 flies. For each toxicant and each mixture three concentrations were used. At least 1800 flies were sprayed for each pair of tests.

All stages of pea aphids, *Macrosiphum pisi* (Harr.), on broad bean plants were sprayed with aqueous suspensions containing 0.05% emulsifier (Atlox 1045A) and various amounts of insecticide with and without 0.05% sesamex [2-(2-ethoxyethoxy)ethyl 3,4-methylenedioxyphenyl acetal of acetaldehyde]. Immediately after spraying, the number of aphids on the plants was counted. After 18 to 20 hours living aphids were again counted. Results were then calculated as apparent mortality (*12*). Two-spotted spider mites, *Tetranychus telarius* (L.), were sprayed with aqueous suspensions with and without 1% sesamex. Only the mortality counts of adult mites were taken.

Before the determination of dosage-mortality curves, a range-finding test was made for each toxicant and its mixture. If the result did not agree with the preliminary finding, the test was repeated.

To evaluate the relative increase or decrease in toxicity on the same basis for a number of mixtures it was necessary to have a constant amount of synergist (or antagonist) as well as the same method for calculating the toxicity results. Several methods have been described by Sun and Johnson (*11*) for comparing the joint toxicity of insecticides in terms of cotoxicity coefficient. When one of the components in a mixture is not toxic at the test concentrations, the toxicity of that component in the mixture becomes insignificant. Then the cotoxicity coefficient, calculated by the following equation (*11*), is the number of times of increase (or decrease) in toxicity.

$$\text{Cotoxicity coefficient} = \frac{LC_{50} \text{ of toxicant alone}}{LC_{50} \text{ of toxicant in mixture}}$$

If the result of calculation is significantly higher than 1, the mixture indicates synergism; if less than 1, the result shows antagonism. Examples in Table II illustrate the data and results of calculation of synergistic combination of SD 3562 and sesamex and of antagonistic combination of methyl parathion and sesamex (*11*).

Because synergists and antagonists can increase and decrease, respectively,

**Table III. Toxicity to Houseflies of Insecticide Mixtures Containing Pyrethrin Synergists**

| Material | $LC_{50}$ of Toxicant, % | Cotoxicity Coefficient with 1% Synergist: Sesamex | Piperonyl butoxide | Sulfoxide | Propyl isome |
|---|---|---|---|---|---|
| Pyrethrins | 0.0605 | 60.5 | 29.5 | 41.3 | 13.8 |
| SD 2966 | 0.089 | 38.7(2)[a] | 9.0 | 5.0 | 3.8 |
| SD 5656 | 0.0425 | 30.4 | 4.0 | 7.7 | 2.5 |
| SD 3562 | 0.059 | 19.7 | 4.1 | 1.7 | 1.6 |
| Methyl parathion | 0.0053 | 0.41(2) | 0.86 | 0.91 | 0.84 |

[a] Number in parentheses indicates number of determinations.

the toxicity of insecticides, different causes of increase or decrease may influence the toxicity. The complexity of their function cannot be analyzed by a simple calculation. Therefore, the results of calculation may be considered as apparent increase or decrease in toxicity. When the results show a cotoxicity coefficient of slightly more or less than 1, the difference in toxicity may not be significant.

### *Results on Syngerism and Antagonism*

**Selection of Synergists.** As is known, the methylenedioxyphenol group in pyrethrin synergists is related to their synergistic action on pyrethrins (*6*, pp. 77–89). Presumably they are common in their mode of action but differ in intensity of synergism. To simplify all possible combinations between synergists and insecticides the most active synergist should be selected.

Series of mixtures containing each of four common pyrethrin synergists, in combination with pyrethrins, SD 2966, SD 5656, SD 3562, and methyl parathion, were tested aginst houseflies. Results in Table III indicate that at 1% concentration sesamex is the best synergist for pyrethrins, SD 2966, SD 5656, and SD 3562 with increases in toxicity of 60.5, 38.7, 30.4, and 19.7 times, respectively. However, the same concentration reduced the cotoxicity coefficient of methyl parathion to 0.41. Piperonyl butoxide, sulfoxide, and propyl isome gave similar effects but were much less potent. Therefore, sesamex was selected as a representative of this group of synergists for further studies.

**Phosphates and Phosphonates.** In addition to SD 2966, SD 3562, and SD 5656, a number of other vinyl phosphates, vinyl phosphonates, and organophosphorus compounds were tested against houseflies with and without 1% sesamex. Results in Table IV showed that 1% sesamex increased the toxicity of vinyl phosphates and vinyl phosphonates 1.4 to 38.7 times. Higher increases in toxicity were found in compounds containing an amino or an amido group. In the nonvinyl organophosphorus compounds, a high increase (29.1 times) was found in Amiton-oxalate, which contains an amino group, but low (1.6 times) in schradan, which contains amido groups. The amino or amido group in almost all members of these three groups of organophosphorus compounds is related to unusually high synergistic action. Reasons for low increases in toxicity of SD 2438 and schradan in combination with synergist are discussed below.

In biological systems the primary and secondary amines are known to be susceptible to oxidation by amine oxidases with the liberation of ammonia (*5*). Schradan (amide) is oxidized in insects and plants to a monophosphoramide oxide (*2*), which is a strong cholinesterase inhibitor. It is reasonable to suspect that the amino or amido groups of the vinyl phosphate and phosphonates (Table IV) may also be susceptible to certain biological oxidations. The resulting products may be less or nontoxic. Therefore, the apparent high increases in toxicity may be largely due to the inhibition of such oxidation by pyrethrin synergists.

**Table IV. Toxicity to Houseflies of Various Insecticides and Their Mixtures Containing Sesamex**

| Material | | $LC_{50}$ of Toxicant, % | | Cotoxicity Coefficient with 1% Sesamex |
|---|---|---|---|---|
| | | Alone | Plus 1% sesamex | |
| Vinyl Phosphates, $R_1 \diagdown N \diagup R_2$ Group | | | | |
| SD 5656 | $N(CH_2CH{=}CH_2)_2$ (amido) | 0.0425 | 0.0014 | 30.4 |
| SD 2244 | $N(CH_3)_2$ (amido) | 0.063 | 0.0025 | 25.2 |
| SD 3562 | $N(CH_3)_2$ (amido) | 0.059 | 0.003 | 19.7 |
| SD 5742 | $N(CH_2CH{=}CH_2)_2$ (amido) | 0.06 | 0.00335 | 17.9 |
| Phosphamidon | $N(C_2H_5)_2$ (amido) | 0.024 | 0.00165 | 14.6 |
| SD 5567 | NH—C$_6$H$_4$—Cl (amido) | 0.034 | 0.0044 | 7.7 |
| SD 4092 | None | 0.0223 | 0.0058 | 3.8 |
| DDVP | None | 0.0055 | 0.00245 | 2.2(2)[a] |
| SD 4239 | None | 0.023 | 0.0091 | 2.5 |
| Phosdrin | None | 0.0054 | 0.0022 | 2.5 |
| SD 2409 | None | 0.0077 | 0.0055 | 1.4 |
| Vinyl Phosphonates | | | | |
| SD 2966 | $N(CH_3)_2$ (amino) | 0.089 | 0.0023 | 38.7(2) |
| SD 2438 | $N(CH_3)_2$ (amido) | 0.029 | 0.0068 | 4.3 |
| Nonvinyl Phosphorus Compounds | | | | |
| Amiton-oxalate | $N(C_2H_5)_2$ (amino) | 0.77 | 0.0265 | 29.1 |
| Schradan | $N(CH_3)_2$ (amido) | 3.1 | 1.95 | 1.6 |
| Phosphorodithioates | | | | |
| Guthion | | 0.020 | 0.0113 | 1.8(2) |
| Malathion | | 0.0845 | 0.0725 | 1.1(2) |
| Phosphorothioates and One Oxygen Analog | | | | |
| Chlorthion | | 0.058 | 0.198 | 0.30(2) |
| Parathion | | 0.0041 | 0.0065 | 0.63 |
| Methyl parathion | | 0.0053 | 0.013 | 0.41(2) |
| Methyl paraoxon | | 0.0039 | 0.0022 | 1.8(2) |
| Phosphonothioate | | | | |
| EPN | | 0.0103 | 0.0147 | 0.70 |
| Cyclodiene Compounds | | | | |
| Isodrin | | 0.016 | 0.0092 | 1.7(2) |
| Endrin[b] | | 0.0118 | 0.0031 | 3.8 |
| Aldrin | | 0.0092 | 0.0153 | 0.60(2) |
| Dieldrin[b] | | 0.0059 | 0.00365 | 1.6 |
| SD 3110 | | 0.0014 | 0.0016 | 0.88 |
| SD 3450[c] | | 0.00105 | 0.0006 | 1.8 |
| Heptachlor | | 0.0064 | 0.0113 | 0.57 |
| Heptachlor epoxide[b] | | 0.004 | 0.003 | 1.3 |
| SD 2642 | | 0.0103 | 0.0062 | 1.7(2) |
| SD 2648[b] | | 0.0072 | 0.0053 | 1.4 |
| Other Chlorinated Insecticides | | | | |
| Lindane | | 0.0067 | 0.0047 | 1.4 |
| DDT | | 0.052 | 0.045 | 1.2(2) |

[a] Number in parentheses indicates number of determinations.
[b] Epoxide.
[c] *N*-oxide of SD 3110.

Based on $LC_{50}$'s, Phosdrin is approximately 1.4 to 16.5 times more toxic to houseflies than its analogs (Table IV). When tested with 1% sesamex the toxicities of some of the vinyl phosphates (SD 5656, SD 2244, SD 3562, SD 2966, and phosphamidon) are raised to about that of Phosdrin. However, this is not true for all analogs tested. The mixtures of those compounds containing a phenyl, chlorophenyl, or nitrophenyl group (SD 4092, SD 4239, and SD 2409) are much less toxic to

**Table V. Toxicity to Houseflies of Insecticide Mixtures Containing Various Concentrations of Sesamex**

| Material | $LC_{50}$ of Toxicant, % | Cotoxicity Coefficient of Mixtures Containing Following Sesamex Concentrations | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.04% | 0.1% | 0.2% | 0.5% | 1% | 2% |
| Methyl parathion | 0.0053 | ... | 0.93 | ... | 0.53 | 0.41(2)[a] | 0.36 |
| Aldrin | 0.0092 | ... | 0.87 | ... | 0.68 | 0.60(2) | 0.69 |
| SD 3562 | 0.059 | 2.1 | ... | 10.3 | ... | 19.7 | ... |

[a] Number in parentheses indicates number of determinations.

**Table VI. Toxicity of Organophosphorus Compounds and Their Mixtures Containing Sesamex to Various Insects**

| Species | Material | Sesamex in Mixture, % | $LC_{50}$ of Toxicant, % | | Cotoxicity Coefficient with Sesamex |
|---|---|---|---|---|---|
| | | | Alone | In mixt. | |
| Pea aphid | SD 2966 | 0.05 | 0.0032 | 0.0009 | 3.6 |
| | SD 3562 | 0.05 | 0.000046 | 0.000031 | 1.5 |
| | Parathion | 0.05 | 0.00038 | 0.00062 | 0.61 |
| Two-spotted spider mite | SD 2966 | 1.0 | 0.015 | 0.0074 | 2.0 |
| | SD 3562 | 1.0 | 0.076 | 0.0165 | 4.6 |
| Housefly | | | | | |
| Susceptible strain | SD 2966 | 1.0 | 0.099 | 0.0024 | 41.3(2)[a] |
| Resistant strain | SD 2966 | 1.0 | 0.314 | 0.0092 | 34.0(2) |

[a] Number in parentheses indicates number of determinations.

houseflies than the sesamex-Phosdrin combination. This may be mainly because the decrease in water solubility by such substitutions changes the partition coefficient and, therefore, may affect the movement of toxicants in insects.

**Thionophosphorus Compounds.** It has been theorized that the increase in toxicity of several organophosphorus compounds (Tables III and IV) containing an amino or an amido group, when mixed with pyrethrin synergists, may be due to the inhibition of detoxification by certain biological oxidations. On the other hand, the activity of some organic thionophosphorus insecticides, such as parathion and methyl parathion (*7*), depends mainly upon their oxidation to oxygen analogs; their combinations with pyrethrin synergists should reduce their toxicity. To test this hypothesis two phosphorodithioates, three phosphorothioates and one oxygen analog, and one phosphonothioate were tested against houseflies with and without 1% sesamex. Results in Table IV indicate that sesamex significantly reduces the toxicity of some thionophosphorus insecticides, such as Chlorthion, parathion, methyl parathion, and EPN.

Although 1% sesamex reduced the cotoxicity coefficient of methyl parathion to 0.41, it increased the toxicity of methyl paraoxon, the oxygen analog of methyl parathion, 1.8 times. This low increase in toxicity indicated that sesamex may not only inhibit the oxidation of methyl parathion but also stabilize its oxygen analog. Other low increases in toxicity for sesamex mixtures containing Guthion, malathion, and some vinyl phosphates (Table IV) may be verified by the same reasoning.

**Chlorinated Insecticides.** It has been indicated that synergistic and antagonistic actions of sesamex on certain organophosphorus compounds were probably due to the inhibition of certain biological oxidations. The increase or decrease in toxicity depends upon the nature of compounds which may be either activated or detoxified by oxidation. Certain cyclodiene insecticides, such as aldrin (*1*) and heptachlor (*3*), are readily oxidized in animals to their respective epoxides, which are generally more toxic than their corresponding precursors. If sesamex inhibits certain biological oxidations, aldrin and heptachlor should be less toxic with than without pyrethrin synergist.

Twelve chlorinated insecticides were sprayed on houseflies with and without 1% sesamex. Their $LC_{50}$'s and cotoxicity coefficients are listed in Table IV. Results indicated that aldrin, heptachlor, and SD 3110 containing 1% sesamex gave lower toxicity than the toxicants alone, and that their corresponding oxides—dieldrin, heptachlor epoxide, and SD 3450—were higher in toxicity with 1% sesamex. However, such change of toxicity did not hold true for isodrin or SD 2642. Stabilization and slight joint toxicity may be related to the absence of apparent decrease in toxicity. Lindane and DDT containing 1% sesamex also showed slight increases in toxicity.

**Concentration Effect.** To estimate the effect of concentration of synergists on different types of compounds, several concentrations of sesamex were added to the kerosine solutions of methyl parathion, aldrin, and SD 3562. Each toxicant and its mixtures were sprayed on houseflies.

Results in Table V indicate that as the concentration of sesamex increased from 0.1 to 2% the toxicity of methyl parathion and aldrin decreased rather slowly. The largest change in cotoxicity coefficient was approximately 0.64 for methyl parathion and 0.40 for aldrin. However, the toxicity of SD 3562–sesamex mixtures increased very rapidly as the concentration of sesamex was increased from 0.04 to 1% to yield a toxicity increase of 19.7 times at this high sesamex level. The rapid increase in toxicity for synergistic action and the slow decrease for antagonistic action indicate that other stabilizing effects and/or some joint toxicity between synergist and toxicant may exist.

**Resistant Houseflies.** It was of interest to see whether synergists have the same effect on resistant as on susceptible strains of insects. Both susceptible and multiple resistant (highly resistant to chlorinated insecticides) houseflies were sprayed with SD 2966 with and without 1% sesamex. Results indicate that the low cross resistance to SD 2966 did not appreciably change the relative synergistic activity of sesamex (Table VI). One per cent sesamex increased the toxicity of SD 2966 41.3 and 34 times against susceptible and resistant strains of houseflies, respectively.

**Aphids and Mites.** To ascertain the extent of joint toxicity of some insecticide-synergist combinations against other species, both peak aphids and two-spotted spider mites were sprayed with aqueous suspensions of SD 2966, SD 3562, and parathion with and without sesamex. Sesamex increased the toxicity of SD 2966 and SD 3562 to pea aphids and two-spotted spider mites 1.5 to 4.6 times (Table VI), less than the increases obtained for the same compounds against houseflies (Table IV). However, 0.05% of sesamex gave a similar lowering of parathion toxicity to aphids as 1% sesamex to houseflies (Table IV). Under the test conditions 0.05 and 1.0% of sesamex were not toxic to aphids and mites, respectively.

## Mode of Action

It has been suspected that the effect of pyrethrin synergists on some organophosphorus and chlorinated insecticides (Table IV) may be due to the inhibition of certain biological oxidations in houseflies in order to produce higher or lower mortalities. To explain their synergistic and antagonistic actions on various insecticides, the effect of SD 2966, SD 3562, and methyl parathion on the ChE activity in homogenates of treated flies was tested with and without

sesamex. If the inhibition of biological oxidation were an important factor affecting the toxicity of these compounds, there should be less conversion of methyl parathion to its active oxygen analog and probably less decomposition of SD 2966 and SD 3562. The results of such changes would not only give large differences in fly mortalities but also would produce different ChE inhibition values for fly extracts.

In each of the three series of experiments the concentrations of methyl parathion (0.005%), SD 2966 (0.02%), and SD 3652 (0.01%) were selected on the basis of previous data, so that the differences in fly mortality would be large between treatments with and without sesamex.

Five cages of flies each containing 100 four-day-old adults of mixed sexes were sprayed with kerosine solutions of each toxicant with and without 1% sesamex. They were then transferred to recovery cages or were at once homogenized with physiological saline at the rate of 10 flies per milliliter of solution in a Waring Blendor. Because of strong ChE inhibitory activity, houseflies treated with SD 2966 were washed with 2-propanol before homogenization. Two milliliters of filtered (through cheesecloth) homogenate were then incubated with a standard solution of bovine ChE using acetylcholine as the substrate. The inhibition was determined by the ΔpH method (*4*). For SD 2966 and SD 3562 the incubation period was 1 hour instead of 30 minutes.

Table VII summarizes the results of above experiments on toxicity and ChE inhibition. Four hours after spraying, 0.005% methyl parathion killed 90% of flies but only 5% with the same concentration of methyl parathion containing 1% sesamex. This indicates that sesamex may inhibit the oxidation of methyl parathion to its oxygen analog which is a potent ChE inhibitor and a more active insecticide. This explanation agrees with the ChE inhibitory data, which show 20.8% inhibition for methyl parathion alone but none for methyl parathion plus 1% sesamex. Sesamex alone did not inhibit the ChE activity in repeated tests under the same conditions. On the other hand 1% sesamex highly increased the toxicity of SD 2966 from 0 to 98% and SD 3562 from 2 to 99% at 1 and 1.75 hours, respectively, after spraying (Table VII), presumably because sesamex prevents detoxification caused by biological oxidation. Although the difference in ChE inhibition was not as sharp as that for methyl parathion, repeated tests indicated the same order of difference. Since SD 2966 and SD 3562 are strong ChE inhibitiors, the relatively high inhibition for the control samples may be due either to their rapid penetration into the flies or to incomplete washing of the toxicant from the surface. In general, the results on ChE inhibition tended to corroborate the theory of biological oxidation of these compounds.

**Table VII. Effect of Sesamex on Toxicity of Organophosphorus Insecticides and on ChE Inhibition in Extracts of Treated Houseflies**

| Toxicant in Spray Solution, % | Housefly Mortality: Time interval after spray, hr. | Housefly Mortality: Mortality, % | ChE Inhibition of Fly Extracts: Incubation time, hr. | ChE Inhibition of Fly Extracts: Inhibition, % |
|---|---|---|---|---|
| 0.005% methyl parathion | 4 | 90 | 0.5 | 20.8 |
| [a] | 4 | 5 | 0.5 | 0 |
| [b] | 0 | 0 | 0.5 | 0 |
| 0.02% SD 2966 | 1 | 0 | 1 | 14.1 |
| [a] | 1 | 98 | 1 | 33.3 |
| [b] | 0 | 0 | 1 | 24.4 |
| 0.01% SD 3562 | 1.75 | 2 | 1 | 10.1 |
| [a] | 1.75 | 99 | 1 | 26.3 |
| [b] | 0 | 0 | 1 | 17.1 |

[a] Plus 1% sesamex.
[b] Control.

To test further the effect of sesamex on the biological oxidation in flies, aldrin was selected as an example for the cyclodiene group of insecticides, the epoxides of which are more toxic.

Twenty cages of houseflies (100 flies per cage) were sprayed with each of the following solutions: 0.01% aldrin, 0.01% aldrin plus 1% sesamex, 1% sesamex, and kerosine only. Immediately after spraying, the flies were transferred to recovery cages and mortality counts made 20 hours later. All the flies in the same treatment were ground with sand and anhydrous sodium sulfate, and extracted four times with a 2 to 1 *n*-hexane–2-propanol mixture. The extracts were then saponified with alcoholic potassium hydroxide and analyzed by colorimetric methods for aldrin (*9*) and dieldrin (*8*). In control samples, 0.0 and 0.6 γ of aldrin and 1.4 and 0.9 γ of dieldrin were obtained for 1% sesamex in kerosine and kerosine treatments, respectively. When known amounts of aldrin and dieldrin were added to the untreated extract, 86% of aldrin and 84% of dieldrin were recovered. All results were corrected for their corresponding blank values.

Results in Table VIII indicate that when flies were treated with aldrin alone more dieldrin (26.5 γ) was found than aldrin (7.4 γ). However, in the treatment with a mixture of aldrin and 1% sesamex as much as 20 γ of aldrin was recovered and only 4.8 γ of dieldrin were detected. This strongly suggests that sesamex inhibits certain biological oxidations. The higher mortality (42%) in the aldrin treatment and the lower mortality (26%) in the aldrin-sesamex treatment also indicate that aldrin is a less toxic form. There is no obvious indication that aldrin is nontoxic unless it is oxidized to dieldrin, because, based on the gross effect of toxicants, the amount of dieldrin (4.8 γ) found in the treatment with aldrin-sesamex is too low to produce a mortality of 26% without the joint action of aldrin.

**Table VIII. Effect of Sesamex on Epoxidation of Aldrin in Treated Houseflies**

| Treatment | Average Mortality, %, 20 Hours | Toxicant Found γ: Aldrin | Toxicant Found γ: Dieldrin |
|---|---|---|---|
| 0.01% aldrin | 42 | 7.4 | 26.5 |
| 0.01% aldrin + 1% sesamex | 26 | 20.0 | 4.8 |

### General Discussion

Results on houseflies indicate that sesamex increased the toxicity of a number of insecticides. A general low increase was found for some organophosphorus and chlorinated insecticides (Table IV); high increases often occurred with organophosphorus compounds containing an amido or an amino group (Tables III, IV, and V). However, the same concentration of synergist significantly reduced the toxicity of several organic thionophosphorus compounds as well as several chlorinated insecticides (Tables III, IV, and V), the epoxides (or *N*-oxide) of which are generally more toxic than their corresponding precursors.

Results on ChE studies indicated that sesamex increased the ChE inhibition of extracts of flies treated with SD 2966 or SD 3562, but decreased the ChE inhibition of similar extracts treated with methyl parathion. Because methyl parathion is readily oxidized to its oxygen analog and SD 2966 and SD 3562 are probably decomposed to less or nontoxic compounds, it is reasonable to believe that by inhibiting certain biological oxidations sesamex acts as an antagonist to methyl parathion and related compounds, but as a synergist to SD 2966, SD 3562, and other organophosphorus compounds containing an amido or

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