The Selective Toxicity of New *N*-Phosphorothioylcarbamate Esters

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The effect of substitution of the proton on the carbamoyl moiety of five commercial carbamate insecticides by dialkylphosphorothioyl groups on toxicity to the housefly and white mouse has been investigated. Derivatization with the *O,O*-dimethylphosphorothioyl group produced compounds which were remarkably less toxic to the white mouse than the original carbamate, but were of equal or increased toxicity to susceptible and carbamate-resistant strains of houseflies.

The design of new insecticidal chemicals possessing favorable properties of selectivity, *i.e.*, chemicals which are toxic to insects but relatively nontoxic to mammals, is a standing goal among insecticide chemists. One of the most fruitful approaches to the discovery of selective insecticides should reside in the exploitation of available information concerning differences in rates of metabolism of specific chemical moieties in insects and mammals. An excellent starting point for this approach is the selectivity of malathion (diethyl mercaptosuccinate, *S*-ester with *O,O*-dimethyl phosphorodithioate) for which it has been postulated that slow oxidation of malathion to the anticholinesterase malaoxon *in vivo* provides the opportunity for detoxifying enzymes to act on malathion or malaoxon in mammals (Krueger and O'Brien, 1959; Metcalf, 1964), in this case primarily a carboxyesterase, rendering the compound nontoxic. In malathion-resistant houseflies, on the other hand, phosphatases appear to be the principal detoxifying enzymes responsible for the degradation of organophosphorus insecticides (Matsumura and Dauterman, 1964). With this rationale in mind, it was anticipated that substitution of the proton on the *N*-methyl moiety of carbamate esters, which are toxic to both mammals and insects, by a substituted phosphorothioyl group might give compounds of reduced mammalian toxicity, owing to the possibilities for alternate routes of detoxication in mammals and insects. Recently, Miskus *et al.* (1969) showed the *N*-acetyl Zectran (4-dimethylamino-3,5-xylyl *N*-acetyl-*N*-methylcarbamate), a derivative of Zectran which is toxic to the spruce budworm but of low toxicity to mice, is metabolized in this insect into relatively large amounts of the carbamate Zectran, but is detoxified by mice through hydrolysis of the carbamate ester moiety to the nontoxic product 4-dimethylamino-3,5-xylenol.

This paper represents part of our continuing endeavor to design molecules which will be selectively toxic to insects, and specifically is concerned with the insecticidal and mammalian toxicity of a series of *N*-(dialkylphosphorothioyl) derivatives of commercially important methylcarbamate insecticides.

MATERIALS AND METHODS

The carbamates used in this study were obtained from commercial sources: carbaryl (1-naphthyl methylcarbamate), *m*-isopropylphenyl methylcarbamate, and aldicarb [2-methyl-2-(methylthio)propionaldehyde *O*-methylcarbamoyloxime] from Union Carbide Corp., carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) from Niagara Chemical Division, FMC Corp., and Baygon (*o*-isopropoxyphenyl methylcarbamate) from Chemagro Corp. Phenols were obtained by alkaline hydrolysis of the above carbamates or from commercial sources. 2-Methyl-2-(methylthio)propionaldehyde oxime was obtained from the Union Carbide Corp. Aryl chloroformates of the phenols obtained from the above carbamates were prepared by treating the phenol with phosgene in the presence of diethylaniline in benzene according to Strain *et al.* (1950). The chloroformates were not characterized and were converted directly to the *N*-dialkylphosphorothioyl-*N*-methylcarbamate derivatives as described below for the synthesis of *o*-isopropoxyphenyl *N*-(*O,O*-dimethylphosphorothioyl)-*N*-methylcarbamate. Physical properties of the chloroformates are as follows: 1-naphthyl chloroformate, b.p. 82–4° C (0.1 mm), n_D^{25} 1.5966; *m*-isopropylphenyl chloroformate, b.p. 50–2° C (0.35 mm), n_D^{25} 1.4969; *o*-isopropoxyphenyl chloroformate, b.p. 66–8° C (0.2 mm), n_D^{25} 1.5011; 2,3-dihydro-2,2-dimethyl-7-benzofuranyl chloroformate, m.p. 82–5° C and 4-nitrophenyl chloroformate, m.p. 80–2° C.

Dialkyl *N*-methylphosphoramidates and amidothioates were prepared from conventional methods by treating the appropriate phosphorochloridates with methylamine in benzene: diethyl *N*-methylphosphoramidate, b.p. 78–80° C (0.4 mm), n_D^{25} 1.4235 (Saunders *et al.*, 1948); *O,O*-diethyl *N*-ethylphosphoramidothioate, b.p. 68° C (0.5 mm), n_D^{25} 1.4693 (Michaelis, 1903); *O,O*-diethyl *N*-methylphosphoramidothioate, b.p. 64–5° C (0.7 mm), n_D^{25} 1.4734, analysis calculated for $C_5H_{14}NO_2PS$, C = 32.78, H = 7.70, found C = 32.55, H = 7.77; *O,O*-dimethyl *N*-methylphosphoramidothioate, b.p. 54–6° C (0.5 mm), n_D^{25} 1.4858, analysis calculated for $C_3H_{10}NO_2PS$, C = 23.21, H = 6.45, found C = 23.62, H = 6.41.

The aryl *N*-(dialkylphosphoryl)- and *N*-(dialkylphosphorothioyl)-*N*-methylcarbamates listed in Table I were prepared by the condensation of the appropriate aryl chloroformates with the lithium derivative of the dialkyl *N*-methylphosphoramidothioate or amidate according to the following procedure for the synthesis of *o*-isopropoxyphenyl *N*-(*O,O*-dimethylphosphorothioyl)-*N*-methylcarbamate (**10**). To phenyllithium prepared from 0.35 g of lithium (0.05 g-atom) and 4.0 g of bromobenzene (0.025 mol) in 80 ml of ether was added gradually 4.0 g of *O,O*-dimethyl *N*-methylphosphoramidothioate (0.026 mol) in 10 ml of ether. After stirring for 10 min, the mixture was chilled in a dry ice-acetone bath and 5.4 g of *o*-isopropoxyphenyl chloroformate in 10 ml of ether were added in one portion and stirring continued at dry ice temperature for 10 min. The mixture was then stirred overnight at room temperature, washed twice with water, dried over anhydrous sodium sulfate, and the product was distilled, b.p. 153–4° C (0.025 mm). The nmr spectrum of the purified product was consistent with the structure with a multiplet centered at δ 6.9 for aromatic protons, multiplet centered at δ 4.5 for the tertiary

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Table I. Physical and Biological Properties of *N*-(Dialkylphosphoryl)- and *N*-(Dialkylphosphorothioyl)-*N*-methylcarbamates

	R	R′	Elemental Analysis Calcd	Elemental Analysis Found	P.B., °C	M.P., °C	n_D^{25}	I_{50} (*M*) Fly ChE	LD_{50} mg/kg Housefly S_{NAIDM} Alone	S_{NAIDM} 1:5 P.B.	R_{MIP} Alone	R_{MIP} 1:5 P.B.	Mouse (oral)
						O ‖ 1-naphthyl-OCN(R′)R							
1	H	CH_3	(carbaryl)		...	140	...	9.0×10^{-7}	900	12.5	>900	95	540
2	$P(S)(OCH_3)_2$	CH_3	C 51.64 H 4.92	C 51.46 H 4.77	...	67–8	...	$>1.0 \times 10^{-4}$	150	>500	225	>500	1650
3	$P(S)(OC_2H_5)_2$	CH_3	C 54.39 H 5.66	C 54.90 H 6.07	174–9 (0.1 mm)	...	1.5713	5.2×10^{-6}	>500	500	>500	500	...
4	$P(O)(OC_2H_5)_2$	CH_3	C 56.97 H 5.94	C 57.48 H 5.70	[a]	...	1.5508	4.5×10^{-7}	>500	145	>500	170	...
						O ‖ *m*-isopropylphenyl-OCN(R′)R							
5	H	CH_3				72–3	...	3.4×10^{-7}	41	11	125	14.5	16
6	$P(S)(OCH_3)_2$	CH_3	C 49.21 H 6.30	C 48.84 H 6.48	154–6 (0.1 mm)	...	1.5232	6.6×10^{-5}	32.5	22.5	33.5	34	760
7	$P(S)(OC_2H_5)_2$	CH_3	C 52.17 H 6.95	C 51.23 H 6.92	144–9 (0.03 mm)	...	1.5105	2.9×10^{-6}	67.5	22.5	165	>500	400–550
8	$P(O)(OC_2H_5)_2$	CH_3	C 54.71 H 7.30	C 55.34 H 7.47	140–4 (0.05 mm)	...	1.4873	1.1×10^{-6}	>500	230	>500	280	>1000
						O ‖ *o*-isopropoxyphenyl-OCN(R′)R							
9	H	CH_3	(Baygon)			85–7		6.9×10^{-7}	22	5.6	45	8.5	24
10	$P(S)(OCH_3)_2$	CH_3	C 46.85 H 6.21	C 46.74 H 6.17	153–4 (0.025 mm)		1.5230	8.4×10^{-6}	32	15	37	21	1400
11	$P(S)(OC_2H_5)_2$	CH_3	C 49.86 H 6.65	C 49.62 H 6.72	155–8 (0.15 mm)	...	1.5117	1.4×10^{-5}	37	19	38	22.5	1700
12	$P(S)(OC_2H_5)_2$	C_2H_5	C 51.20 H 6.93 S 8.53	C 52.40 H 7.34 S 8.28	154–7 (0.13 mm)	...	1.5090	2.1×10^{-5}	500	>500	500	>500	...
						O ‖ $CH_3SC(CH_3)_2CH{=}NOCN(R')R$							
13	H	CH_3	(aldicarb)	...	...	99–100	...	8.4×10^{-5}	5.5	3.4	30	13.5	0.3–0.5
14	$P(S)(OCH_3)_2$	CH_3	C 34.39 H 6.05	C 35.29 H 6.11	[b]	...	1.5100	2.9×10^{-4}	95	>500	170	360	170
						O ‖ 2,2-dimethyl-2,3-dihydrobenzofuranyl-7-OCN(R′)(R)							
15	H	CH_3	(carbofuran)		...	147	...	2.5×10^{-7}	6.7	2.5	500[c]	12.5[c]	2
16	$P(S)(OCH_3)_2$	CH_3	C 48.71 H 5.79	C 49.03 H 6.10	...	63–5	...	9.3×10^{-6}	13	5.5	500[c]	45[c]	150–190
17	$P(O)(OCH_3)(SCH_3)$	CH_3	C 48.71 H 5.79	C 48.87 H 5.53	[b]		1.5353	...	>500	29	>500	300	...
						O ‖ *p*-nitrophenyl-OCN(R′)(R)							
18	$P(S)(OCH_3)_2$	CH_3	C 37.50 H 4.06	C 37.24 H 3.97	...	62–5	...	3.8×10^{-4}	>500	>500	>500	>500	...

[a] Sample was purified by preparative glc. [b] Compound could not be distilled and was purified according to procedure for **17** under Materials and Methods. [c] These values were obtained from a resistant strain of houseflies which were approximately 9-fold more resistant to compound **3** than the resistant houseflies used for the other evaluations.

isopropoxy proton, a doublet centered at δ 3.8 (J = 15 Hz) for the methoxy protons, a doublet centered at δ 3.3 (J = 10 Hz) for *N*-methyl protons, and a doublet centered at δ 1.3 (J = 6 Hz) for the methyl protons on isopropyl. Infrared spectrum showed strong carbonyl absorption at 1740 cm^{-1}. Elemental analysis and physical properties are given in Table I.

In many cases diaryl carbonates were obtained as an impurity which could not be removed by distillation. Although the formation of this side product could be minimized by carrying out the reaction at low temperature and by adding the chloroformate rapidly to the lithium salt, complete avoidance of this substance was not possible. Purification was accomplished by allowing the carbonate contaminated product to stand in 10 ml of ethyl alcohol containing 0.5 g of potassium hydroxide for 15 min at room temperature. The mixture was diluted with water, product extracted with ether, dried and distilled.

2-Methyl-2-(methylthio)propionaldehyde *O*-[*N*-*O*′,*O*′-dimethylphosphorothioyl)-*N*-methylcarbamoyl]oxime (**14**) was prepared as follows. A solution of 3.2 g 4-nitrophenyl *N*-(*O*-, *O*-dimethylphosphorothioyl)-*N*-methylcarbamate (**18**) in 20 ml of acetone was added in one portion to the sodium salt

of 2-methyl-2-(methylthio)propionaldehyde oxime prepared from 1.5 g of oxime and an equivalent amount of sodium ethoxide in 50 ml of ethanol. The mixture was allowed to stand overnight at room temperature and the product was isolated according to the procedure above for **10**. No attempt was made to distil the product because of its thermal instability. Elemental analysis is given in Table I. Nmr spectrum showed the following absorptions: a singlet at δ 7.5 for N=CH—, a doublet centered at δ 3.8 (J = 15 Hz) for $P(OCH_3)_2$, a doublet centered at δ 3.2 (J = 10 Hz) for $PN—CH_3$, a singlet at δ 2.0 for $—SCH_3$, and a singlet at δ 1.5 for $—C(CH_3)_2$. Analysis of the absorption integrals indicated a purity of about 90%. Infrared spectrum showed strong carbonyl absorption at 1740 cm^{-1}.

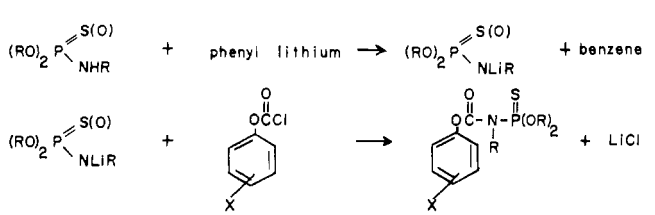
2,2-Dimethyl-2,3-dihydrobenzofuranyl-7 *N*-(*O*-methyl-*S*-methylphosphorothioyl)-*N*-methylcarbamate (**17**) was prepared by demethylation of the corresponding *N*-(*O*,*O*-dimethylphosphorothioyl)-*N*-methylcarbamate (**16**) and remethylation as follows: **16** was monodemethylated with sodium benzenethiolate in acetone according to Miller (1962). The acetone was removed and the solid residue was dissolved in water and extracted several times with ether. After removal of the water, the residue was realkylated with dimethyl sulfate in acetone. The product was taken up in ether, washed several times with water, concentrated, washed with ligroin, and dried under high vacuum. The product, a viscous clear oil, was not distilled. Elemental analysis is given in Table I. Nmr spectrum showed the following absorptions: a multiplet centered at δ 6.8 for aromatic protons, a doublet centered at δ 3.8 (J = 14 Hz) for $POCH_3$, a doublet centered at δ 3.2 (J = 8 Hz) for $PNCH_3$, a singlet at δ 3.0 for furanyl-ring methylene, a doublet centered at δ 2.4 (J = 16 Hz) for $PSCH_3$, and a singlet at δ 1.2 for furanyl-ring $C(CH_3)_2$. Analysis of the absorption integrals indicated a purity of greater than 95%.

Nmr spectra were obtained in carbon tetrachloride or deuteriochloroform using tetramethylsilane as the internal standard on either the Varian A-60 or T-60 spectrometer.

The techniques for the preparation of fly-head cholinesterase and determination of anticholinesterase activity (I_{50} values) have been described (Fukuto and Metcalf, 1956). The incubation time for inhibitor and cholinesterase was 15 min. The method for determination of insecticidal activity against susceptible female houseflies (*Musca domestica* L., S_{NAIDM} strain) and carbamate-resistant houseflies (R_{MIP} strain) have been described (Metcalf and March, 1949; Georghiou *et al.*, 1961). Mammalian toxicity was determined orally on 3- to 6-month-old Swiss white mice reared from a strain originally purchased from Curd's Caviary, La Puente, Calif. The test compound was dissolved in olive oil and applied as previously described (Hollingworth *et al.*, 1967). Mortalities were recorded after 3 days.

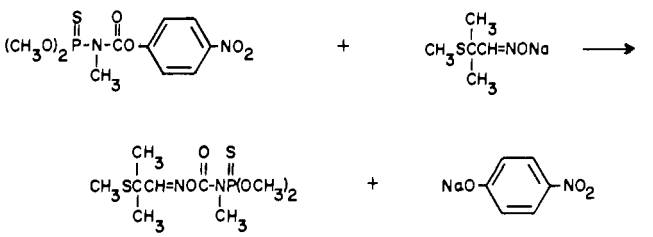
RESULTS AND DISCUSSION

Synthesis. Aryl *N*-(dialkylphosphorothioyl)- and *N*-(dialkylphosphoryl)-*N*-methylcarbamates were prepared according to the equation below.



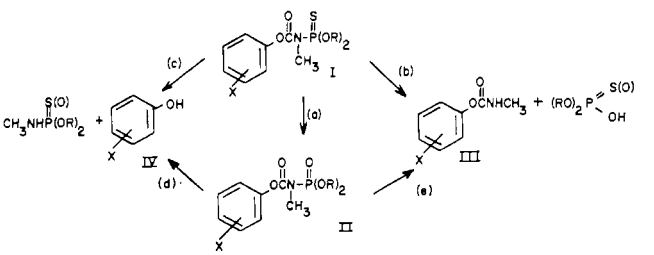
The reaction proceeded in good yields and exclusively by carbonylation on nitrogen rather than on sulfur (or oxygen), as substantiated by nmr and infrared spectra. The course of the reaction was similar to the alkylation of phosphoramidothioate anion which has been shown to be preferentially methylated and carbonylated on nitrogen by treatment with methyl iodide (Miller and O'Leary, 1962) and phthalic anhydride (Osborne *et al.*, 1966). Earlier attempts to prepare the *N*-(phosphorothioyl)-*N*-methylcarbamates by direct phosphorochloridate or tetraalkyl phosphoric anhydride were not successful. With the anhydride, the only product isolated was the substituted aryl dialkyl phosphorothioate or phosphate.

For the preparation of the *N*-(dimethyl phosphorothioyl) derivative of aldicarb (**14**), an alternate method of synthesis was employed because of the instability of 2-methyl-2-(methylthio)propionaldehyde oxime chloroformate (Payne *et al.*, 1966). Synthesis of **14** was accomplished according to the following equation



The reaction evidently is feasible because of greater nucleophilicity of oxime anion toward the carbonyl carbon compared to the phosphorus atom.

Biological Activity. The rationale used in the study of the toxicological properties of the *N*-(dialkylphosphorothioyl) derivatives is presented by the equations given below, indicating the major possibilities for alternate pathways of metabolism which may occur in the insect or mammal, exclusive of ring hydroxylation or dealkylation reactions. Alternate pathways are indicated by steps (a) through (e).



The intact P(O) carbamate may act either as a phosphate or carbamate ester in inhibiting cholinesterase at the site of action.

Slow conversion of I to II would provide opportunity for an esterase(s) to act either on the carbonyl function or on the phosphoramide function.

By analogy with malathion, steps (c) and (d) may be faster in mammals than in insects, and conversely steps (b) and (e) may be faster in insects than in mammals. P—N Bond cleavage [steps (b) and (e)] are activation steps and would release the toxic *N*-methylcarbamate (III) at or near the site of action.

The greater occurrence of steps (b) and (e) in insects and steps (c) and (d) in mammals would result in reduced mammalian toxicity. Derivatization consequently should lead to products with favorable properties of selectivity.

Supporting evidence for this rationale is found in Table I, where toxicological data are given for a number of new com-

pounds obtained by derivatizing the *N*-methyl moiety of five commercial carbamate insecticides with dimethyl or diethyl phosphorothioyl groups. The results clearly show that the *N*-(dialkylphosphorothioyl)-*N*-methylcarbamates were substantially less toxic to the white mouse and at the same time (with the exception of aldicarb analog) were of equal toxicity, or in some cases even more toxic, to houseflies than the parent carbamates. Further, the derivatized carbamates were, in most cases, more toxic to the carbamate-resistant (R_{MIP}) flies than the parent carbamates. Derivatization with the dimethyl phosphorothioyl moiety particularly reduced mouse toxicity of the more toxic carbamates, *e.g.*, *m*-isopropylphenyl *N*-methylcarbamate 48-fold, aldicarb approximately 400-fold, carbofuran about 80-fold, and Baygon 58-fold.

Although an explanation for the much lower mammalian toxicity of the derivatized carbamates seems plausible in terms of alternate routes of metabolism, *i.e.*, detoxication *via* steps (c) and (d) predominating over activation steps (b) and (e) in the white mouse, the variation of toxicity of these compounds to houseflies is less readily explained. Examination of the data in Table I shows that the toxicities of the four dimethyl phosphorothioyl analogs (**2**, **6**, **10**, and **16**) to susceptible houseflies are roughly parallel to the toxicities of the corresponding aryl *N*-methylcarbamates (**1**, **5**, **9**, and **15**). For example, the relative toxicities of **16**, **10**, **6**, and **2**, respectively, are 1 (taking the value for **16** as unity), 0.41, 0.40, and 0.088 compared to 1, 0.30, 0.16, and 0.0075 for the respective carbamates **15**, **9**, **5**, and **1**. The parallel of toxicities in the two series suggests that the actual toxicant in the case of the derivatized compounds is the parent *N*-methylcarbamate after its liberation, *in vivo*, by direct hydrolysis of the P(S) compound by step (b), or P(S) to P(O) desulfuration [step (a)] and subsequent hydrolysis to the carbamate [step (e)]. Support for the hypothesis that the parent aryl *N*-methylcarbamate is the actual toxicant was found in the complete absence of toxicity, with and without piperonyl butoxide, of the *N*-ethyl analog of **11** [*o*-isopropoxyphenyl *N*-(*O*,*O*-diethylphosphorothioyl)-*N*-ethylcarbamate (**12**)] which, *in vivo*, would be expected to produce *o*-isopropoxyphenyl *N*-ethylcarbamate, a nontoxic carbamate (Kolbezen *et al.*, 1954).

The variation in the action of piperonyl butoxide on toxicity of the derivatized carbamates is difficult to explain. For example, the toxicities of **2**, **7**, and **14** to susceptible flies were reduced 3- to 5-fold by cotreatment with five parts of piperonyl butoxide, compared to increases in toxicities of 1.4- to 2.4-fold for **6**, **10**, **11**, and **16**. The fact that both synergistic and antagonistic actions are exerted by piperonyl butoxide suggests that the derivatized carbamates are being metabolized at different rates and/or by different routes to the aryl *N*-methylcarbamates. The antagonistic action demonstrated with **2**, **7**, and **14** suggests that P(S) to P(O) conversion [step (a)] may be an important activation step to the eventual aryl or oxime *N*-methylcarbamate, and in these cases this step is inhibited by piperonyl butoxide. Others have shown that piperonyl butoxide type synergists occasionally act as antagonists of phosphorothionates by reducing P(S) to P(O) conversion (Sun and Johnson, 1960). As a simple test for this hypothesis, the P(O) analog **8** of **7** was prepared and examined. However, **8** proved to be nontoxic to houseflies, although it was synergized by piperonyl butoxide. Apparently **8**, when applied directly to the insect, is rapidly metabolized and detoxified. Explanation of synergism with **6**, **10**, **11**, and **16** with both susceptible and resistant houseflies is similarly complicated. Apparently, synergism occurs with compounds which are innately more toxic to houseflies (compare **6**, **10**, **11**, and **16** with **2**, **7**, and **14**) and the major action of piperonyl butoxide in these cases appears to be the prevention of detoxication of the aryl *N*-methylcarbamate after rapid formation of the latter, probably by step (b).

Although the anticholinesterase activities of the derivatized carbamates against housefly-head cholinesterase are consistently lower than the parent carbamates, the significant levels of inhibition obtained by these derivatives indicate that cholinesterase inactivation may be due in part to direct phosphorylation of the enzyme. That phosphorylation may contribute to inhibition is supported by the fact that the dialkyl phosphoryl derivatives are significantly stronger inhibitors than the corresponding dialkyl phosphorothioyl derivatives (compare **3** and **7** with **4** and **8**). In this regard, the overall mechanism of inhibition would be complex, since the leaving group after phosphorylation, *i.e.*, the carbamate ester, is also an active anticholinesterase. However, the role which phosphorylation may play in the intoxication process is not clearly understood, particularly in light of the much lower insecticidal activity of **8** compared to **7**.

In brief summary, derivatization of insecticidal carbamate esters with dimethyl or diethyl phosphorothioyl groups gave products which generally proved to be highly insecticidal but were considerably less toxic to the white mouse than the parent carbamate. Although preliminary hypotheses for the favorable properties of selectivity shown by these compounds have been suggested, further comments concerning mode of action, particularly in insects, must await elucidation of their metabolism in the housefly and the white mouse. Work along these lines is contemplated shortly. The toxicological results reported here strongly support our approach for the design of selective insecticides and the examination of other derivatives of carbamate esters is currently in progress.

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