A Baird Model 4-55 infrared spectrophotometer was employed at 2*N* slit program and 2*X* abscissa. Solutions for calibration were prepared using binary mixtures (by weight) of pure isomers made up to 5 ml. with carbon disulfide. The solutions were placed in a 0.2-mm. sodium chloride cell and the spectrum was scanned from 7.7 to 8.3 microns at the rate of 0.5 micron per minute. A base line was drawn between the transmittance maxima at 7.8 and 8.2 microns, absorbances were calculated, and graphs were plotted of absorbance at the respective wave lengths *vs.* concentration of the individual isomers. Both plots gave a straight line through all points.

Samples of Thiodan for analysis were treated in the same manner as the calibration standards.

L. Crystallization of Radio-Tagged Thiodan. Small samples of Thiodan-5a,9a-C_2^{14} were dissolved in warm hexane, filtered, and crystallized by cooling with ice water. Another crop was obtained in each case by further cooling with carbon dioxide ice, and the remaining mother liquor was evaporated to dryness. Each crop and residue were assayed chemically for Thiodan, a radio-count was determined, and the isomer ratio was estimated. The original samples assayed 85 to 93% Thiodan with a low melting isomer–high melting isomer ratio of about 2 to 1. Upon crystallization, the purer first two crops had higher radioassays than the residues. These data indicated that the non-Thiodan portion was not radioactive. The various crops had different isomer ratios from the original samples, but no generalization could be made as to the direction of enrichment. When recrystallized Thiodan-5a,9a-C_2^{14} was crystallized with recrystallized commercial Thiodan of different isomer composition, the radioactivity of the resulting crops, which differed in isomer ratios, followed as would be expected. When radio-Thiodan and commercial Thiodan, neither of which had been recrystallized, were mixed and then crystallized from hexane, the radioactivity varied with the purity of the resulting crops. These data indicated that radio-Thiodan and commercial Thiodan had the same isomer content, and that the impurities in radio-Thiodan were not radioactive.

Because crystallization of Thiodan-5a,9a-C_2^{14} would alter the isomer content, it was preferable to use the unpurified material, and because the impurities did not appear to be radioactive, it was feasible to use the radio-Thiodan in biological experiments.

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INSECTICIDE TOXICITY

Preparation and Biological Activity of a Series of Halogenated Ethyl and Vinyl Dimethyl Phosphate Esters

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Practically all of the work reported in the synthesis (*1*, *4–7*) and biological activity (*4*, *5*, *7*) of insecticidally active halogenated ethyl or vinyl dialkyl phosphates has been done on chloro-substituted compounds with passing mention of the possibility of preparing mixed bromo-chloro compounds (*6*, *7*). However, recently a report on the insecticidal properties of dimethyl 1,2-dibromo-2,2-dichlorophosphate was given by Gojemerac and Waples (*2*). This compound is available commercially under the name "Ortho-Dibrom" (California Spray Chemical Corp., Moorestown, N. J.).

The present report deals with the preparation and comparative toxicity to flies and rats of a series of tertiary phosphate esters with two methyl groups as constant constituents. The variations in the tertiary group consisted of a series of bromo, chloro, and bromo-chloro–substituted ethyl or vinyl groups.

Compounds Synthesized

The compounds synthesized are shown in Table I, together with the method of synthesis. The tetrahalogenated compounds were prepared by bromination or chlorination of the dimethyl dichlorovinyl and dibromovinyl esters. The dichlorovinyl compound (I) was a specially purified commercial product (Montrose Chemical Co., Newark, N. J.). Dimethyl 2,2-dibromoethyl phosphate (IV), the bromine analog of DDVP (I), was prepared by Perkow's reaction (*7*). The reaction was run in an ether solution at a lower reaction temperature (−10° to 0° C.) than that given by Perkow because the reaction was extremely vigorous. The reaction mixture was washed with aqueous sodium bicarbonate and several times with water. After drying with anhydrous sodium sulfate, the ether was removed under reduced pressure. Compounds II and V were obtained by bromination of I and IV. Brominations were done in carbon tetrachloride solutions with a slight excess of the calculated amount of bromine for a 24-hour period. Compounds III and VI were obtained by chlorinating I and IV. Chlorination was done by bubbling dry chlorine gas through carbon tetra-

A series of dimethyl phosphate esters was prepared in which the third ester substituents were a series of bromo, chloro, and mixed bromo-chloro–substituted ethyl and vinyl groups. Among these compounds were the 1,2,2,2-tetrabromoethyl, 1,2,2,2-tetrachloroethyl, 1,2-dibromo-2,2-dichloroethyl, 2,2-dibromo-1,2-dichloroethyl, and 2,2-dibromovinyl esters of dimethyl phosphate. Tests were made on their insecticidal activity toward houseflies and their toxicity to white rats. When compared with 2,2-dichlorovinyl dimethyl phosphate (DDVP), several compounds showed equal or greater effectiveness against houseflies, with a lesser toxicity to white rats.

chloride solutions for 3 to 4 hours while cooling the reaction mixture in a water bath. The carbon tetrachloride solutions of the reaction products were treated in the same manner as the ether solutions discussed in the previous paragraph.

Refractive indices and chemical analyses (Table I) showed a satisfactory degree of purity of these compounds. All but one of the compounds were liquids at room temperature. The tetrabromo compound (V) solidified after refrigeration, and stayed solid at room temperature. The melting point was very broad with complete liquefaction at approximately 45° C.

Toxicity to Rats and Chickens

Acute oral LD_{50} values were found for male rats using peanut oil solutions. The compounds are listed in Table II in order of increasing toxicity. Of the compounds containing only one kind of halogen, the bromine compounds are much less toxic to rats than their chlorine-containing analogs. Two of the compounds (II and VI) have both bromine and chlorine and differ only in the position of the halogen atoms. The compound (II) which has the bromine on the 1-ethyl carbon atom shows markedly less toxicity than does the compound (VI) which has chlorine in this position. However, on the basis of these limited data, no generalizations can be made regarding this phenomenon. Comparison of the toxicities on a molar basis does not change the order of the compounds, even though there is such a wide difference in molecular weights.

A dermal LD_{50} value of about 800 mg. per kg. was found for compound II using male rats. Severe edema was produced in rats at 400 mg. per kg. and above. The lesions healed, however, on surviving rats. Dermal toxicity studies were not made on the other compounds. The toxicity of this same compound (II) seemed to be greater to chickens when given subcutaneously in peanut oil in the abdominal region than would be expected from oral LD_{50} values found for rats. At a dermal dose of 100 mg. per kg. both of two chickens treated died, while at 50 mg. per kg. one of two chickens treated died. At dosages ranging from 200 to 600 mg. per kg., none of six chickens tested survived. No evidence of paralysis, which occurs with some organic phosphorus compounds, was found. Severe lesions occurred at sublethal dosages. Lesions of varying severity occurred in 10 chickens treated with 40 mg. per kg., although all the fowls tested survived until discontinued (29 days). Perforation of the abdominal wall at the place of injection occurred in three chickens at this level. These chickens seemed otherwise unaffected. The severity of these lesions caused by dermal application on rats and subcutaneous injection in chickens seems to be noteworthy, even though the levels of dosage were relatively high. It is possible that some impurities were responsible for this effect. However, the chemical analysis indicates that this material had a purity of at least 95%. Any impurities responsible for these lesions must be very active.

Table I. Methods of Synthesizing Halogenated Ethyl and Vinyl Esters of Dimethyl Phosphate and Values for Chemical Analyses

General formula. $(CH_3O)_2P(O)OR$

	R Groups[1]					
	I $CH{=}CCl_2$	II $CHBrCBrCl_2$	III $CHClCCl_3$	IV $CH{=}CBr_2$	V $CHBrCBr_3$	VI $CHClCBr_2Cl$
	Source					
	Commercial	I + 2 Br	I + 2 Cl	$(CH_3O)_3P$ + Bromal	IV + 2 Br	IV + 2 Cl
Mol. wt.	221.0	380.8	291.9	309.9	469.7	380.8
Ref. index, 25° C.	1.4516	1.5083	1.4714	1.5005	1.5530	1.5180
Carbon, %						
Theory	...	12.6	16.4	15.5	10.2	12.6
Found	...	12.1	15.7	14.6	10.4	12.3
Halogen, %						
Theory	...	60.6	48.6	51.6	68.1	60.6
Found	...	59.6	46.7	51.4	68.0	58.7
Phos., %						
Theory	...	8.14	10.6	10.00	6.60	8.14
Found	...	8.33	11.5	9.28	6.62	8.29

Table II. Acute Oral Toxicity[a] to Rats[b] of Halogenated Ethyl and Vinyl Tertiary Esters of Dimethyl Phosphate

General Formula. $(CH_3O)_2P(O)OR$

Compound	R Groups	LD_{50} Mg./kg.	LD_{50} Confidence (19/20) limits[c]	Relative Toxicity[d]
IV	$CH{=}CBr_2$	253	202–316	1
II	$CHBrCBrCl_2$	250	219–285	1
V	$CHBrCBr_3$	184	156–217	$1^1/_2$
I	$CH{=}CCl_2$	80	62–104	3
VI	$CHClCBr_2Cl$	49	45–54	5
III	$CHClCCl_3$	14.3	11.7–17.4	18

[a] Given in peanut oil.
[b] Sherman strain male rats.
[c] Method of Litchfield and Wilcoxon (*3*).
[d] Based on value of 1 for compounds IV and II.

Fly Toxicity Studies

The toxicity tests toward houseflies (Roberds strain) were done by topical applications of acetone solutions of the compounds. The LD_{50} and LD_{90} values shown in Table III indicate that all the compounds had insecticidal activity great enough to be of interest as insecticides if their other properties warranted such use. The compounds are listed in order of increasing toxicity. The order of toxicities for LD_{50} and LD_{90} values is the same, except for interchanging of the positions of I and V. The relative toxicities of these compounds, based on a value of 1.0 for the least effective (compound III), vary

Table III. Toxicity to Houseflies (Roberds) by Topical Application of Halogenated Ethyl and Vinyl Tertiary Esters of Dimethyl Phosphate

General formula. $(CH_3O)_2P(O)OR$

Compound	R Group	Micrograms per Fly		Relative Toxicity[a]	
		LD_{50}	LD_{90}	LD_{50}	LD_{90}
III	$CHClCCl_3$	0.147	0.325	1.0	1.0
IV	$CH{=}CBr_2$	0.123	0.243	1.2	1.3
I	$CH{=}CCl_2$	0.076	0.100	1.9	3.3
V	$CHBrCBr_3$	0.068	0.163	2.2	2.0
VI	$CHClCBr_2Cl$	0.059	0.093	2.5	3.5
II	$CHBrCBrCl_2$	0.048	0.082	3.0	4.0

[a] Based on value of 1.0 for compound III.

Table IV. Toxicities Toward Houseflies and Rats of Halogenated Ethyl and Vinyl Esters of Dimethyl Phosphate

General formula. $(CH_3O)_2P(O)OR$

Compound	R Group	LD_{50}, Mg./Kg.		LD_{50} Ratio, Rat/Fly
		Rat, oral	Fly[a], topical	
II	$CHBrCBrCl_2$	250	2.7	93
V	$CHBrCBr_3$	184	3.8	48
IV	$CH{=}CBr_2$	253	6.8	37
I	$CH{=}CCl_2$	80	4.2	19
VI	$CHClCBr_2Cl$	49	3.3	15
III	$CHClCCl_3$	14	8.2	2

[a] Average fly weight = 18 mg.

over the range 1 to 3 for LD_{50} values and 1 to 4 for LD_{90} values.

Comparison of Rat and Fly Toxicities

A comparison of the toxicities of these compounds toward rats and flies can be made by comparing the values for the rat-fly LD_{50} ratios (Table IV). The higher the figure the more specificity the compound has for flies and the more favorable should be its use as an insecticide. The compounds are listed in descending order of these ratios. The compounds containing only bromine give more favorable ratios than those having only chlorine, and the mixed bromo-chloro compound having the bromine on the 1-carbon atom of the ethyl group has a better ratio than its bromo-chloro isomer. This order is similar to that found for the relative toxicities toward rats. The variation in ratios showing a gradation of from 2 to 93 represents a 45-fold difference in relative rat-fly toxicities.

One interesting point is the relative toxicity of the mixed bromo-chloro compounds to rats. The two compounds (II and VI) have similar LD_{50} values for flies but differ by a factor of 5 in their LD_{50} values for rats. This large difference in rat toxicity could be due to differences in the rate of absorption of the compounds from the intestinal tract or in the chemical stability of the compounds in the digestive tract, or to differences in specificity of these compounds toward various enzymes found in the rat but not common to the fly. Thus these compounds may be of potential use in comparing the enzyme systems of rats and flies in relation to inhibition by organic phosphorus compounds.

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INSECTICIDE POTENTIATION

Effect of EPN on in Vivo Metabolism of Malathion by the Rat and Dog

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The phenomenon of "potentiation" in organophosphates, where certain combinations of compounds display an unexpectedly high toxicity, was first described in 1957 (*5*) and has been frequently confirmed (*4*). One of the best combinations for potentiation is EPN, *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothionate, with malathion, *S*-[1,2-bis-(ethoxycarbonyl)ethyl] *O,O*-dimethyl phosphorodithioate, and one of the most susceptible animals is the dog.

The low toxicity of malathion to the mammal is due to its extensive degradation by carboxyester hydrolysis (*6*). Cook *et al.* (*3*) showed that EPN in vitro inhibited the degradation of malathion by liver in vitro, and Murphy and DuBois (*4, 7*) found that it inhibited the degradation by liver in vitro of malaoxon (the oxidized form of malathion which is the actual toxicant). One would, therefore, suspect that potentiation results from an increased level of malaoxon at some target site as a result of reduced destruction of malathion and/or malaoxon. However, Seume and O'Brien (*9*) showed for several rat tissues in vitro that EPN inhibited the oxidation as well as the degradation of malathion, so that EPN led to reduced levels of malaoxon in spite of increased levels of malathion. They suggested that EPN produced potentiation by increasing the persistence of malaoxon at some target site, rather than by raising its short-term concentration. In support of this, they showed that with the rat EPN in vivo or in vitro

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