THE INSECTICIDAL PROPERTIES OF SOME ESTERS OF PHOSPHORUS ACIDS

BY

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A.B., University of Illinois, 1941
A.M., University of Illinois, 1947

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ENTOMOLOGY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1949

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THE GRADUATE COLLEGE

January 8, 1949

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† Required for doctor's degree but not for master's.

3M—1-48—37560

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I. FOREWORD AND ACKNOWLEDGEMENTS

Interest in certain phosphorus acid esters as insecticides and acaricides has developed quite rapidly in the United States since the toxic ingredient of the German nicotine substitute Bladan, was reported to be hexaethyl tetraphosphate (Anonymous, 1945). In part, this interest has been due to the need for a nicotine substitute in this country (Bishopp, 1946a) since the present demand exceeds the available supply. Also, a considerable degree of control of certain species of phytophagous mites of considerable economic importance is obtained by the use of either of the three phosphorus acid esters now in commercial production.

The purpose of this investigation was to determine the insecticidal properties of a relatively large group of phosphorus acid esters in order to obtain an indication as to the scope of their toxicities and possible usefulness, and so that an attempt may be made to relate their toxicity to chemical structure.

This investigation was conducted under the able and invaluable guidance of Dr. C. W. Kearns of the Department of Entomology and Dr. G. C. Decker of the Illinois Natural History Survey. Mrs. Jeane C. Warner ran the contact spray tests against the house fly and gave valuable assistance in other phases of the investigation. The Monsanto Chemical Company supported the project and furnished the great majority of the materials tested. The author expresses his gratitude to these persons for their aid and counsel.

II. INTRODUCTION

Following the end of hostilities in Europe in 1945, various Allied scientific investigation teams entered Germany to obtain all manner of technical information and data. As a result of these activities numerous reports have been issued by the British and United States governments on all phases of German scientific endeavor before and during the war. Among these were included reports on German insecticide research and development (Smadel and Curtis, 1945, Hall, 1945, Kilgore, 1945a). Additional information is given by Kilgore (1945b, 1946a,b) on German insecticides and insecticide testing. He found German testing methods to be quite crude and haphazard as compared to our Peet-Grady and other methods.

Among the insecticides reported, Bladan, a formulated nicotine substitute, attracted considerable attention because its active ingredient, hexaethyl tetraphosphate (HETP), was a unique synthetic organic insecticide. This chemical was discovered and patented by Schrader (1942, 1943). Bishopp (1946b) mentioned that HETP was claimed to be an excellent substitute for nicotine in the control of aphids, but in this country little else was known regarding its insecticidal properties until the flow of information from government agencies increased, and the reporting of insecticidal tests began. Later Bishopp (1946a) reported on preliminary results with the material against several insects and pointed out that it hydrolysed readily in water, was highly toxic to warm-blooded animals, that it was somewhat disagreeable to apply and that concentrated formulations might produce plant injury. Subsequently, Blauvelt (1947) found that it caused an injury to rose blooms called "bull

headedness" and warned growers to exercise caution when using the material.

Hoark (1947) summarized the information available on hexaethyl tetraphosphate to April, 1947. His summary covered its preparation, physical and chemical properties, manufacture, formulation, stability, effect on metals, effect on plants, toxicity to mammals, patents, and insecticidal value.

Ludvik and Decker (1947) considering the uniqueness of hexaethyl tetraphosphate as an economic poison, undertook a study of various phosphorus acid nuclei with differing substituent groups in an effort to circumscribe the toxicity of organic phosphorus compounds in general. Their work resulted in the discovery of seven phosphorus esters exceeding nicotine in toxicity. Two of these, tetraethyl pyrophosphate and tetra-n-propyl pyrophosphate, exceeded hexaethyl tetraphosphate in toxicity. Interest immediately developed in the insecticidal potentialities of tetraethyl pyrophosphate. It should be pointed out that Schrader (1947) had prepared and investigated the insecticidal properties of tetraethyl pyrophosphate but that the results of his work were not released by the British Intelligence Objectives Sub-Committee until after the work of Ludvik and Decker appeared. The physics and chemistry, insecticidal and phytotoxic properties, toxicity to higher animals, and effects on containers and equipment of tetraethyl pyrophosphate have been reviewed by Harris (1947).

The first American publication on diethyl-p-nitrophenyl thiophosphate is that of Gleissner (1947) which outlines its comparative toxicity, physical and chemical properties, insecticidal activity, and toxicity to warm-blooded animals.

III. REVIEW OF THE LITERATURE

A. CHEMISTRY

That hexaethyl tetraphosphate actually consists of a mixture of chemicals, the toxic ingredient being tetraethyl pyrophosphate, was reported by Hansen (1947a,b). Hall and Jacobson (1948a) made detailed chemical studies on hexaethyl tetraphosphate which confirm Hansen's report. Subsequently, Rohwer and Haller (1948) in reporting on industry-government cooperative studies on tetraethyl pyrophosphate, briefly outline its chemistry, toxicology, labeling, naming, and tentative procedures for its analysis. The decision was made that materials containing up to 20 per cent of the active ingredient would be designated hexaethyl tetraphosphate, while materials containing more than 35 per cent would be designated tetraethyl pyrophosphate (TEPP), with the stipulation that the per cent of active ingredients be clearly indicated on the package label.

Probably the most important single account to date of research on the chemistry of phosphorus acid esters and their insecticidal properties is that of Schrader (1947). It is a summary of his research done in Germany, largely during the period 1938-1945, and covers the three phosphorus acid esters now quite well known to American economic entomologists; hexaethyl tetraphosphate, tetraethyl pyrophosphate, and diethyl p-nitrophenyl thiophosphate (parathion). Undoubtedly, several more German compounds will receive considerable attention from American entomologists and chemists.

Bronson and Hall (1946) have reported on the preparation and properties of hexaethyl tetraphosphate, but the most important and detailed account to date of the chemistry of hexaethyl tetraphosphate and tetraethyl pyrophosphate is that of Hall and Jacobson (1948). They show that the latter chemical is the insecticidally active ingredient of the former. They found ethyl metaphosphate

and triethyl orthophosphate to be present in hexaethyl tetraphosphate and suggest that pentaethyl triphosphate may be another constituent of the mixture.

Methods for the preparation of hexaethyl tetraphosphate (I) and tetraethyl pyrophosphate (II) are indicated by the following equations:

$POCl_3 + 3\ (C_2H_5)_3PO_4 \longrightarrow 3\ C_2H_5Cl + (C_2H_5)_6P_4O_{13}$ (I) (Schrader, 1942)

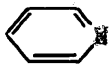
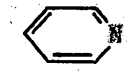
$4\ POCl_3 + 9\ C_2H_5OH \longrightarrow 9\ HCl + 3\ C_2H_5Cl + (C_2H_5)_6P_4O_{13}$ (I) (Thurston, 1946)

$2\ (C_2H_5)_3PO_4 + P_2O_5 \longrightarrow (C_2H_5)_6P_4O_{13}$ (I) (Woodstock, 1946)

$5\ (C_2H_5)_3PO_4 + POCl_3 \longrightarrow 3\ C_2H_5Cl + 3\ (C_2H_5)_4P_2O_7$ (II) (Hall, 1948a)

$4\ (C_2H_5)_3PO_4 + P_2O_5 \longrightarrow 3\ (C_2H_5)_4P_2O_7$ (II) (Woodstock, 1946)

$(C_2H_5O)_2POCl + (C_2H_5)_3PO_4 \longrightarrow C_2H_5Cl + (C_2H_5)_4P_2O_7$ (II) (Hall, 1948a)

$(C_2H_5O)_2P(O)Cl + H_2O +$  $\longrightarrow (C_2H_5O)_2P(O)OH +$ 

$(C_2H_5O)_2P(O)Cl + HOP(O)(OC_2H_5)_2 +$  $\longrightarrow$

$(C_2H_5O)_2P(O)OP(O)(OC_2H_5)_2$ (II) + 

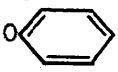
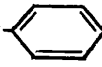
Schrader (1947) prepared diethyl p-nitrophenyl thiophosphate (III) as indicated by the following equations:

$2\ C_2H_5ONa + PSCl_3 \longrightarrow (C_2H_5O)_2P(S)Cl + 2\ NaCl$

 $+ (C_2H_5O)_2P(S)Cl \longrightarrow (C_2H_5O)_2P(S)O-$  $+ NaCl$

German activity in this field stemmed from research on organic fluorine compounds having sulfur as the central atom. The possibilities, according to Schrader, of preparing new compounds were exhausted when the following linkages were explored: N-S, C-S, and O-S, as typified by $(CH_3)_2NSO_2F$, CH_3SO_2F, and $C_6H_5OSO_2F$. Since phosphorus is adjacent to sulfur in the periodic table of elements, the decision was made to use phosphorus as a new central atom and to investigate the properties of a series of compounds similar to those studied with sulfur. The compound $(CH_3)_2NPOCl_2$, analogous to $(CH_3)_2NSO_2F$, was converted to $(CH_3)_2NPOF_2$ which showed mild insecticidal action. This led to a study of compounds of the type $(CH_3)_2NP(O)(OR_1)OR_2$.

Further work led to a patent (German Pat. 155/39, Top Secret) of compounds of the general type $R_1(R_2)NP(O)(OR_3)$ Acyl, for insecticidal purposes (R_1, R_2, and R_3 are alkyl while the acyl can be an inorganic or organic acid residue). Next, $R_1(R_2)NP(O)F_2$ was converted to $((R_2)N)_2P(O)F$ with several derivatives showing considerable insecticidal activity. When the molecule $((CH_3)_2N)_2P(O)F$ was doubled, the previously unknown pyroester, $((CH_3)_2N)_2P(O)OP(O)(N(CH_3)_2)_2$, was obtained.

A third group of compounds of general type $R_1O(R_2O)P(O)F$ was investigated next, with the diethyl derivative exhibiting remarkable activity. Evidently the preparation of hexamethyl tetraamido pyrophosphate and the discovery of its insecticidal properties led to the investigation of tetraethyl pyrophosphate and to its mono- and dithio derivatives, all of which possess high insecticidal activity. The replacement of one oxygen by one sulfur atom made the pyrophosphate ester stable to water, while the replacement of both oxygen atoms with sulfur atoms imparted stability to lime. Substitution of acidic organic groups in the radical $(C_2H_5O)_2P(O)-$ led to the preparation of diethyl p-nitrophenyl phosphate $(C_2H_5O)_2P(O)O$  NO_2 and its thio derivative $(C_2H_5O)_2P(S)O$  NO_2. As with the pyrophosphate ester, the sulfur atom provided stability toward lime.

The physical properties of hexaethyl tetraphosphate, tetraethyl pyrophosphate, and diethyl p-nitrophenyl thiophosphate are:

	HETP	TEPP	Parathion
Refractive Index, n_D^{25}	1.4265	1.4170	1.5360
Specific gravity, d_{25}^{25}	1.2833	1.1845	1.26
Viscosity, 25°C.	51.5	5.1	--
Boiling point	Decomposes 165°C.	104-110°C. 0.08mm.	375°C., 760 mm.

Contrary to reports from German sources, Hall and Jacobson (1948a) found that the hexaethyl tetraphosphate mixture hydrolyzes to a mixture of diethyl and monoethyl orthophosphoric acids and not to ethanol and orthophosphoric acid. Tetraethyl pyrophosphate hydrolyzes to diethyl orthophosphoric acid.

Hall and Jacobson (1948b) present a method of analysis for tetraethyl pyrophosphate based on its hydrolysis to diethyl phosphoric acid, which can then be titrated with standard alkali.

A proposed method for the analysis of parathion is based on its reduction to diethyl p-aminophenyl thiophosphate followed by diazotization and coupling with a chromphoretic chemical. Colorimetric comparisons with previously prepared standards can then be made to determine the amount of parathion present. This method is claimed to detect parathion down to a level of 0.1 ppm. (Averell, private communication).

III. B. INSECTICIDAL RESULTS

Bronson and Hall (1946) published the first data on the insecticidal activity of hexaethyl tetraphosphate. They showed that it was superior to nicotine against the cabbage aphid, Brevicoryne brassicae (L.) and the pea aphid, Macrosiphum pisi (Kltb.).

Fayette, et al, (1946) reported good results from the use of a hexaethyl tetraphosphate (HETP 1 to 1600 parts of water) spray formulation against the two-spotted spider mite, Tetranychus bimaculatus (Harvey) on roses. They observed that though some eggs were affected, many subsequently hatched, necessitating at least two sprays to give satisfactory control.

In experiments with insecticides on honeybees, Eide (1947) found that a hexaethyl tetraphosphate residue dried for 18 hours possessed considerable toxicity with some residual activity remaining for at least nine days.

Ludvik and Decker (1947) report that hexaethyl tetraphosphate at 1 to

10,000 gave a 63 per cent kill of Myzus porosus (Sanderson), an aphid infesting roses, while tetraethyl pyrophosphate at 1 to 40,000 gave a kill of 52 per cent of the same species. They tested 44 other phosphorus acid esters against either M. porosus or M. persicae (Suls.).

Hexaethyl tetraphosphate at 1 to 1600 killed 82-98 per cent of pear psylla nymphs, Psylla pyricola (Foerst) according to Hamilton (1947). Treatments made in August, 1946, did not give as good control as nicotine-sulfate at 1 to 800, but later in the season when the temperature was lower, the effectiveness of the two materials was reversed. Control of pear psylla adults could be effected only when an oil was added to the spray mixture. Tests by Hamilton (1948) showed during the following season that although sprays of nicotine sulfate, hexaethyl tetraphosphate, tetraethyl pyrophosphate killed high percentages of pear psylla nymphs present at the time of application, the populations built back rapidly. Parathion, however, exhibited a high initial kill and also held the population to a low level for a considerable period of time.

Smith, et al. (1947) tested hexaethyl tetraphosphate aerosols in greenhouses. They used a 10 per cent mixture of commercial grade material in methyl chloride at dosages ranging from 0.3 to 6.0 grams of HETP per 1,000 cubic feet of space. Applications were made at dusk. A dosage of one gram per 1,000 cubic feet was found to be toxic to the two-spotted spider mite, the greenhouse white fly, two species of mealy bugs, and seven species of aphids, but not to the broad mite. Lower dosages were also effective against aphids and white flies. During fumigation, the temperature was held at 70°F., and the ventilators were not opened until the morning following treatment. A varietal susceptibility to injury was found with tomatoes and chrysanthemums, with the injury tending to be more severe when treatments were made at lower temperatures. Over 130 other plant species were unaffected, however. Smith et al. (1948) discuss further the

use of hexaethyl tetraphosphate aerosols in the greenhouse and also report on similar experiments with tetraethyl pyrophosphate. Smith et al. (1948b) have tested parathion aerosols for this purpose.

Hinman (1947) reports on the use of HETP for the control of grasshoppers. Two pounds of HETP in 160 gallons of water per acre gave 100 per cent kill, but also severely burned quarter-bloom-stage alfalfa.

A DDT aerosol (1 gallon of 25 per cent in 4 gallons of kerosene) was more effective than an HETP aerosol (1 pint of HETP in 5 gallons of water) against the melon aphid, Aphis gossypii (Glov.) in experiments carried out by Wene (1947), using a Todd insecticidal fog applicator.

Siegler and Hall (1947) found freshly prepared HETP solutions to be toxic to newly hatched codling moth larvae, Carpocapsa pomonella (L.) at concentrations of one pound and one-half pound per 100 gallons of water, but concluded that the material would have little or no practical use for the control of this pest because it lacks necessary residual properties. They also reported the mortalities produced by HETP on a number of aphids and mites.

Lange and Smith (1947) exposed adult pea leaf miners, Liriomyza orbona (Meig.) to one, two, and three microgram deposits per 10 square inches of surface (glass) of chlordan, benzene hexachloride, DDT, and hexaethyl tetraphosphate. The materials were most effective in the order enumerated.

Ivy and Ewing (1947) found 1.25% hexaethyl tetraphosphate dusts to be extremely effective against cotton aphids. Five per cent HETP in pyrophyllite was approximately as toxic to adult and nymphal cotton fleahoppers as 5% DDT-sulfur, but was ineffective against boll weevils and bollworms. Gaines and Dean (1948) report that good control of these aphids was also obtained by the use of one per cent parathion dust.

Zimmerman and Hartzell (1947) reported the toxic effects of hexaethyl

tetraphosphate and tetraethyl pyrophosphate to a number of plants. Thermal evaporation of both materials caused rose leaves to drop and produce a burning and epinasty of tomato leaves. It was assumed that ethylene was formed upon decomposition and that this gas was responsible for the injuries observed. Hexamethyl tetraphosphate did not give this injury, presumably because ethylene would not be formed upon decomposition. Varying concentrations of the two materials when applied to the soil of potted plants produced varying degrees of injury.

Gaines (1947) found parathion to show promise in the control of grasshoppers on cotton.

Questel and Connin (1947) report the translocation of parathion from treated soil to the tissues of growing corn seedlings. This plant tissue was toxic to newly hatched corn borer larvae, Pyrausta nubilalis (Hbn.) for at least two weeks.

Blauvelt and Hoffman (1948) report parathion to be the most important material yet discovered for greenhouse pest control. Aerosols containing 10% parathion by weight and 90% liquified methyl chloride gave the best results when applied at the rate of one pound of aerosol to 50,000 cubic feet, and remained effective for at least one week both as a contact and stomach poison. This aerosol mixture has proved to be effective against a wide variety of greenhouse pests.

Brett and Rhoades (1948) report that two per cent parathion dust at the rate of 10 pounds per acre killed 95% of the grasshoppers in alfalfa in eight hours.

Dills and Odland (1948) have obtained poor results with hexaethyl tetraphosphate and tetraethyl pyrophosphate against the cabbage maggot.

Jones and Rosentiel (1948) tested 0.25% parathion dust against the two-spotted

spider mite on field grown beans and found it to be highly effective for two days but ineffective after 16 days. It was the most toxic of seven materials tested against the black bean aphid, Anuraphis persicae-niger Smith. In laboratory tests, the cherry fruit fly, Rhagoletis cingulata Loew., was found to be susceptible to parathion, also.

Huckett(1948) reported hexaethyl tetraphosphate sprays and parathion dusts as giving very good control of the two-spotted spider mite on lima beans. These treatments also exhibited a minimum degree of bean aphid and Mexican bean beetle damage as compared with other materials tested.

According to Harman (1948), two applications of 15% wettable parathion give much better control of second-brood red-banded leafroller on apples than do DDT and lead arsenate.

Two applications of 15% wettable parathion likewise gave better control of the plum curculio, Conotrachelus nenuphar (Hbst.), than lead arsenate in tests made by Dewey and Van Geluwe (1948). Snapp (1948) also found parathion promising for the control of this pest.

Anderson and Hofmaster (1948) obtained poor results with both parathion and hexaethyl tetraphosphate against the pea aphid.

Soil treatments by Schread (1948) against Japanese beetle larvae showed parathion to be more rapid in action than chlordan, benzene hexachloride, chlorinated camphene, and DDT, but the experiment was not of sufficient duration to determine the ultimate soil residual toxicities.

Gersdorff and Nelson (1948) report tetraethyl pyrophosphate to be about five times and parathion to be about 17 times more toxic than hexaethyl tetraphosphate to the house fly, Musca domestia L., in contact spray tests. The mean concentrations producing 50% mortality are 0.095, 0.030, and 0.52 mg./ml., respectively.

Carruth and Howe (1948) obtained effective control of the squash vine borer, Melittia cucurbitae (Harr.), with one per cent parathion dust.

From aerosol tests with hexaethyl tetraphosphate and tetraethyl pyrophosphate, Hoffman (1948) concludes that three grams of each per thousand cubic feet is the optimum dose to obtain control of the two-spotted spider mite. HETP was found to be about one-third as toxic as TEPP to this species.

Scales and Smith (1948) report two per cent parathion dust as giving a mortality of the tarnished plant bug, Lygus oblineatus (Say), on cotton, comparable with that of 20% chlorinated camphene and 2.3% gamma benzene hexachloride.

Considerable promise was shown by a one per cent parathion - 55% sulfur dust against the grape leaf roller, Desmia funeralis (Hbn.), in trials made by Frazier and Barnes (1948).

Eckert (1948) determined the approximate LD-50 of hexaethyl tetraphosphate, tetraethyl pyrophosphate, and parathion to the honeybee to be 0.29, 0.075, and 0.07 micrograms per bee, respectively.

Chamberlin et al. (1948) and Peterson and Sherman (1948) report that hexaethyl tetraphosphate and tetraethyl pyrophosphate give little or no control of meadow spittlebug nymphs, Philaenus leucophthalmus (L.).

Boll weevil larvae developing in cotton squares were unaffected by 5 per cent parathion dust, according to Gaines and Scales (1948).

In experiments on the control of the greenbug, Toxoptera graminum (Rond.), Dahms (1948) found gamma benzene hexachloride to give excellent control. The initial control obtained with hexaethyl tetraphosphate was good, but after three weeks, the infestation was greater than when treatment was made. The yield of the treated oats, however, was significantly greater than that of the check plot.

Ewing and Parencia (1948) reported that parathion dusts are ineffective against the boll weevil on cotton.

Ginsburg (1948) reports data which indicate that parathion is considerably more toxic than DDT to Aedes aegypti larvae and pupae.

Jefferson and Pence (1948) report that preliminary tests with parathion against the leaf miner, Liriomyza flaveola (Fallen), infesting asters show sufficient promise to warrant further testing.

Other phosphorus acid esters that have shown considerable insecticidal activity are tetra-n-propyl pyrophosphate, tetra-n-butyl pyrophosphate, sym. phenyl tetra-n-propyl triphosphate and sym. tetra-n-butyl phenyl triphosphate, which were tested by Ludvik and Decker (1947) against the aphid Myzus porosus Sanderson.

Wolcott (1947) found triphenyl phosphate to be of considerable interest as a repellent for the West Indian dry-wood termite, Cryptotermes brevis Walker. This compound was not toxic to the green peach aphid in the tests of Ludvik and Decker.

Tri-o-cresyl phosphate showed only slight effectiveness while tri-n-butyl phosphate appeared to be quite promising in small-plot field tests against chiggers, according to Linduska, et al (1948).

Knipling, et al (1947) report the results of screening tests with some 7000 chemicals as insecticides, miticides, and repellents. Included are results from 37 phosphorus acid esters, one of which, tri-n-butyl phosphate, showed considerable promise as an insecticide against the body louse, Pediculus humanus corporis Deg., showed some promise as a repellent to chiggers and considerable promise as a repellent to Aedes aegypti L. when applied either to the skin or clothing.

Schrader (1947) gives chemical preparations for various organic

phosphates and their insecticidal value insofar as his war-scattered notes permit. Further reference will be made to Schrader's work as it applies to certain compounds included in this study.

III C. MODE OF ACTION

Present data indicate that the effectiveness of the several organic phosphate esters is due to their inhibition of choline esterase, an enzyme thought to be intimately concerned with the transfer of electrical nerve impulses across neuromuscular synapses.

Stotz and Hastings (1937) found 6 x 10^{-5} M pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$) to give a 38% inhibition of the oxidase activity of beef heart extract with the dehydrogenase activity remaining unaffected. Hadidian and Hoagland (1941) found however upon buffering the enzyme-substrate solution to pH 7.4, that concentrations up to 6.0 x 10^{-5} M pyrophosphate did not produce inhibition of the oxidase component as indicated by the oxidation of p-phenylenediamine. Lower concentrations of pyrophosphate though, did produce a 90% inhibition of the oxidation of succinate by the enzyme extracts. Since Stotz and Hastings had shown that pyrophosphate did not poison the dehydrogenase component, and since the lack of inhibition of the p-phenylenediamine reaction indicated that pyrophosphate did did not poison the oxidase component, only one conclusion could be drawn - that a further step must exist in the sequence besides the two which involve succino-dehydrogenase and cytochrome-c-cytochrome oxidase in the oxidation of succinate to fumarate. Temperature studies of pyrophosphate-poisoned reactions yielded further support for the presence of this third step. The new reaction gave an Arrhenius u (energy of activation) of 17,500 calories as compared with us of 11,200 and 16,000 for the succino-dehydrogenase and cytochrome-c-cytochrome oxidase steps, respectively. These authors postulated that pyrophosphate might act on cytochrome a or b, but did not limit the possibilities to these reactions

er components only.

Dubois and Mangum (1947) in an attempt to determine the mode of action of hexaethyl tetraphophate, obtained a 50% inhibition of rat brain cholinesterase activity from a 1.6×10^{-8} M concentration of hexaethyl tetraphosphate as compared to a 6.3×10^{-8} M concentration of di-iso-propyl fluorophosphate. Concentrations of 1×10^{-7} M hexaethyl tetraphosphate produced an inhibition of 47% and 58% of rat brain and cockroach thoracic cholinesterase activity, respectively. Chadwick and Hill (1947) have obtained results similar to those of DuBois and Mangun. Hansen (1947) speculates from a study (unreported) of a large number of phosphate compounds that diethyl phosphoric acid is the break-down product formed in the insect body which is responsible for the toxic action of tetraethyl pyrophosphate. This conclusion was based on the observation that only those phosphate esters having two alkyl groups per phosphorous atom are toxic, while mono- and trialkyl compounds are relatively non- toxic. This postulation was not borne out, however, in experiments made by the author, who injected and applied topically approximately one cubic millimeter of diethyl phosphoric acid to a number of male, large milkweed bugs, Oncopeltus fasciatus Dallas. No mortality resulted from either treatment, whereas approximately one cubic millimeter of a 5 mg.-per-milliliter solution of tetraethyl pyrophosphate produced 100% mortality. Unpublished data of the author show, however, that diethyl phosphoric acid does exhibit a marked in vitro inhibition of American-roach thoracic and abdominal nerve cord cholinesterase. The author is not prepared to explain this apparent discrepancy. This might tend, however, to support Hansen's postulation that the mechanism of action of tetraethyl pyrophosphate may be an interference with a phosphate enzyme similar to adenosine triphosphate.

III D. TOXICITY TO HIGHER ANIMALS

Early reports on hexaethyl tetraphosphate stressed that the material was highly toxic to higher animals as well as insects, and that due care and caution should be exercised in handling it. Rohwer (1947) reported the minimum lethal dose by oral ingestion to be as low as five mg./kg. and the fatal dose to rabbits by skin absorption to be 5-10 mg./kg. He described the symptoms of poisoning in experimental animals as progressing from gastro-intestinal tract upset, anorexia, and severe diarrhea to a characteristic "head-drop", a great weakness and apathy, depending on the severity of the poisoning. Rohwer and Haller (1948) report the LD 50 of distilled tetraethyl pyrophosphate to white mice as being 0.80 mg./kg., but do not give the method of application.

Krop (1948) reports that 200 to 500 micrograms per kilogram of undiluted tetraethyl pyrophosphate dropped into the conjunctival sac of dogs may be lethal. Assuming that conjunctival absorption of and susceptibility to tetraethyl pyrophosphate are of the same order of magnitude in man and the dog, 0.015 to 0.035 cubic centimeters might therefore prove lethal to man.

Lehman (1948) reports the following data for selected agricultural chemicals:

Oral toxicity (no test animal cited)

Insecticide	Mean Lethal Dose, mg./kg.
TEPP	2
Parathion	3.5
HETP	7
Nicotine	10

Dermal Toxicity (no test animal cited)

	Skin Irritation	Dangerous Quantities			Quantities dangerous to man (Est'd)	
		Single Exposure		Multiple Exposure	Single Exposure	Multiple Exposure
		Dry	Solution	Solution		
		mg./kg.	mg./kg.	mg./kg.	grams	grams/day
TEPP	Slight		10	5	0.6	0.3
Parathion	Slight	40	50	5	3	0.3
HETP	Slight			5		0.3
Nicotine	Moderate	60	50	40	3	2.4

Chronic Toxicity in Rats

Insecticide	Lowest level producing gross effects	Duration	Remarks
	p.p.m.	Weeks	
Parathion	25	4	
HETP		12	1,000 p.p.m., no effect
Nicotine	60	43	

Pathology in chronically poisoned animals

Insecticide	Predominant Injury
TEPP	Enterocolitis and gall bladder necrosis
Parathion	" " " " "
HETP	Gall bladder necrosis
Nicotine	Inanition

In discussing laboratory toxicological tests, Smyth and Carpenter (1948) reported that tri-(2-ethylhexyl) phosphate when administered in food to groups of 10 rats for 30 days at the rate of 1.55 mg./kg. caused a reduction in growth. The maximum dose having no effect was 0.43 mg./kg., and the single oral LD 50 dose was reported as being 37.08 mg./kg..

IV. EXPERIMENTAL PROCEDURES

A. TEST INSECTS

Myzus persicae Sulzer

Cultures of the green peach aphid, Myzus persicae, were maintained on potted eggplants grown in the greenhouse. Individual leaves infested with 100 to 200 aphids of various stages of development were cut from the plants and sprayed. The stem of the sprayed leaf was inserted through a paper disk, wrapped in cotton, and set into a small bottle of water. The unit was then placed within a heated wire barrier (Bruce, 1947) to prevent the loss of unaffected aphids.

Myzus porosus Sanderson

Cultures of the aphid Myzus porosus were maintained on potted rose plants grown in the greenhouse. Individual leaves infested with approximately 100 aphids of various stages of development were cut from the plants and sprayed. The petiole of a sprayed leaf was trimmed and inserted into the tube of a watering vial described by Ludvik and Decker (1947) and the unit placed within a heated wire barrier.

Attagenus piceus Oliv.

Larvae of the black carpet beetle, Attagenus piceus, were reared in the laboratory in a medium consisting of two per cent Brewers' yeast in chick starter mash. Larvae about three-eights of an inch in length were selected for testing.

Musca domestica L.

Five day old adults of the standard N.A.I.D.M. strain of the house fly, Musca domestica, were used in the present work. The rearing procedure used was that described for the Peet-Grady method in Soap Blue Book (1946).

IV B. TESTING TECHNIQUES

Contact Sprays

Myzus persicae, M. porosus, and Musca domestica were used as test insects for determining the effects of the chemicals as contact sprays. An atomizer-type, compressed air paint sprayer of one-pint capacity operating at pressure of 30 pounds per square inch was used to spray excised leaves infested with either Myzus persicae or M. porosus. Solutions were prepared by pipetting 0.1 milliliter of chemical into a measured volume of stock solution containing one gram of sodium decylbenzenesulfonate per 5,000 cubic centimeters of distilled water. Water insoluble liquids were thoroughly shaken in a graduated cylinder to obtain an homogeneous emulsion and sprayed as rapidly as possible so that a uniform spray would result. Weighed quantities of solid chemicals were dissolved in a known volume of dioxane and their subsequent water mixtures handled in the manner described for water insoluble liquid compounds. In the case of M. persicae, tests were made only at a concentration of 0.2 per cent, whereas with M. porosus, sufficient subsequent dilutions were made to determine the range of effectiveness of the chemical under consideration.

Contact spray tests against adult house flies were carried out in accordance with the small chamber method developed by Kearns and March (1943). All materials were tested at a concentration of one per cent by weight in 50 per cent benzene - 50 per cent mineral seal oil or in 50 per cent methyl alcohol - 50 per cent benzene, depending upon the solubilities of the chemicals. Chemicals which exhibited toxicity were tested at lower concentrations to determine the range of their effectiveness. Each contact spray test was run five times.

Residual Activity.

Tests for residual activity were made by exposing five day old adult house flies for 30 minutes to deposits on glass of 50 milligrams of chemical per

square foot of surface area. The flies were reared as described for the contact spray tests, but the puparia were placed in 7.5 x 7.5 x 1.25 inches wooden cages having screen tops and sliding metal bottoms. The sliding bottom facilitated removal of the empty puparia, and also allowed the flies to have free access to the treated surface during the period of exposure. For exposure, the cage was inverted and covered with a glass plate (treated surface down). These units were stacked 10 high and the metal slides pulled out for 30 minutes. The treated surface was prepared by pipetting two milliliters of an absolute ethyl alcohol solution, made up to contain 12.5 milligrams of chemical per milliliter, onto a one-half square foot area of a nine inch square glass plate. The solution was spread over the surface as evenly as possible with a small piece of glass.

Circles of wool (Fletcher, 1943) two square inches in area were treated with one milliliter of the solution used for the preparation of the glass plates for the house fly tests. After the alcohol evaporated from the wool, the circles were placed in 35 x 50 millimeter crystalizing dishes and manually infested with 10 Attagenus piceus larvae. When all 10 larvae were killed by a particular chemical, 10 more larvae were placed upon the wool. Three glass plates and three wool circles were prepared for each chemical.

Observations of mortality in all insects except Attagenus piceus were made the day after treatments or exposures were completed.

V. EXPERIMENTAL RESULTS

In so far as possible, all chemicals were tested as contact sprays against the green peach aphid, Myzus persicae, and the house fly, Musca domestica. Chemicals which exhibited marked insecticidal activities against these two species were subsequently tested to determine the susceptibilities of different species, to establish toxicity ranges, and to investigate residual activities.

Certain data from the work of Ludvik and Decker (1947) are included in the presentation of the results of the present study to facilitate the comparison of toxicities, and because certain compounds tested by them were evaluated against another insect species.

The results of this investigation are presented in the following tables:

Table 1. Toxicity of certain phosphorus acid esters applied as contact sprays to Myzus persicae, M. porosus, and Musca domestica.

Compound		Per Cent Kill		
		Myzus[1] persicae	Myzus[1] porosus	Musca[2] domestica
Alkyl Acid Phosphate Esters: ($(HO)_2P(O)OR$ and $HOP(O)(OR)_2$)[3]				
Methyl	R = CH_3	51.4*[4]		1.3 (m)[5]
Ethyl	R = C_2H_5	37.8*		3.2 (m)
Propyl	R = C_3H_7	55.1*		1.7 (m)
Isopropyl	R = $(CH_3)_2CH$	73.6*	46.5*	3.4 (m)
Butyl	R = C_4H_9	62.2*		4.6 (m)
Isoamyl	R = mixed primary C_5H_{11}	56.6*		3.2 (m)
Octyl	R = C_8H_{17}	79.4*	66.0*	9.3
Decyl	R = $C_{10}H_{21}$	65.9*		6.0 (m)
Loralkyl	R = mixture of 10 to 18 carbon chains	4.5*		8.3
Dialkyl Acid Phosphate Esters: ($HOP(O)(OR)_2$)				
Diethyl	R = C_2H_5	13.4		4.1 (m)
Diethyl thionothio[6]	R = C_2H_5	17.2		6.7
Dibutyl	R = C_4H_9	22.4		7.8
Trialkyl and Triaryl Phosphate Esters: ($(RO)_3PO$)				
Trimethyl	R = CH_3	52.2*		5.3
Triethyl	R = C_2H_5	14.7*		4.9
Triethyl thio-	R = C_2H_5	17.7		0.2
Tri-(2-chloroethyl)	R = ClC_2H_4	45.6*		9.4
Tripropyl	R = C_3H_7	22.2		5.1

Table 1, continued

Compound		Per Cent Kill: Myzus persicae	Myzus perosus	Musca domestica
Triallyl	R = CH_2=$CHCH_2$	28.8*		9.9
Trimethallyl	R = CH_2=$C(CH_3)CH_2$	33.9*		2.0 (m)
Tri-(2,3-dichloropropyl)	R = $ClCH_2CH(Cl)CH_2$	3.5		4.2
Tributyl	R = C_4H_9	59.8		6.0
Triisobutyl	R = $(CH_3)_2CHCH_2$	23.7		8.8
Triamyl	R = C_5H_{11}	66.6		7.0
Triisoamyl	R = $(CH_3)_2CH(CH_2)_2$	66.0		6.8
Tri-(2-methylamyl)	R = $C_2H_7CH(CH_3)CH_2$	90.2		9.9
Tri-(2-ethylhexyl)	R = $C_4H_9CH(C_2H_5)CH_2$	87.1*	51.1*	4.1
Tridodecyl	R = $C_{12}H_{25}$	41.8		3.7 (m)
Triphenyl	R = C_6H_5	21.2*		3.3
Tricresyl	R = $CH_3C_6H_4$	20.3*		6.6
Tri-(o-chlorophenyl)	R = ClC_6H_4	5.3*		4.2
Tri-(2,4,6-trichlorophenyl)	R = $Cl_3C_6H_2$	8.2*		2.6 (m)
Mixed Alkyl-Aryl Phosphate Esters: $((RO)_2P(O)OR')$				
Diethyl octyl	R = C_2H_5 R' = C_8H_{17}	97.7	98.9	99.0
Acetyl diethyl	R = C_2H_5 R' = CH_3COO	100.0		
Diethyl phenyl	R = C_2H_5 R' = C_6H_5	99.1	9.8	39.8
Diethyl phenyl thio-	R = C_2H_5 R' = C_6H_5	30.9		5.0

Table 1, continued.

Compound	Per Cent Kill: Myzus persicae	Myzus porosus	Musca domestica
Diethyl p-nitrophenyl thio- R = C_2H_5 R' = $O_2NC_6H_4$	99.8		
Diethyl benzyl R = C_2H_5 R' = $C_6H_4CH_2$	24.3		11.7
Di-(2-chloroethyl) Loralkyl R = ClC_2H_4 R' = mixture of 10 to 18 carbon chains	40.8		9.7
Dibutyl ethyl R = C_4H_9 R' = C_2H_5	55.1		6.7
Mixed butyl 2-ethylhexyl	95.0		5.5
Dibutyl phenyl R = C_4H_9 R' = C_6H_5	58.7		2.3
Di-(2-ethylhexyl) phenyl R = $C_4H_9CH(C_2H_5)CH_2$ R' = C_6H_5	80.5		1.4
Methyl diphenyl R = C_6H_5 R' = CH_3	70.0		11.5
Ethyl diphenyl R = C_6H_5 R' = C_2H_5	27.0		1.2
2-Methoxyethyl diphenyl R = C_6H_5 R' = $CH_3OC_2H_4$	17.9		5.4
2-Butoxyethyl diphenyl R = C_6H_5 R' = $C_4H_9OC_2H_4$	39.1		5.0
Butyl diphenyl R = C_6H_5 R' = C_4H_9	49.1		1.2
5-Butoxyamyl diphenyl R = C_6H_5 R' = $C_4H_9OC_5H_{10}$	48.0		6.0

Table 1, continued.

Compound	Per Cent Kill		
	Myzus persicae	Myzus porosus	Musca domestica
6-Methylheptyl diphenyl R = C_6H_5 R' = $(CH_3)_2CH(CH_2)_5$	62.1		8.7
Nonyl diphenyl R = C_6H_5 R' = C_9H_{19}	74.8		6.0
Decyl diphenyl R = C_6H_5 R' = $C_{10}H_{21}$	59.8		5.9
Decyl diphenyl R = C_6H_5 R' = branched $C_{10}H_{21}$	62.7		4.5
Cyclohexyl diphenyl R = C_6H_5 R' = C_6H_{11}	26.2		3.8
Cresyl diphenyl R = C_6H_5 R' = $CH_3C_6H_4$	8.3		8.7
p-tert. Butylphenyl diphenyl R = C_6H_5 R' = $(CH_3)_3CC_6H_4$	7.4		3.7
p-tert. Amylphenyl diphenyl R = C_6H_5 R' = $C_2H_5C(CH_3)_2C_6H_4$	10.0		10.0
o-Chlorophenyl diphenyl R = C_6H_5 R' = ClC_6H_4	6.6		5.1
Diphenyl o-xenyl R = C_6H_5 R' = $C_6H_5C_6H_4$	6.0		6.4
Butyl dicresyl R = $CH_3C_6H_4$ R' = C_4H_9	49.2		4.8
Dicresyl 2-ethylhexyl R = $CH_3C_6H_4$ R' = $C_4H_9CH(C_2H_5)CH_2$	30.9		4.8
Phosphonate Esters: $((RO)_2P(O)R')$			
Diethyl trichloromethane R = C_2H_5 R' = Cl_3C	28.1*		11.3

Table 1, continued.

Compound	Per Cent Kill		
	Myzus persicae	Myzus poresus	Musca domestica
Triethyl phosphonoacetate R = C_2H_5 R' = $C_2H_5OOCCH_2$	55.4*		10.0
Diethyl p-chlorobenzene R = C_2H_5 R' = ClC_6H_4	55.0*		8.2
Diethyl 3,4-dichlorobenzene R = C_2H_5 R' = $Cl_2C_6H_3$	66.8*		7.4
Di-(2-chloroethyl)2-chloroethane R = R' = ClC_2H_4	20.7		7.9
Dibutyl trichloromethane R = C_4H_9 R' = Cl_3C	24.3*		12.4
Dibutyl 2-propene R = C_4H_9 R' = $CH_2=CHCH_2$	70.9*		9.8
Dibutyl 2-methyl-2-propene R = C_4H_9 R' = $CH_2=C(CH_3)CH_2$	76.1*		4.3
Dibutyl β-styrene R = C_4H_9 R' = $C_6H_5CH=CH$	90.3*	38.7*	10.7
Di-(2-ethylhexyl) benzene R = $C_4H_9CH(C_2H_5)CH_2$ R' = C_6H_5	94.8		5.8
Di-(2-ethylhexyl) toluene R = $C_4H_9CH(C_2H_5)CH_2$ R' = $CH_3C_6H_4$	95.5		3.7
Di-(2-ethylhexyl) ethylbenzene R = $C_4H_9CH(C_2H_5)CH_2$ R' = $C_2H_5C_6H_4$	85.6		5.5
Di-(2-ethylhexyl) p-chlorobenzene R = $C_4H_9CH(C_2H_5)CH_2$ R' = ClC_6H_4	83.4		3.7
Di-(2-ethylhexyl) α-toluene R = $C_4H_9CH(C_2H_5)CH_2$ R' = $C_6H_5CH_2$	96.9		4.7

Table 1, continued.

Compound	Per Cent Kill		
	Myzus persicae	Myzus porosus	Musca domestica
Di-(p-chlorophenyl) ethane R = ClC_6H_4 R' = C_2H_5	26.2*		11.5
Dialkyl Acid Phosphite Esters: ($(RO)_2POH$)			
Diethyl R = C_2H_5	12.2*		1.4 (m)
Di-(2-cyanoethyl) R = NCC_2H_4	12.0		
Dipropyl R = C_3H_7	20.4		1.2
Dibutyl R = C_4H_9	23.8*		5.3
Trialkyl and Triaryl Phosphite Esters: ($P(OR)_3$)			
Triethyl R = C_2H_5	13.7*		10.3
Tributyl R = C_4H_9	30.6*		3.1 (m)
Tricyclohexyl R = C_6H_{11}	8.4		4.6
Triphenyl R = C_6H_5	63.8		7.2
Tricresyl R = $CH_3C_6H_4$	63.3		7.6
Alkyl and Aryl Phosphonic Acids: ($(HO)_2P(O)R$)			
α-Toluene R = $C_6H_5CH_2$	5.3		2.3 (m)
(Diphenylmethane) R = $(C_6H_5)_2CH$			2.8 (m)
Butane R = C_4H_9			0.8 (m)
Pentane R = C_5H_{11}			1.4 (m)
Hexane R = C_6H_{13}			1.0 (m)
p-Chlorobenzene R = ClC_6H_4	6.1*		6.8 (m)
3,4-Dichlorobenzene R = $Cl_2C_6H_3$	4.8*		9.3 (m)

Table 1, continued.

Compound	Per Cent Kill		
	Myzus persicae	Myzus porosus	Musca domestica
Tetraalkyl Pyrophosphate and Related Esters: $((RO)_2P(O)OP(O)(OR)_2)$			
Ethyl butanephosphonodiethoxy phosphine oxide, $C_2H_5O(C_4H_9)P(O)OP(O)(OC_2H_5)_2$			29.2
Ethyl 2-ethylhexanephosphonodiethoxy phosphine oxide, $C_2H_5O(C_4H_9CH(C_2H_5)CH_2)P(O)OP(O)(OC_2H_5)_2$	100.0	20.4	18.5
Ethyl p-chlorobenzenephosphonodiethoxy phosphine oxide, $C_2H_5O(ClC_6H_4)P(O)OP(O)(OC_2H_5)_2$			83.8
unsym. Diethyl diphenyl pyrophosphate, $(C_2H_5O)_2P(O)OP(O)(OC_6H_5)_2$			86.7
Tetrabutyl R = C_4H_9			97.6
Ethyl tributyl pyrophosphate, $C_2H_5O(C_4H_9O)P(O)OP(O)(OC_4H_9)_2$			91.9
Butyl butanephosphonoethoxybutoxy phosphine oxide, $C_4H_9O(C_4H_9)P(O)OP(O)(OC_2H_5)OC_4H_9$			81.0
Octamethyltetraamido pyrophosphate, $((CH_3)_2N)_2P(O)OP(O)(N(CH_3)_2)_2$	99.1		12.0
Diethoxyphosphino ethyl acid phosphate, $(C_2H_5O)_2POP(O)(OH)OC_2H_5$		98.4	9.7 (m)
Alkyl Amido Phosphate Esters:			
Hexamethyl phosphoramide, $((CH_3)_2N)_3PO$	10.5		6.7
Ethyl tetramethyldiamido phosphate, $C_2H_5OP(O)N(CH_3)_2)_2$	5.0		2.6
Diethyl N, N-dimethylamido phosphate, $(C_2H_5O)_2P(O)N(CH_3)_2$	9.1		3.1
Diethyl N-ethylamido phosphate, $(C_2H_5O)_2P(O)NHC_2H_5$	8.7		3.3

Table 1, continued.

Compound	Per Cent Kill: Myzus persicae	Myzus porosus	Musca domestica
bis-(2-Methoxyethyl) N,N-diethylamido phosphate, $(CH_3OC_2H_4O)_2P(O)N(C_2H_5)_2$	6.3		4.4
Tetraalkyl-Aryl Triphosphate Esters: $((RO)_2P(O)OP(O)(OR')OP(O)(OR)_2)$			
Phenyl tetrapropyl R = C_3H_7, R' = C_6H_5			24.1
Tetrapropyl p-cresyl R = C_3H_7, R' = $CH_3C_6H_4$	9.5		3.5
Miscellaneous Compounds:			
Ethyl metaphosphate, $C_2H_5OP(O)O$		87.8	69.4 (m)
Poly-(dibutoxyphosphonitrile), $(N{=}P(OC_4H_9)_2)_x$	48.7*		4.9
Tetraphenyl diethyleneglycol diphosphate, $((C_6H_5O)_2P(O)OC_2H_4)_2O$	33.8*		3.5
Di-(cyclohexylamino)-phosphonitrile, $N{=}P(NHC_6H_{11})_2$			3.1
Triphenyl phosphine, $(C_6H_5)_3P$	31.6		6.6
Tri-(p-chlorophenyl) phosphine oxide, $(ClC_6H_4)_3PO$	13.4*		4.7
Triphenyl phosphine sulfide, $(C_6H_5)_3PS$			5.9
Difference required for significance at the five per cent level	28.8	9.4	8.2
50% Benzene-50% mineral seal oil			2.3[7]
50% Benzene-50% methyl alcohol			2.6[8]
Sodium decylbenzenesulfonate at 0.02%	7.1[9]	7.1[10]	
Nicotine, 99%, at 0.05%	42.4[9]	44.1[10]	

[1] At concentrations of 0.2 per cent.

Table 1, concluded.

Compound	Per Cent Kill		
	Myzus persicae	Myzus porosus	Musca domestica

2/ At concentrations of one per cent.

3/ Commercial products containing a constant ratio of the di- to the mono-ester.

4/ Data from Ludvik and Decker (1947).

5/ (m) indicates that the compound was soluble in 50% benzene-50% methyl alcohol.

6/ Obtained from Victor Chemical Works, Chicago, Illinois.

7/ Average of 143 tests.

8/ Average of 25 tests.

9/ Average of 31 tests.

10/ Average of 37 tests.

Table 2. Toxicity of certain phosphorus acid esters applied as contact sprays to *Myzus porosus*.

Compound		Per Cent Kill at Parts Per Million Concentration									
		1000	500	200	100	50	25	12.5	6.25	3.13	1.56
Trialkyl Phosphate Esters: $((RO)_2P(O)OR')$											
Diethyl octyl	$R = C_2H_5$ $R' = C_8H_{17}$	95.7	91.5	56.0							
Acetyl diethyl	$R = C_2H_5$ $R' = CH_3COO$		92.3	60.7	31.5	10.0					
Dialkyl-Aryl Phosphate Esters: $((RO)_2P(O)OR')$											
Diethyl o-nitrophenyl[1/]	$R = C_2H_5$ $R' = O_2NC_6H_4$		97.9	93.7	68.2	33.6					
Diethyl p-nitrophenyl[1/]	$R = C_2H_5$ $R' = O_2NC_6H_4$							99.4	94.1	84.9	48.1
Diethyl p-nitrophenyl thio	$R = C_2H_5$ $R' = O_2NC_6H_4$						99.5	93.0	76.6	47.5	
Tetraalkyl Pyrophosphate and Related Esters: $((RO)_2P(O)OP(O)(OR)_2)$											
Tetraethyl	$R = C_2H_5$				97.4	97.2	95.8	74.5	32.1	15.0	
Tetraethyl monothio	$R = C_2H_5$					99.0	71.0	36.6	11.4		

Table 2, continued.

Compound	Per Cent Kill at Parts Per Million Concentration									
	1000	500	200	100	50	25	12.5	6.25	3.13	1.56
Ethyl methanephosphonodiethoxy phosphine oxide, $C_2H_5O(CH_3)P(O)OP(O)(OC_2H_5)_2$			96.2	89.6	80.8	60.9				
Ethyl ethanephosphonodiethoxy phosphine oxide, $C_2H_5O(C_2H_5)P(O)OP(O)(OC_2H_5)_2$				98.9	88.0	74.7	35.8	17.8		
Ethyl butanephosphonodiethoxy phosphine oxide, $C_2H_5O(C_4H_9)P(O)OP(O)(OC_2H_5)_2$	81.4	60.7	27.2							
Ethyl benzenephosphonodiethoxy phosphine oxide, $C_2H_5O(C_6H_5)P(O)OP(O)(OC_2H_5)_2$			94.5	70.6	53.5	29.6				
Ethyl p-chlorobenzenephosphonodiethoxy phosphine oxide, $C_2H_5O(ClC_6H_4)P(O)OP(O)(OC_2H_5)_2$			88.3	79.1	51.4	48.7				
unsym. Diethyl dibutyl pyrophosphate, $(C_2H_5O)_2P(O)OP(O)(OC_4H_9)_2$					99.0	97.0	74.2	40.2	15.1	
unsym. Diethyl di-(2-ethylhexyl) pyrophosphate, $(C_2H_5O)_2P(O)OP(O)(OCH_2CH(C_2H_5)C_4H_9)_2$				99.5	73.9	35.1	14.3			
unsym. Diethyl diphenyl pyrophosphate, $(C_2H_5O)_2P(O)OP(O)(OC_6H_5)_2$				97.6	87.9	71.8	44.8	11.1		
Tetrapropyl[2/] R = C_3H_7				91.6	75.4	50.9				
Tetraisopropyl R = $(CH_3)_2CH$						95.8	69.6	60.3	21.3	

Table 2, continued.

Compound	Per Cent Kill at Parts Per Million Concentration								
	1000	500	200	100	50	25	12.5	6.25	3.13	1.56
Di-(isopropoxy)phosphino diisopropyl phosphate, $((CH_3)_2CHO)_2POP(O)(OCH(CH_3)_2)_2$				97.9	90.2	65.6	48.3	30.8		
Tetrabutyl R = C_4H_9				99.5	81.6	50.8	11.8			
Ethyl tributyl pyrophosphate, $C_2H_5O(C_4H_9O)P(O)OP(O)(OC_4H_9)_2$		100.0	84.3	71.8						
Butyl butanephosphonoethoxybutoxy phosphine oxide, $C_4H_9O(C_4H_9)P(O)OP(O)(OC_2H_5)OC_4H_9$	97.6	95.4	89.9	68.3	24.8					
Diethoxyphosphino ethyl acid phosphate, $(C_2H_5O)_2POP(O)(OH)OC_2H_5$	92.7	72.8	20.5							
Tetraalkyl-Aryl Triphosphate Ester: $((RO)_2P(O)OP(O)(OR')OP(O)(OR)_2)$										
Phenyl tetrapropyl R = C_3H_7, R' = C_6H_5			99.5	85.9[2]	66.4[2]	16.3				
Alkyl Metaphosphate Ester: $(ROP(O)O)$										
Ethyl R = C_2H_5	79.8	68.3	32.2							
Difference required for significance at the five per cent level	14.1	19.4	19.7	16.4	18.4	22.4	18.6	13.7	14.8	

1/Obtained from Victor Chemical Works, Chicago, Illinois.

2/Data from Ludvik and Decker (1947).

Table 3. Toxicity of certain phosphorus acid esters applied as contact sprays to *Musca domestica*.

Compound	Per Cent Kill at Parts Per Million Concentration									
	5000	2500	1250	625	313	156	78	39	18.5	9.25
Trialkyl Phosphate Esters: $((RO)_2P(O)OR')$										
Diethyl octyl R = C_2H_5, R' = C_8H_{17}	85.0	39.7								
Acetyl diethyl R = C_2H_5, R' = CH_3COO	99.8	96.4	73.8	33.7						
Dialkyl-Aryl Phosphate Esters: $((RO)_2P(O)OR')$										
Diethyl o-nitrophenyl R = C_2H_5, R' = $O_2NC_6H_4$			99.8	98.3	75.9	84.3	54.2	11.5		
Diethyl p-nitrophenyl R = C_2H_5, R' = $O_2NC_6H_4$					100.0	99.8	97.6	71.4	35.7	8.2
Diethyl p-nitrophenyl thio R = C_2H_5, R' = $O_2NC_6H_4$					99.1	99.7	83.4	36.0	21.4	
Tetraalkyl Pyrophosphate and Related Esters: $((RO)_2P(O)OP(O)(OR)_2)$										
Tetraethyl R = C_2H_5			99.2	97.9	93.1	82.1	61.2	42.0		
Tetraethyl monothio R = C_2H_5				95.9	91.7	47.7				

Table 3, continued.

Compound	Per Cent Kill at Parts Per Million Concentration									
	5000	2500	1250	625	313	156	78	39	18.5	9.25
Ethyl methanephosphonodiethoxy phosphine oxide, $C_2H_5O(CH_3)P(O)OP(O)(OC_2H_5)_2$	96.3	91.6	70.7	21.5						
Ethyl ethanephosphonodiethoxy phosphine oxide, $C_2H_5O(C_2H_5)P(O)OP(O)(OC_2H_5)_2$	94.8	96.0	29.5							
Ethyl butanephosphonodiethoxy phosphine oxide, $C_2H_5O(C_4H_9)P(O)OP(O)(OC_2H_5)_2$	20.2	11.4								
Ethyl benzenephosphonodiethoxy phosphine oxide, $C_2H_5O(C_6H_5)P(O)OP(O)(OC_2H_5)_2$	97.4	86.9	50.8	33.5						
Ethyl p-chlorobenzenephosphonodiethoxy phosphine oxide, $C_2H_5O(ClC_6H_4)P(O)OP(O)(OC_2H_5)_2$	73.1	44.1	30.1							
unsym. Diethyl dibutyl pyrophosphate, $(C_2H_5O)_2P(O)OP(O)(OC_4H_9)_2$		100.0	95.2	87.4	63.4	43.8				
unsym. Diethyl di-(2-ethylhexyl) pyrophosphate, $(C_2H_5O)_2P(O)OP(O)(OCH_2CH(C_2H_5)C_4H_9)_2$	99.6	81.7	51.8	28.5						
unsym. Diethyl diphenyl pyrophosphate, $(C_2H_5O)_2P(O)OP(O)(OC_6H_5)_2$	35.7									

Table 3, continued.

Compound	Per Cent Kill at Parts Per Million Concentration									
	5000	2500	1250	625	313	156	78	39	18.5	9.25
Tetrapropyl R = C_3H_7			93.8	79.7	51.4	19.4				
Tetraisopropyl R = $(CH_3)_2CH$						96.0	84.0	42.0	23.5	
Di-(isopropoxy)phosphine diisopropyl phosphate, $((CH_3)_2CHO)_2POP(O)(OCH(CH_3)_2)_2$			99.6	90.6	58.3	29.9				
Tetrabutyl R = C_4H_9	90.4	58.4	22.1							
Ethyl tributyl pyrophosphate, $C_2H_5O(C_4H_9O)P(O)OP(O)(OC_4H_9)_2$	61.4	41.1	17.1							
Butyl butanephosphonoethoxybutoxy phosphine oxide, $C_4H_9O(C_4H_9)P(O)OP(O)(OC_2H_5)OC_4H_9$	76.2	58.6								
Alkyl Metaphosphate Ester: (HOP(O)O)										
Ethyl R = C_2H_5	17.5									
Difference required for significance at the five per cent level	19.1	20.9	9.8	28.8	14.0	22.0	28.2	34.2		

Table 4. 24 Hour mortality of *Musca domestica* after exposure to aged treated surfaces.

Compound		Age of Surface: Two Hours	Five Days	Ten Days
Trialkyl Phosphate Esters: $((RO)_2P(O)OR')$				
Diethyl octyl	$R = C_2H_5$, $R' = C_8H_{17}$	62.7	7.4	
Diethyl acetyl	$R = C_2H_5$, $R' = CH_3COO$	54.4	4.8	
Dialkyl-Aryl Phosphate Esters: $((RO)_2P(O)OR')$				
Diethyl phenyl	$R = C_2H_5$, $R' = C_6H_5$	2.2		
Diethyl o-nitrophenyl	$R = C_2H_5$, $R' = O_2NC_6H_4$	99.4	95.7	86.4[1]
Diethyl p-nitrophenyl	$R = C_2H_5$, $R' = O_2NC_6H_4$	100.0	100.0	100.0[2]
Diethyl p-nitrophenyl thio	$R = C_2H_5$, $R' = O_2NC_6H_4$	100.0	100.0	100.0[3]
Tetraalkyl Pyrophosphate and Related Esters: $((RO)_2P(O)OP(O)(OR)_2)$				
Tetraethyl	$R = C_2H_5$	97.5	2.2	
Tetraethyl monothio	$R = C_2H_5$	100.0	2.8	
Ethyl methanephosphonodiethoxy phosphine oxide, $C_2H_5O(CH_3)P(O)OP(O)(OC_2H_5)_2$		61.6	1.4	
Ethyl ethanephosphonodiethoxy phosphine oxide, $C_2H_5O(C_2H_5)P(O)OP(O)(OC_2H_5)_2$		68.8	0.9	
Ethyl butanephosphonodiethoxy phosphine oxide, $C_2H_5O(C_4H_9)P(O)OP(O)(OC_2H_5)_2$		0.4		
Ethyl benzenephosphonodiethoxy phosphine oxide, $C_2H_5O(C_6H_5)P(O)OP(O)(OC_2H_5)_2$		72.8	3.1	
Ethyl p-chlorobenzenephosphonodiethoxy phosphine oxide, $C_2H_5O(ClC_6H_4)P(O)OP(O)(OC_2H_5)_2$		72.3	1.0	
unsym. Diethyl dibutyl pyrophosphate, $(C_2H_5O)_2P(O)OP(O)(OC_4H_9)_2$		92.7	3.5	

Table 4, concluded

Compound		Age of Surface: Two Hours	Five Days	Ten Days
unsym. Diethyl di-(2-ethylhexyl) pyrophosphate, $(C_2H_5O)_2P(O)OP(O)(OCH_2CH(C_2H_5)C_4H_9$		81.3	2.8	
unsym. Diethyl diphenyl pyrophosphate, $(C_2H_5O)_2P(O)OP(O)(OC_6H_5)_2$		95.2	3.0	
Tetrapropyl	R = C_3H_7	98.2	3.5	
Tetraisopropyl	R = $(CH_3)_2CH$	100.0	74.0	1.2
Di-(isopropoxy)phosphino diisopropyl phosphate, $((CH_3)_2CHO)_2POP(O)(OCH(CH_3)_2)_2$		22.6	3.1	
Tetrabutyl	R = C_4H_9	97.8	0.7	
Ethyl tributyl pyrophosphate $C_2H_5O(C_4H_9O)P(O)OP(O)(OC_4H_9)_2$		78.6	2.0	
Butyl butanephosphonoethoxybutoxy phosphine oxide, $C_4H_9O(C_4H_9)P(O)OP(O)(OC_2H_5)OC_4H_9$		65.6	2.2	
Diethoxyphosphino ethyl acid phosphate, $(C_2H_5O)POP(O)(OH)OC_2H_5$		0.0		
Tetraalkyl-Aryl Triphosphate Ester: $((RO)_2P(O)OP(O)(OR')OP(O)(OR)_2)$				
Phenyl tetrapropyl	R = C_3H_7, R' = C_6H_5	0.8		
Alkyl Metaphosphate Ester: (ROP(O)O)				
Ethyl	R = C_2H_5	34.2	2.8	
Ethyl Alcohol, Absolute		0.0	0.7	0.6
Untreated Glass		0.7	0.8	1.1

1/ 62% kill after 23 days, and 20% kill after 37 days.

2/ 54% kill after 86 days.

3/ 56% kill after 29 days, and 21% kill after 37 days.

Table 5. Degree of damage resulting from continuous exposure of Attagenus piceus larvae to treated wool, and mortality of the initial infestation of A. piceus larvae following continuous exposure to treated wool.

Compound		Degree of Damage[1] Age of Treatment, Days					Per Cent Kill Age of Treatment, Days				
		10	22	35	57	79	10	22	35	57	79
Trialkyl Phosphate Esters: $((RO)_3P(O)OR')$											
Diethyl octyl	$R = C_2H_5$ $R' = C_8H_{17}$	2	2	2	2	2.7	13	33	100		
Diethyl acetyl	$R = C_2H_5$ $R' = CH_3COO$	2.3	3	3	3	3	0	23	67	77	80
Dialkyl-Aryl Phosphate Esters: $((RO)_3P(O)OR')$											
Diethyl phenyl	$R = C_2H_5$ $R' = C_6H_5$	3	3.7	3.7	3.7		7	17	43	53	
Diethyl o-nitrophenyl	$R = C_2H_5$ $R' = O_2NC_6H_4$	1	1.3	1.3	1.7	1.7	50	87	100		
Diethyl p-nitrophenyl	$R = C_2H_5$ $R' = O_2NC_6H_4$	1	1	1	1	1	63	87	100		
Diethyl p-nitrophenyl thio	$R = C_2H_5$ $R' = O_2NC_6H_4$	1	1	1	1	1	13	93	100		
Tetraalkyl Pyrophosphate and Related Esters: $((RO)_3P(O)OP(O)(OR)_2)$											
Tetraethyl	$R = C_2H_5$	1.7	2	2.7	3	3	43	90	100		
Tetraethyl monothio	$R = C_2H_5$	2	2	2	2	2	57	83	100		

Table 5, continued.

Compound	Degree of Damage, Age of Treatment, Days					Per Cent Kill, Age of Treatment, Days				
	10	22	35	57	79	10	22	35	57	79
Ethyl methanephosphonodiethoxy phosphine oxide, $C_2H_5O(CH_3)P(O)OP(O)(OC_2H_5)_2$	2.3	2.3	2.3	2.7	3	10	63	87	100	
Ethyl ethanephosphonodiethoxy phosphine oxide, $C_2H_5O(C_2H_5)P(O)OP(O)(OC_2H_5)_2$	2.7	3	3	3	3.3	0	7	30	50	53
Ethyl butanephosphonodiethoxy phosphine oxide, $C_2H_5O(C_4H_9)P(O)OP(O)(OC_2H_5)_2$	4	4	4			0	0	0		
Ethyl benzenephosphonodiethoxy phosphine oxide, $C_2H_5O(C_6H_5)P(O)OP(O)(OC_2H_5)_2$	1.7	2.3	3	3.3	3.7	40	73	77	77	90
Ethyl p-chlorobenzenephosphonodiethoxy phosphine oxide, $C_2H_5O(ClC_6H_4)P(O)OP(O)(OC_2H_5)_2$	1.3	1.7	2.3	3.3	3.7	13	83	100		
unsym. Diethyl dibutyl pyrophosphate, $(C_2H_5O)_2P(O)OP(O)(OC_4H_9)_2$	1.3	2	2.7	3	3.7	73	97	100		
unsym. Diethyl di-(2-ethylhexyl) pyrophosphate, $(C_2H_5O)_2P(O)OP(O)(OCH_2CH(C_2H_5)C_4H_9)_2$	2	2.7	2.7	3	3	23	27	30	63	93
unsym. Diethyl diphenyl pyrophosphate, $(C_2H_5)_2P(O)OP(O)(OC_6H_5)_2$	2	2	3	3.3	3.3	47	97	100		
Tetrapropyl R = C_3H_7	3	3	3	3	3.3	13	27	53	73	90
Tetraisopropyl R = $(CH_3)_2CH$	1.3	1.3	1.3	1.3	1.3	53	100			

Table 5, continued.

Compound	Degree of Damage, Age of Treatment, Days					Per Cent Kill, Age of Treatment, Days				
	10	22	35	57	79	10	22	35	57	79
Di-(isopropoxy)phosphino diisopropyl phosphate, $((CH_3)_2CHO)_2POP(O)(OCH(CH_3)_2)_2$	3.7	4	4			0	0	0		
Tetrabutyl R = C_4H_9	3.7	4	4			3	3	3		
Ethyl tributyl pyrophosphate, $C_2H_5O(C_4H_9O)P(O)OP(O)(OC_4H_9)_2$	3.3	3.3	3.3	3.3	3.3	13	57	90	100	
Butyl butanephosphonoethoxybutoxy phosphine oxide, $C_4H_9O(C_4H_9)P(O)OP(O)(OC_2H_5)OC_4H_9$	3.7	4	4			0	7	7		
Diethoxyphosphino ethyl acid phosphate, $(C_2H_5O)_2POP(O)(OH)OC_2H_5$	4	4	4			0	0	3		
Tetraalkyl-Aryl Triphosphate Ester: $((RO)_2P(O)OP(O)(OR')OP(O)(OR)_2)$										
Phenyl tetrapropyl R = C_3H_7, R' = C_6H_5	4	4	4			3	3	3		
Alkyl Metaphosphate Ester: $(ROP(O)O)$										
Ethyl R = C_2H_5	3	3	3	3.3	3.7	3	3	3	27	80
Ethyl Alcohol, Absolute	4	4	4	4	4	0	0	0	3	3
Untreated Wool	4	4	4	4	4	0	0	0	0	0

1/ 1, no apparent feeding

Table 5, concluded.

Compound	Degree of Damage, Age of Treatment, Days					Per Cent Kill, Age of Treatment, Days				
	10	22	35	57	79	10	22	35	57	79

1/ Degrees of damage (continued):

2, nap feeding only
3, definite fiber damage
4, fibers cut so that holes made in the wool.

VI. DISCUSSION

In general, the results of the tests against Myzus persicae revealed minor differences in toxicity among the various chemicals. Tests against the house fly were essentially quantal at the one per cent concentration, and minor differences in toxicity were not apparent. By the methods used, the house fly was found to be more resistant to this series of chemicals than was Myzus porosus. Tests against both species showed diethyl p-nitrophenyl phosphate to be more toxic than diethyl p-nitrophenyl thiophosphate, which in turn proved to be more toxic than tetraethyl pyrophosphate. Though residues on glass plates of several of the more toxic compounds gave high kills of house flies two hours after the plates were prepared, only three compounds exhibited definite residual activity. Tetraisopropyl pyrophosphate gave a kill of 74 per cent five days after the plates were prepared. This was surprising since this compound would be expected to hydrolyze quite rapidly. The relatively high volatility and/or rapid hydrolysis of these compounds must be considered as the explanation for their lack of residual activity. Diethyl o-nitrophenyl phosphate and diethyl p-nitrophenyl thiophosphate were 62 per cent effective after 23 days and 56 per cent effective after 29 days, respectively. Diethyl p-nitrophenyl phosphate was 54 per cent effective 86 days following the treatment of the plates.

The following compounds were most effective in protecting wool from damage by black carpet beetle larvae in the order enumerated: diethyl p-nitrophenyl phosphate, diethyl p-nitrophenyl thiophosphate, tetraisopropyl pyrophosphate, diethyl o-nitrophenyl phosphate, tetraethyl monthiopyrophosphate, diethyl octyl phosphate, and tetraethyl pyrophosphate. No apparent feeding on the wool occurred 79 days after treatment with the first two compounds. The third and fourth compounds permitted only a trace of nap feeding after the same period. Definite nap feeding was apparent on the wool treated with the

fifth and sixth compounds, while the seventh, tetraethyl pyrophosphate, though giving high initial protection, permitted definite fiber damage to occur upon subsequent infestation.

As a group, the alkyl acid phosphate esters tested are non-toxic to aphids and the house fly. It is possible that a few isolated species of insects might be found which would be highly susceptible to one or more members of this series. This is to be doubted however, since experience with other organic phosphates, e.g., tetraethyl pyrophosphate and parathion, indicates a general rather than a specific toxicity of these compounds to insects and other members of the same phylum. As Kearns and Flint (1937) have previously shown with other homologous series, the toxicity within the group rises as the number of carbon atoms in the aliphatic chain is increased. Maximum toxicity is reached with the eight carbon chain, with further increases in length producing lower toxicity. This trend is apparent, but significant only between the ethyl and the octyl derivatives, in the results obtained against Myzus persicae. No support for this trend can be gained from the results obtained against Musca domestica.

The dialkyl acid phosphates are likewise non-toxic to aphids and the house fly, though the trend of the results against both insects indicates the same relationship between length of carbon chain and toxicity. The replacement of the acidic oxygen by sulfur and the replacement of the double-bonded oxygen by sulfur would seem to increase the toxicity of the diethyl derivative. The differences between the results from the three compounds tested, however, are too small to permit a valid conclusion to be drawn.

Two compounds in the series of trialkyl phosphate esters studied, exhibit considerable toxicity to Myzus persicae, but are non-toxic to Musca domestica. They are the 2-methylamyl and the 2-ethylhexyl derivatives. The

two, three, four, five, and twelve carbon atom, straight chain derivatives were available for study in this series. Here again there is a trend toward maximum toxicity as the length of carbon atom chain increases followed by a decrease in toxicity as the chain exceeds a certain length. The increase in toxicity proceeding from the ethyl and propyl to the butyl and amyl derivatives is statistically significant, while the toxicity of the dodecyl derivative is intermediate in relation to these four derivatives. In general, the chemical activity of iso-aliphatic compounds closely parallels that of the next lower normal (straight chain) homologue. A definite indication that insecticidal activity is similarly related to molecular structure is obtained by comparing the results obtained against Myzus persicae from tripropyl, tributyl, and triisobutyl phosphate. The differences in toxicity between tributyl and tripropyl, and between tributyl and triisobutyl phosphate are statistically significant, while the values obtained for tripropyl and triisobutyl phosphate are essentially equal. Kearns and Flint found the toxicity of N- substituted isopropyl and isobutyl derivatives of cyclohexylamine to be similar to that of the N-n-alkyl derivatives, however. It is difficult to account for the apparent high toxicity obtained from tri-(2-methylamyl) phosphate since the difference in toxicity between it and triamyl phosphate is not quite great enough to be significant. Unsaturation in an aliphatic chain had little effect on the toxicity of the molecules involved, e.g., tripropyl and triallyl phosphate. Replacement of hydrogen by chlorine on the number two carbon atom of triethyl phosphate gave a significant difference in toxicity, while chlorine atoms on the two and three positions of the propyl group, and chlorine on the ortho position of the phenyl group produced a marked but insignificant depression of toxicity. Alkyl groups apparently have greater effect upon the toxicity of the compound than do aryl groups, since the derivatives studied of the latter are relatively non-toxic. The replacement of the double-bonded oxygen by sulfur has very

little effect on toxicity in this group of compounds.

In the light of the results discussed above, the toxicity of individual compounds in the groups mentioned thus far, must be attributed essentially to the effects of the particular substituents of the phosphate radicals and not to some inherent toxicity in the radical per se.

Exceedingly remarkable increases in toxicity result from the substitution of any acyl, aryl, or larger alkyl group for one of the ethyl groups of triethyl phosphate. The toxicity imparted by such substitution increases from phenyl (aryl) to octyl (alkyl) to acetyl (acyl) to nitrophenyl (subustituted aryl). In general, this conclusion is in good agreement with the work of Schrader (1947). Schrader proceeded farther, however, in determining the effects of various phenyl substituents. He found p-nitrophenyl to have a greater effect upon toxicity than p-ethyl benzoate which was more active than p-chlorophenyl. Among these three groups, ortho substitution reduced the toxicity somewhat, but m-nitrophenyl imparted only slight toxicity to the molecule. The present investigation further demonstrated the same relationship with the two derivatives studied. However, p-nitrophenyl phosphate was found to be about six times as toxic to Myzus persicae as to Schrader's "aphides", while the ortho compound was found to be about one-eighth as toxic as Schrader reports. Diethyl benzyl phosphate was found to be significantly less toxic than diethyl phenyl phosphate. The change in toxicity may be due to the change in molecular architecture or to a change in lipoid solubility. Increases in length of the carbon chains of the di-substituent groups also decrease the toxicity. Diarylalkyl compounds likewise possess only slight degrees of toxicity, though here again, the toxicity tends to increase with the length of the normal alkyl chain. Diarylalkaryl phosphates were found to be insecticidally inert. The replacement of the double-bonded oxygen by sulfur definitely lowers toxicity as shown by the results

obtained with diethyl phenyl phosphate and diethyl phenyl thiophosphate, and with diethyl p-nitrophenyl phosphate and diethyl p-nitrophenyl thiophosphate.

The phosphonate esters, as a group, possess considerable toxicity to aphids but not to the house fly. Their toxicity, however, does not approach that of the substituted diethyl phosphate esters. The most toxic compound of this group studied was di-(2-ethylhexyl) a-toluenephosphonate which might be compared with the essentially non-toxic diethyl benzyl phosphate. Unfortunately, little comparison can be made between this and other groups of compounds since the derivatives studied are not parallel in structure. The toxic properties of diisopropyl fluorophosphate, which may be considered as a member of the general group of phosphonates, have been investigated by McCombie and Saunders (1946) against mice. Though highly toxic, it does not possess the extreme toxicity of diethyl p-nitrophenyl phosphate, for example. These workers found that when F was replaced by H, OH, Cl, ethyl, ethoxy, or other groups, the toxicity disappeared completely. In the light of the toxicities herein reported for this group, it might be of interest to investigate several of the analogous derivatives which were found to influence the toxicity of the substituted diethyl phosphate esters.

The present results indicate that dialkyl acid phosphite and trialkyl phosphite esters are essentially non -toxic.

The alkyl and aryl phosphonic acids studied proved to be insecticidally inert.

The tetraalkyl pyrophosphate group is without a parallel in exhibiting high toxicity to insects. However, as the number of carbon atoms in the normal chain is increased, decreased toxicity results. As was the case with the trialkyl phosphates, the activity of the iso-derivative studied was equal to that of the next lower homologue and greater than its normal derivative. The activity

of the unsym.diethyl dibutyl derivative is of the same order of magnitude as tetraethyl pyrophosphate. The ethyl tributyl derivative was found to be even less toxic than tetrabutyl pyrophosphate. The activity of unsym.diethyl diphenyl pyrophosphate was less than that of tetraethyl pyrophosphate. The phosphono-phosphine oxide derivatives are less toxic than tetraethyl pyrophosphate also, with the toxicity decreasing as the size of the substituent alkyl group increases. Therefore, the alkyl-O-P linkage must be of considerable importance to the toxicity of these compounds. That the P=O linkage is important can be readily seen by a comparison between the results obtained for tetraisopropyl pyrophosphate and di-(isopropoxy)-phosphino diisopropyl phosphate. Comparison of results obtained between tetraethyl pyrophosphate and diethoxyphosphino ethyl acid phosphate shows that the presence of two alkoxy groups upon the P=O is of considerable importance to the toxicity. The results obtained for octamethyl-tetraamido pyrophosphate tend to support the latter conclusion also. The replacement of one of the double-bonded oxygen atoms by sulfur in tetraethyl pyrophosphate definitely depresses the toxicity of the molecule.

The alkyl amidophosphates tested were found to be inactive. Schrader (1947) found that compounds of the dialkyl N,N-dimethylamidophosphate type were inactive also.

Phenyl tetrapropyl triphosphate was quite toxic to Myzus porosus but not to the house fly.

Ethyl metaphosphate is another compound which showed some activity toward Myzus porosus and the house fly.

Comparisons of chemical structures in relation to toxicity indicate that certain essential chemical components are necessary to impart high toxicity in organic phosphate esters. The first of these requirements is that the phosphorus atom must be pentavalent, and that two of the valences must be linked

with those of an oxygen atom. This is shown by comparing the results obtained from tributyl phosphate and tributyl phosphite but is more clearly demonstrated in the case of tetraisopropyl pyrophosphate vs. di-(isopropoxy) phosphino diisopropyl phosphate. A second requirement is that two of the remaining valences must be occupied by OR groups. This is established by the following relationships in toxicity: tetraethyl pyrophosphate equal to tetraisopropyl pyrophosphate, both being more active than di-(isopropoxy)phosphino diisopropyl phosphate which in turn is more active than di-(ethoxy)phosphino ethyl acid phosphate. That rigid restrictions as to the constitution of R must be imposed, is shown by the relationships existing between diethyl phenyl, dibutyl phenyl, diphenyl butyl, and diphenyl ethyl phosphate, and by those between tetraethyl, tetraisopropyl, tetrapropyl, and tetrabutyl pyrophosphate. Therefore, R must be either ethyl or isopropyl. The third and final necessary component is that which must occupy the fifth phosphorus valence. That this valence cannot be occupied by a P-R linkage is shown by the relative inactivity of the phosphonate esters tested. The previously cited work of McCombie and Saunders supports this conclusion. Further support stems from the comparison of diethyl p-chlorobenzenephosphonate, as herein reported, with diethyl p-chlorophenyl phosphate, which was reported by Schrader (1947) as possessing a high degree of activity. As illustrated, the P-R linkage is essentially a P-C-R linkage. Evidence that the P-N-R linkage is likewise ineffective is demonstrated by the gross inactivity of diethyl N,N-dimethylamido phosphate. Again, Schrader's work supports this conclusion. Consequently, the fifth phosphorus valence must be occupied by an O-R group. However, certain restrictions must be placed upon the radical involved. Triethyl phosphate is inactive, so the R under present consideration must now be designated as R'. Data obtained during the present investigation and from the literature (Schrader) indicate that R' may be a relatively large

(within limits) alkyl group, an acyl group, an aryl group, or an arylacyl group. The generalized structure necessary for high toxicity in an organic phosphate ester is therefore $(RO)_2P(O)OR'$, where R and R' must conform to certain designated limits. These conditions account for the toxicity of all highly active compounds studied. They do not account however, for the toxicity of the less active ethyl metaphosphate. Also, tetrapropyl p-cresyl triphosphate should be toxic, but is not. The sample tested may not have been pure, or may have decomposed before testing was carried out, however.

VII. SUMMARY

132 organic esters of phosphorus acids, namely metaphosphoric, orthophosphoric, pyrophosphoric, triphosphoric, and phosphorous acid, were examined for possible activity as contact insecticides against the green peach aphid, Myzus persicae and the house fly, Musca domestica. 26 compounds were found to possess at least a fair degree of insecticidal activity and were tested further as contact sprays against a more resistant aphid, Myzus porosus and against the house fly. 24 of these compounds exhibited fair to extreme toxicity to M. porosus, while only 19 showed varying degrees of toxicity to the house fly. Of the 26, only three compounds proved to be active residual toxicants to the house fly. Seven compounds were shown to give good to fair protection of wool from the feeding of the larvae of the black carpet beetle, Attagenus piceus.

Comparisons of chemical structures in relation to toxicity indicated that certain essential chemical components were necessary to impart high toxicity in organic phosphate esters. The first requirement was that the phosphorus atom must be pentavalent, and that two of the valences must be linked with those of an oxygen atom. The second requirement was that two of the remaining valences must be occupied by OR groups. The limits of R were established as being ethyl or isopropyl. The third and final requirement was that an OR' group must occupy the remaining valence. It was shown that R' may be a relatively large alkyl group, an acyl group, an aryl group, or an arylacyl group. The generalized structure necessary for high toxicity in an organic phosphate ester was therefore established as $(RO)_2P(O)OR'$, with definite limits determined for R and R'. This essential structure accounted for the toxicity of all compounds studied except for that of ethyl metaphosphate. Tetrapropyl p-cresyl triphosphate should be toxic since its structure satisfies the conditions cited above, but was not

found to be so in this investigation. The compound might have undergone deterioration before testing, however. The replacement of P=O by P=S resulted in decreased toxicity of toxic compounds. The toxicity of certain phosphoric esters was shown to increase as the length of the normal alkyl chain was increased to an optimum of about eight carbon atoms. Iso-derivatives were shown to be of the same activity as their next lower normal homologues.

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IX. VITA

George Franklin Ludvik was born August 12, 1919 in Dearborn, Michigan, the son of Benjamin Edward and Olga Thal Ludvik. He completed his grammar school education at Dearborn Junior High School in June, 1933 and his high school education at Dearborn High School in June, 1937. In September, 1937 he enrolled at the University of Illinois. In June, 1941 he graduated from the University of Illinois, receiving a Bachelor of Arts degree in Entomology. He continued his education at the University of Illinois in September, 1941 and was appointed a Special Research Assistant with the Illinois Agricultural Experiment Station and the Illinois Natural History Survey. In October, 1942 his education was interrupted when he was inducted into the Army of the United States. He served mainly with medical units and was honorably discharged in January, 1946 with the rating of Technician Fourth Grade. He re-entered the University of Illinois in February, 1946 and was appointed Special Research Assistant with the Illinois Agricultural Experiment Station and the Illinois Natural History Survey. He received a Master of Arts degree in Entomology in February, 1947 and continued his graduate studies in Entomology at the University of Illinois.

He was married to Virginia Ellen Walsh in Albion, Illinois, June 13, 1942. Their daughter, Nancy Ellen, was born August 16, 1947 in Urbana, Illinois.

He is a member of Phi Sigma, Gamma Sigma Delta, and an associate member of Sigma Xi. He belongs to the American Association of Economic Entomologists and the Illinois Academy of Science.

Publication: Ludvik, G. F. and G. C. Decker (1947). Toxicity of certain esters of phosphorus acids to aphids, J. Econ. Ent., 40(1):97-100.