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Chromatography and residue analysis of an organic phosphate insecticide (demeton)

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CHROMATOGRAPHY AND RESIDUE ANALYSIS
OF AN ORGANIC PHOSPHATE INSECTICIDE (DEMETON)

by

William J. Magee

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOYHY

Major Subject: Entomology

Approved:

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1955
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It was believed that methode standard

equate course in plant physiology. In fact, the intermediates of photosynthesis as preparation for a
tation of the intermediates of photosynthesis as preparation for a

The writer first became interested in paper chromatographic separa-

ference.

As a result of this experience and the experimental results in a small
mixture of water and the oxidized enzyme in the dark, the relationship of tosconol to the derivative from complex
should "show" some minute amounts of tosconol or the derivative from complex.

Such a method of testing on plants tested with transposed materials was attempted to determine the amount
phased great hardship on the interesting industry with respect to deter-

sensitive to small differences of test results, analysis for the interest.

The reactions of the food and drug administration to accept the therapy
residues of transposed toxocans, to test the test methods were developed.

The possibilities. In the absence of suitable chemical methods for establishment
that amount of determination of the quantity and structure were fixed.

From plant extracts transferred present in such small amounts

the treated with transposed toxocans, however, the

their visualization. The possibility of extracts from plant extracts for tech-
suitable methods for testing the toxicant from plant extracts for tech-

some methods related to nhexanols have been greatly hindered. By the lack of

Fundamental studies on compounds that are transposed by plants in

INTRODUCTION
to those described by Benson et al. (1950) could be used to good advantage in a graduate research fellowship investigation of demeton and related compounds. The writer expressed these views in the fall of 1951 in a term paper required for a course in radiotracer technique. This paper (Magee, 1951) closed with the following statement:

The combined use of radioautography and paper chromatography has been a useful tool in plant and animal biochemical research with radioactive labeled compounds. It is felt that the application of these methods to the problems met in the study of systemic insecticides would be as successful as their usage in photosynthesis research. These methods would also find application in other problems confronting the insect physiologist and insect toxicologist.

At the national meetings of the Entomological Society of America in Cincinnati, Ohio, December, 1951, the writer suggested to Mr. W. Scott James of the Pittsburgh Agricultural Chemical Company that chromatographic methods be explored by chemists who were attempting to develop a chemical method of demeton residue analysis acceptable to the Food and Drug Administration.

After returning to Ames, further study of the literature indicated that successful chromatographic investigations with demeton might be made without the necessity of using tracer labeled insecticide. Accordingly, the investigations described herein were initiated at Ames, January, 1952 and continued without interruption to September, 1953.
The studies of certain translocated isocitrate lyases have been undertaken in the United Kingdom and the United States. An excellent demonstration of the developed isocitrate lyase in Germany, the United Kingdom, and the United States has been presented in a paper by R. H. (1963). From these began the study of certain translocated isocitrate lyases in the United Kingdom and the United States. The translocated isocitrate lyases were discovered when the enzyme was present in the United States in 1972 and 1973, and in 1974, they were observed in the United Kingdom. This study of certain translocated isocitrate lyases has led to the present preparation of translocated isocitrate lyases by the present author. A comprehensive discussion of the numerous investigations of the field of plant pathology for comprehension of plant disease caused by translocated isocitrate lyases has received little attention to the present day. Because the translocated isocitrate lyase is a center of interest to many scientists, the translocated isocitrate lyase has been under unusual scrutiny of plants as a center of interest to many scientists, and the translocated isocitrate lyase has been under unusual scrutiny of plants as a center of interest to many scientists. Most early attempts to utilize the sap of the plant by the translocated isocitrate lyase from the site of infection on all parts of the plant would be injurious to the plant. The desire to protect crops of economic importance from damage by

REVIEW OF LITERATURE
a recent monograph by Schrader (1952) and in reviews by Ripper (1952) and Ivy (1953). Additional reviews which cite both current and older literature are given in doctoral dissertations by Abramitis (1951), Ivy (1951) and Johansen (1952).

The compound, \( O, O\)-diethyl 0-2(ethylmercapto)ethyl thionophosphate, was prepared by Schrader (1952) sometime between 1948 and 1950 by the reaction at elevated temperature of diethyl chlorothionophosphate and 2-hydroxyethylthioethane in an inert solvent and in the presence of an acid combining material. Formulations of this material are known by the trade name "Systox" and by the approved common name "demeton."

According to Hartley (1952) most formulations of demeton are an isomeric mixture of \( O, O\)-diethyl 0-2(ethylmercapto)ethyl thionophosphate and \( O, O\)-diethyl S-2(ethylmercapto)ethyl thiophosphate. He attributes the activity of demeton as a translocated insecticide to the presence of the thiophosphate isomer. The isomerization of demeton at high temperatures was discussed by Schrader (1952) who also demonstrated that the two isomers could be prepared by varying the reacting mixtures. Apparently a mixture of the two isomers could be obtained in any desired proportion by using different reactants or by using different inert solvents. Schrader (1952) described the physical constants of the two isomers and showed that these do not widely differ, however, the water solubility of \( O, O\)-diethyl S-2(ethylmercapto)ethyl thiophosphate was 1 part in 500 parts of water while that of \( O, O\)-diethyl 0-2(ethylmercapto)ethyl thionophosphate was 1 part in 5000 parts of water. Experiments using the two pure isomers labeled with \(^{32}\) indicate that these compounds are converted within treated plants to several more water soluble compounds with no apparent
loss in toxicity (Hartley, 1952).

No attempt has been made to prepare a comprehensive review of the use of paper chromatography as a preparative tool in analytical and biological chemistry. Excellent reviews have been published by Balston and Talbot (1951), Block et al. (1952) and Cramer (1952). It appeared more important to discuss only those papers which had a direct relationship to the development of the present study or were of similar nature to it.

Hanes and Isherwood (1949) have reported an intensive and methodical study of paper chromatographic separations of metabolically important organophosphorus compounds. Results of these experiments indicated that no single solvent system would give adequate resolution of complex mixtures of phosphates and that it might be necessary to use two dimensional procedures to effect separation of these compounds. A total of 60 systems was tested and seven were found to be useful. Either a strongly acidic or a strongly basic system gave the best separations. Interfering impurities in the filter paper were removed by washing prior to use in acid followed by washing in alcoholic 8-hydroxy-quinoline solution. To detect the compound on the dried chromatograph, the sheet of filter paper was sprayed at the rate of 1 ml. per 100 sq. cm. with a solution containing 5 ml. 60 per cent perchloric acid, 10 ml. 1N hydrochloric acid, 25 ml. 4 per cent ammonium molybdate and 60 ml. distilled water. The chromatograph was then dried for several minutes in warm air and heated at 85°C for 7 minutes. This treatment hydrolyzed the phosphate esters to o-phosphate. After regaining moisture from air, the filter paper was fumed with hydrogen sulfide. The position of the esters appeared as blue
spots. Quantitative estimations were made by digestion of the spots and subsequent colorimetric analysis for o-phosphate as molybdenum blue.

Calvin and Benson (1949) and Benson et al. (1950) have used paper chromatography to isolate and identify the phosphate esters of sugars in studies on the intermediates of sucrose formed during photosynthesis. For the most part these investigations utilized radioactive materials, and detection of the esters on the chromatograph was obtained by radioautography. Solvent systems composed of butanol-acetic acid-water, butanol-propionic acid-water and water saturated phenol were used to develop chromatographs of alcoholic extracts of algae which had been supplied with C¹⁴H or P³². The spray reagent of Feigl (1947) was used by Benson et al. (1950) to identify some of the easily hydrolyzed phosphate esters on the developed chromatograph.

Walker and Warren (1951) suggested that "Versene" (ethylenediamine tetra-acetic acid) be substituted for the alcoholic 8-hydroxyquinoline wash of Hanes and Isherwood (1949). Phosphoric esters tended to move more slowly when "Versene" was present but the order of movement was preserved.

Carter (1950) reported the separation of nucleic acid derivatives by two dimensional chromatography. He obtained good separations using a solvent system of 5 per cent disodium phosphate saturated with iso-amyl alcohol followed by development in the second direction with n-butanol saturated with 10 per cent aqueous urea. The second solvent was used in an atmosphere saturated with water vapor. The compounds were located on the chromatograph by fluorescence in ultra-violet light.

Bandurski and Axelrod (1951) and Mortimer (1952) found that the
In the chromatographic systems described above, the proper phase was

• the stationary phase (2) and followed in a second direction with other systems.

The chromatographic system was developed in one direction with other systems.

- methanol-water, 2 (methanol-water, 1) ethanol-water, 2 (methanol-water, 1) ethanol-water.

- the chromatographic system was followed by development of chromatographic systems (1950) and Hans and Isherwood (1970).

- the chromatographic system was followed by development of chromatographic systems (1950) and Hans and Isherwood (1970).

- the chromatographic system was followed by development of chromatographic systems (1950) and Hans and Isherwood (1970).

After application with Hands-Isherwood's reagent, the chromatography was

- further improved over the method of Hands and Isherwood (1970).
held stationary by the supporting substance, paper, while the nonpolar phase was the mobile part of the system. For chromatography of compounds which partition in favor of the nonpolar phase, it has been suggested that the two phases be reversed. In a reverse phase system the supporting substance (paper, glass, silica or kieselguhr) is treated in some manner to hold the nonpolar phase stationary. The mobile phase is then the more polar constituent of the system.

Boldingh (1948) first conducted experiments with reversed phase paper chromatographic systems by treating filter paper strips with dilute vulcanized rubber latex. The strips were dried in air, rinsed with alcohol and acetone, and stored in acetone. The paper contained up to 30 per cent rubber by weight. Esters of the higher fatty acids were separated with either methanol or a one to one mixture of methanol and acetone.

Moynihan and O’Colla (1951) utilized a reversed phase system to separate the constituents of crude hexachlorocyclohexane (HCH) which was composed of five isomers and heptacyclohexane. Crude DDT did not show any separation of isomers, but it ran farther than any of the hexachlorocyclohexane constituents. The paper was impregnated with acetic anhydride by immersion or by allowing the acetic anhydride to rise by capillary ascent. The solvent system was either n-hexane or petroleum ether saturated with acetic anhydride. All compounds gave a brown color when heated after spraying with an aqueous ferrous sulfate solution suspended in glacial acetic acid.

Kritchevsky and Tiselius (1951) applied a reversed phase chromatographic system to obtain resolution of complex mixtures of steroids. Filter paper was rendered hydrophobic by immersing strips of paper in a
5 per cent solution of Dow Corning Silicones 1107 in cyclohexane. The excess solution was removed by blotting and the paper was dried at 110° C. for 1 hour. The treated paper readily adsorbed vapors of nonpolar compounds such as chloroform, and its properties were not changed by washing with organic solvents. The upper, more polar phase from a mixture of 10 parts chloroform, 10 parts methanol and 6 parts water was used as the mobile phase. The chromatographs were developed in an airtight chamber in which the atmosphere was saturated with the stationary phase, i.e. the lower, less polar phase of the mixture.

Winteringham et al. (1950 and 1951) have separated radioactive bromine analogues of DDT isomers and their detoxicated derivatives on filter paper impregnated with petroleum jelly. The paper was drawn through a 2.5 per cent ether solution of petroleum jelly, drained and dried. The solvent system was composed of 80 parts ethanol, 15 parts water and 5 parts ammonium hydroxide. The compounds were located radiometrically with a Geiger-Mueller counter.

O'Colla (1952) extended the methods of Moynihan and O'Colla (1951) to chromatographic studies of several chlorinated insecticides. The constituents of BHC and chlordane were separated and detected. The behavior of DDT and toxaphene on chromatographs was observed. The method was also applied to the quantitative estimation of gamma-BHC.

Mitchell (1952a) modified the impregnation methods of Moynihan and O'Colla (1951) to obtain reproducible results through more uniform distribution of the stationary solvent. Acetic anhydride was diluted 1 part to 9 parts ethyl ether and sprayed on the filter paper in rapid and uniform horizontal strips. Immediately after spraying, the chromatogram was
developed in a mobile solvent of mixed octanes. Reproducible separations of the isomers of BHC were obtained. Mitchell (1952b) developed a spray test which is positive on the paper chromatogram for all chlorines containing insecticides that he has tested. The paper is sprayed with 0.05 N ethanolic silver nitrate, dried in air for 0.5 hour, sprayed with 37 per cent formaldehyds solution, dried in air for 0.5 hour, sprayed with 1N methanolic potassium hydroxide, dried in air for a few minutes to remove excess methanol, heated in an oven at 130-133° C. for 0.5 hour, sprayed with a solution containing equal parts of concentrated nitric acid and 30 per cent hydrogen peroxide, dried in air overnight and exposed to sun or bright daylight until spots are fully developed. Aldrin, isodrin, dieldrin, endrin, BHC isomers, chlordane, DDT isomers, heptachlor, methoxychlor and toxaphene gave a positive test to the treatment. Mitchell and Patterson (1953) resolved mixtures of aldrin and dieldrin using reversed phase chromatography. The stationary solvent, scybean oil, was sprayed on the paper in an ether solution. A number of miscible organic solvents would resolve the mixture. Four parts acetone to 1 part water or 7 parts acetonitrile to 3 parts water were the preferred solvents.

Lord et al. (1950) separated pyrethrum extracts, purified by the nitromethane technique, into pyrethrins I and II on alumina impregnated paper using light petroleum-bensene mixtures. The 2,4-dinitrophenyl-hydrasine derivatives of these extracts were separated also into pyrethrins I and II on unimpregnated paper using light petroleum as a solvent.

Winteringham (1952) investigated the separation of synthetic
radioactive and non-radioactive pyrethrins-type insecticides and their derivatives by reversed phase chromatography. Paper strips were washed for 30 minutes with a solution of 4:5 parts ethanol, 50 parts water and 5 parts hydrochloric acid. The paper was then washed with dilute aqueous ammonium hydroxide and rinsed with distilled water. After drying the paper was impregnated by drawing it through a 3 per cent ether solution of petroleum jelly, draining off the excess and drying. A solvent system of 4:5 parts ethanol, 50 parts water and 5 parts ammonium hydroxide was used to develop the chromatograph in an atmosphere of nitrogen saturated with water vapor. With this method the keto-alcohol and acid portions of allethrin hydrolysis ran together on the chromatograph. To resolve these compounds, washed but nonimpregnated strips containing the mixture were developed in an ascending solution of petroleum ether saturated with 10 per cent aqueous hydrochloric acid for 10 minutes. The strips were dried and rechromatographed with an ascending solution of petroleum ether saturated with ammonium hydroxide for 30 minutes. To locate resolved compounds on all chromatographs, the strips were sprayed with neutral aqueous 0.1 per cent potassium permanganate solution, washed immediately with distilled water, dried partially and sprayed while still damp with 0.5 per cent benzidine solution of dilute aqueous acetic acid. Zeid et al. (1953) used this method to study radioactive and non-radioactive pyrethrins and their derivatives.

Metcalf and March (1953a) used a technique similar to that of Kritchevsky and Tiselius (1951) to separate several organophosphorus insecticides and their isomers. Filter paper was impregnated with Dow Corning Silicone 550 from a 5 per cent hexane solution. The solution
used to develop the chromatograph was the same one used to resolve steroids in the earlier investigation. Phosphate esters containing p-nitrophenyl groups were determined by conversion to the intense yellow p-nitrophenate ion with a 5 per cent alcoholic potassium hydroxide spray solution and subsequent heating in an oven for a few minutes at 105° C. Hanes-I's herwood reagent was satisfactory for location of organophosphate esters which contained no p-nitrophenyl group. This method was used by Metcalf and March (1953b) to study the isomerization of pure organophosphate insecticides at high temperatures. Metcalf and March (1953c) have also used this technique to demonstrate the conversion of parathion and methyl parathion to their corresponding oxygen analogues by an enzyme system in the American cockroach.

Many methods are available for colorimetric analysis of o-phosphate by measuring the deep color of the molybdenum blue complex obtained when phosphomolybdic acid is reduced by a suitable agent. Apparently the color arises from selective reduction of phosphomolybdic acid to a mixture of lower oxides of molybdenum. Most of the described procedures of phosphate analysis by molybdenum blue differ principally in the reducing agent used. Probably the most widely used method is that of Fiske and Subbarow (1925) in which 1,2,4-aminonaphtholsulfonic acid is the reducing agent. Stannous chloride has the advantage over aminonaphtholsulfonic acid in that the stock reagent is quite stable and color production with phosphomolybdate is much more intense, permitting the estimation of smaller amounts of phosphorus. Time control is essential when stannous chloride is used, for color changes continuously with time. To stabilize the color when stannous chloride was used, Berenblum and Chain
(1938) suggested that the solution be extracted with iso-butanol before the reducing agent is added. Sideris (1942) proposed the use of a reducing agent prepared by the action of hydrochloric acid on metallic tin and extraction of the colored solution by n-butanol. A microgram and submicrogram method of phosphorus estimation, using stannous chloride as a reducing agent, has been described by Shaffer et al. (1953). Allen (1940) suggested the use of 2,4-diaminophenol as a reducing agent. The blue color was quite stable, and the procedure required few reagents. The stability of the colored solution was increased by addition of 10 per cent oxalic acid and extraction with iso-butanol.

Pons et al. (1953) described analytical methods for the determination of total, inorganic, acid-soluble, phosphatide and phytin phosphorus in plant materials. Colorimetric methods of phosphate analysis were reported in detail. Amounts of total, phytin and acid-soluble phosphorus were determined after oxidation to o-phosphate by a reduced molybdate procedure in which the readings could be taken any time within 24 hours after color development. Inorganic and phosphatide phosphorus determinations utilized extraction of the molybdenum blue complex with iso-butanol. The color was stable and readings could be made at any time from 40 minutes to 19 hours after development.

A procedure for analysis of the isomers of demeton has recently been described by Gardner and Heath (1953). The isomers were labeled with P$^{32}$ and separated on a column of kieselguhr by a solvent system of iso-octane saturated with methanol. An eluent fraction of the first 25 to 35 ml. contained the O,O-diethyl O-2(ethylmercapto)ethyl thiophosphate. A second fraction of the 45 to 100 ml. eluent contained the
0,0-diethyl S-2(ethylmercapto)ethyl thionophosphate. The eluent fractions were concentrated and analyzed for phosphate colorimetrically.

Shortly after the first draft of this manuscript was typed and undergoing revision, a popular article describing similar techniques to those used herein was published by Metcalf (1954). Orange trees, apple trees, walnut trees, potatoes and cotton plants were sprayed with tracer labeled demeton and schradan. The plant samples were extracted by chloroform or other suitable solvent and chromatographed with the chloroform–ethanol–water solvent of Kritchevsky and Tiselius (1951). The developed chromatograph was assayed by counting under a Geiger-Mueller tube or by radioautography. The counting technique is said to give a residue analysis accurate to within 0.001 p.p.m. which is considerably more accurate than the colorimetric analyses employed in the following study. Metcalf (1954) reports that 0,0-diethyl S-2(ethylmercapto)ethyl thiophosphate penetrates plant tissues more rapidly than 0,0-diethyl O-2(ethylmercapto)ethyl thionophosphate and is translocated without conversion. Within 24 to 48 hours, 0,0-diethyl S-2(ethylmercapto)ethyl thiophosphate was converted to a more toxic compound. 0,0-diethyl O-2(ethylmercapto)ethyl thionophosphate penetrated plant tissues more slowly and was immediately metabolized therein to a more toxic metabolite. This metabolite was more readily translocated than the parent compound.
OBJECTIVES

Initially it was necessary to show by preliminary bioassay that the insecticide chosen for study (demeton) was translocated within the plant selected for this investigation (bell pepper) in amounts lethal to a test insect (aphids).

The primary prerequisite of the investigation was to find a solvent system or combination of solvent systems which would resolve any components present in demeton formulations from each other and other phosphate compounds likely to be present in plant extracts. It then became essential to determine whether the insecticide remained unchanged within the plant or was converted by the metabolic activity of the plant to a different compound or group of compounds. This would be indicated by comparison of chromatographs of plant extract with chromatographs of the original compound.

If the introduced compounds were metabolized by the plant to different materials, it became a secondary objective of the investigation to attempt chromatographic identity of the metabolites within the limits imposed by the equipment available.

Finally it was desirable to develop a procedure which might be used for residue analysis of demeton and, with suitable modification, other phosphate insecticides which are translocated by plants.
In addition to the following oxidation were assumed to occur:

\[ \text{H}_2\text{SO}_4 + \text{H}_2\text{SO}_4 \rightarrow \text{H}_2\text{SO}_4 + \text{H}_2\text{SO}_4 \]

Process occurring to the formation scheme:

It was believed that the compounds were a simple oxidation activity. It was believed that these compounds in the presence of the reagents of different compounds were formed. As the mixture of these compounds (I), it was assumed that demethylation was a mixture of these compounds.

- Pepper plants under greenhouse and laboratory conditions.
- Pepper plants under greenhouse and laboratory conditions.
- Pepper plants under greenhouse and laboratory conditions.

As the species culture reachion on bell pepper plants under greenhouse and laboratory conditions, the red pepper might be produced. The pepper plants in the bell pepper plants in the Gulf Coast and in the Southern states, it was supposed that demethylation would be translocated from the best of previously tested compounds in Germany and in the

HYPOTHESES AND ASSUMPTIONS

16
suitable to the corresponding derivatives of the parent compounds. The above scheme would support the suggestion of Harrington (1967) that the separation of the compounds by the paper chromatographic method might be more water soluble than the parent compounds. The above scheme would also support the suggestion of the parent compounds, without the presence of other reagents, that the compounds would appear that the compounds are quite soluble in water. According to common compound conditions, according to common conditions, the low motronic water could be removed by the corresponding derivatives of the parent compounds.
conversion. Connor (1949) states that sulfones activate alpha-hydrogen and are electron-attracting. Accordingly the sulfone derivatives of demeton (V, VII, IX and XII) should be very effective antienzymes.

Finally it was presumed that the parent compounds and their derivatives could be separated chromatographically and the relative amounts of each determined quantitatively.
MATERIALS AND APPARATUS

Bioassay

Insecticides

Samples of technical demeton, 1/4 pounds active ingredient per gallon water miscible demeton, and 25 per cent wettable powder demeton dust, were furnished by the Chemagro Corporation through the Pittsburgh Agricultural Chemical Company. A parathion formulation containing 2 pounds active ingredient per gallon was also obtained from this source. A sample of schradan containing 1/4 pounds active ingredient per gallon was supplied by the Dow Chemical Company. For labeling purposes the following chemical names were considered correct for the active ingredients of these preparations: octamethylpyrophosphoramide for schradan, 0,0-diethyl 0-p-nitrophenyl thionophosphate for parathion and 0,0-diethyl 0-2(ethyl-mercapto)ethyl thionophosphate (I) for demeton.

Plants

Seeds of bell pepper plants were obtained from the W. Atlee Burpee Company. Plants for contact toxicity tests were grown in sand culture flats then transferred for testing to shell vials containing nutrient solution. While growing in sand culture, the plants were watered with nutrient solution. Plants for all other tests were started in flats containing soil then transferred when 2 inches high to soil in pots having a diameter of 6 inches. The plants in 6 inch pots were 4 months of age and were fruiting at the time of testing.
**Nutrient solution**

The nutrient solution for growing plants in sand and shell vials contained 738.45 mg. calcium nitrate tetrahydrate, 566.93 mg. magnesium sulfate heptahydrate, 313.01 mg. potassium acid phosphate, 5.00 mg. ferric chloride, 5.00 mg. tartaric acid, 2.86 mg. boric acid, 1.81 mg. manganese tetrahydrate, 0.11 mg. zinc chloride and 0.05 mg. cupric chloride dihydrate per liter of solution.

**Spray apparatus**

Sprays for the contact toxicity tests were applied through a DeVin-biss atomizer with an air pressure of 15 pounds which was maintained by feeding from a high pressure air line through a pressure regulator valve. Sprays for tests of residual action were applied with an air pressure of 40 pounds from a portable aircraft oxygen cylinder fitted with a pressure regulator valve. These sprays were discharged through an atomizer fitted with a Delavan WDA 10 spray nozzle.

**Spray towers**

The contact toxicity tests were conducted with a bell jar spray tower having a diameter of 7.5 inches and a height of 12 inches. The tower was mounted on a stand containing a revolving turn-table which held one to eight shell vials in an upright position. These shell vials contained aphid-infested plants growing in nutrient solution. The turn-table was revolved at constant speed by a 0.25 H. P. electric motor through a pulley system.
Sprays for residual action tests were applied in an aluminum spray tower having a diameter of 30 inches and a height of 54 inches. This tower was mounted on a stand which would cover one centrally placed plant. Bottom board halves of the stand were then placed so that only the aerial parts of the plant were wet by the spray.

Test animals

The green peach aphid was the principal test animal for all bioassay experiments. Greenhouse cultures were maintained by allowing the aphids to culture freely on untreated plants. Plants for testing were selected from these infested plants. When necessary, plants were infested by placing infested leaves in the terminals. The aphids then transferred to the clean plant as the leaf dried. Red spider mite infestations were provided for the later tests in similar manner.

Chromatography

Solvent systems

The solvent systems listed below were composed of G. P. reagent grade chemicals or chemicals of the highest degree of purity obtainable. The numbers following each ingredient were the amounts used in milliliters to make up the solutions.

- Acetic acid, 19; n-butanol, 7h; distilled water, 50.
- Acetic acid, 20; n-butanol, 80; distilled water, 20.
- Acetic acid, 60; ethyl acetate, 60; distilled water, 20.
- Benzene, 100; dioxane, 100; distilled water, 60.
- Benzene, 100; methanol, 100; distilled water, 60.
Benzene, 100; methyl cellosolve, 100; distilled water, 60.

Benzene, 100; methyl ethyl ketone, 100; distilled water, 60.

n-Butanol saturated with 10 per cent aqueous urea, 100.

Chloroform, 100; dioxane, 100; distilled water, 60.

Chloroform, 100; ethanol, 100; distilled water, 60.

Chloroform, 100; methanol, 100; distilled water, 60.

Chloroform, 100; methanol, 100; formamide, 60.

Chloroform, 100; methyl cellosolve, 100; distilled water, 60.

Chloroform, 100; methyl ethyl ketone, 100; distilled water, 60.

Ethanol, 45; ammonium hydroxide, 5; distilled water, 50.

Ethanol, 60; ammonium hydroxide, 10; distilled water, 30.

Ethyl acetate, 60; formamide, 40; pyridine, 10.

Ethyl acetate, 60; formamide, 40; triethanolamine, 10.

Formic acid, 80; methanol, 15; distilled water, 5.

n-Hexane, 100; dioxane, 100; distilled water, 60.

n-Hexane, 100; methanol, 100; distilled water, 60.

n-Hexane, 100; methyl cellosolve, 100; distilled water, 60.

n-Hexane, 100; methyl ethyl ketone, 100; distilled water, 60.

Methanol, 45; ammonium hydroxide, 5; distilled water, 50.

Methanol, 60; ammonium hydroxide, 10; distilled water, 30.

Methyl cellosolve, 70; methyl ethyl ketone, 20; 3 N ammonium hydroxide, 30.

iso-Octane, 100; dioxane, 100; distilled water, 60.

iso-Octane, 100; methanol, 100; distilled water, 60.

iso-Octane, 100; methyl cellosolve, 100; distilled water, 60.

iso-Octane, 100; methyl ethyl ketone, 100; distilled water, 60.
Petroleum ether, 100; methanol, 100; distilled water, 60.
Petroleum ether, 100; methanol, 100; formamide, 60.
Phenol saturated with distilled water, 100.

Filter paper

The chromatographic separations were conducted on 25 cm. square sheets of Whatman No. 1 filter paper. Early separations were attempted on unpregnated papers. In later runs reversed phase systems were used. Papers, impregnated with petroleum jelly according to the methods of Winteringham et al. (1950), were not found to be as satisfactory as those impregnated with silicones (Dow Corning 550 or General Electric 9992) by the methods of Kritchevsky and Tiselius (1951).

Chromatographic chambers

Glass cylindrical jars (Corning 850) with ground glass rims and dimensions of 6 inches diameter and 12 inches height were used for the separations of plant extracts. Preliminary separations were made in these jars, museum jars (1½ inches high and 10 inches in diameter), two-gallon fruit jars and large-sized dessicators. Glass covers were sealed to the chamber rims with Dow Corning stopcock grease.

Constant temperature cabinet

Separations with acid solvent systems were conducted in a temperature controlled cabinet at 4°C. Separations with other solvent systems were conducted at 15°C.
Spray indicator reagents

The following spray reagents were used in tests to find a suitable reagent to detect demeton on filter paper chromatograms.

Concentrated nitric acid.
Concentrated sulfuric acid.
Concentrated hydrochloric acid.

Feigl's spot test reagent for phosphate. The spot test of Feigl (1947) was performed by spraying consecutively with: a) a solution of 5 g. ammonium molybdate dissolved in 100 ml. cold distilled water and poured into 35 ml. concentrated nitric acid, followed by drying at 70° C., b) a solution of 50 mg. benzidine hydrochloride dissolved in 10 ml. concentrated acetic acid and diluted to 100 ml. with distilled water, followed by drying at room temperature and c) saturated sodium acetate aqueous solution followed by drying at room temperature. The presence of easily hydrolyzed phosphate was indicated by a deep blue spot.

Ammoniacal silver nitrate. Equal parts of a 0.1 N silver nitrate solution and a 5 N ammonium hydroxide solution were mixed just before use as a spray on the dried chromatograph. Immediately after spraying the dried chromatograph was heated for 10 minutes at 100° C. Presence of reducing substance was indicated by a dark spot on the faint buff background.

Potassium permanganate. The dried chromatograph was sprayed with a solution of 1 per cent potassium permanganate and 2 per cent sodium carbonate in distilled water. Presence of reducing substance was indicated immediately by a brilliant yellow spot against a wine colored
background. The position of the spot was marked immediately for it could not be easily distinguished from background when dry.

Hanes-Isherwood reagent. The dried chromatograph was sprayed at the rate of 1 ml. per 100 cm.² with a solution composed of 5 ml. 60 per cent perchloric acid, 10 ml. N hydrochloric acid, 25 ml. 4 per cent ammonium molybdate and 60 ml. distilled water. The chromatograph was then heated in the water saturated atmosphere of an oven at 85⁰ C. for 2 to 6 hours. Usually the blue spot of reduced phosphomolybdate showed without further treatment. The intensity of the spot could be increased in some cases by autoclaving at 8 to 10 pounds for a few minutes or spraying with stannous chloride solution. The stannous chloride solution was prepared by dissolving 10 g. stannous chloride in 25 ml. concentrated hydrochloric acid and diluting 1 ml. of this stock solution to 200 ml. with distilled water. Autoclaving and the stannous chloride spray increased the background blue. The blue background was selectively bleached by spraying the chromatograph with a solution containing equal parts of concentrated ammonium hydroxide and distilled water.

Drying oven

The sprayed chromatographs were dried in a DeKhotinsky constant temperature drying oven with adjustable temperature control. The atmosphere of the oven was kept saturated with water vapor by evaporating distilled water from deep petri dishes placed on the bottom and top shelves.
Analysis

Glassware

The following items of glassware were used in this study:

Beakers, with capacities of 200 and 500 ml.

Cylinders, graduated, with capacities of 5, 10, 25 and 100 ml.

Micropipettes, Alfred Bichnell Associates adjustable, automatic "micropette", with capacity of 0.7 ml.

Pipettes, Alfred Bichnell Associates adjustable, automatic, with capacity of 5 ml.

Funnels, separatory, with capacities of 250, 500 and 1000 ml.

Blendor

A Waring blendor was used to macerate plant material for extraction with solvents.

Water bath

A Technicon constant temperature water bath with adjustable controls was used to concentrate the plant extract by solvent evaporation. The water bath was set to operate at 50° C.

Reagents for mercaptan and thiono-sulfur analysis

The following reagents were used in tests and analyses for mercaptans and thiono-sulfur:

Three N sodium hydroxide solution containing 126 g. of C. P. sodium hydroxide in 1 l. of solution.
Three N potassium hydroxide solution containing 126 g. of C. P. sodium hydroxide in 1 l. of solution with absolute methanol.

One-tenth N standard sodium hydroxide containing 4.2 g. C. P. sodium hydroxide in 1 l. of solution. The solution was standardized with N hydrochloric acid.

Three N hydrochloric acid containing 258 ml. of 11.6 M acid (36 per cent hydrochloric acid) in 1 l. of solution.

One-tenth N standard iodine aqueous solution containing 13.5 g. pure sublimed iodine and 24 g. potassium iodide in 1 l. of solution. The solution was standardized with standard thiosulfate.

One-tenth N iodine solution containing 13.5 g. of iodine and 24 g. of potassium iodide dissolved in 1 l. of solution with three N hydrochloric acid.

One-tenth N sodium thiosulfate solution containing 25 g. of sodium thiosulfate in 1 l. of solution. The solution was standardized with potassium dichromate.

Starch indicator solution containing 3 g. starch in 500 ml. saturated sodium chloride solution (filtered), 80 ml. glacial acetic acid and 20 ml. water.

Reagents for phosphate analysis

The following reagents were used to determine phosphorus by the method of Allen (1940):

Ammonium molybdate solution containing 8.3 g. ammonium molybdate dissolved in distilled water and diluted to 100 ml.

A 60 per cent solution of perchloric acid (sp. gr. 1.54).
Amidol reagent containing 2 g. 2,4-diaminophenol dihydrochloride and 40 g. sodium bisulfite in glass distilled water and diluted to 200 ml. This solution was kept in a well stoppered, black bottle and prepared fresh every 10 days.

Hydrogen peroxide solution containing 30 per cent hydrogen peroxide free of phosphate.

Standard phosphate solution containing 1 mg. phosphorus per ml. solution. This was a commercial preparation by the Hartman-Leddon Company.

Digestion apparatus

A micro-Kjeldahl digestion apparatus was used for digesting the organophosphate insecticide to inorganic phosphate for colorimetric estimation. Digestions were also made in micro-Kjeldahl flasks suspended over a hot plate in a forced draft hood.

Colorimeter

A Klett-Summerson photoelectric colorimeter with deep red filter was used to make the phosphorus determinations. Readings of solutions in Klett-Summerson colorimeter tubes were taken at 660 to 720 mu.
METHODS OF PROCEDURE

Bioassay

Contact toxicity tests

Aphid-infested bell pepper plants were taken from sand cultures, washed to remove excess sand from the roots, and transferred to shell vials containing nutrient solution. The number of aphids per plant was recorded. The plants were then sprayed with varying dosages of demeton, parathion and schradan in the bell jar spray tower. The sprays were applied at the rate of 10 gallons per acre and allowed to settle for 30 seconds. Immediately after the settling period had elapsed, the plants were transferred to clean shell vials containing nutrient solution and placed at random on racks in the greenhouse. Each treatment dosage and the untreated check were replicated four times in each of seven test series. Survival records were taken 2½ hours after treatment and converted to percentage mortalities by the formula of Abbott (1925) for calculation of dose-effect curves.

Residual toxicity tests with insecticides applied to plant foliage

Heavily aphid-infested bell pepper plants in 6 inch pots were used in these tests. The fourth and fifth leaves from the top of the plant were marked and the aphid populations were recorded before and after treatment. Survival records were taken at the end of 2, 7, 1½ and 21 days after treatment and converted to percentage mortalities by Abbott's
formula. Spray applications were made with varying dosages of demeton, schradan or parathion in the large aluminum spray tower. The sprays were applied at the rate of 10 gallons per acre and allowed to settle for 30 seconds. Each treatment dosage was replicated four times. Four untreated plants were included in the test as check replicates. After treatment the plants were placed at random on benches in the greenhouse.

Residual toxicity tests with insecticides applied to plant roots

These tests were conducted with plants growing in 6 inch pots. The plants had been transplanted from flats and were heavily infested with green peach aphids. The fourth and fifth leaves from the top of the plant were marked to determine aphid infestation before and after treatment. Survival records were taken 2, 7, 11, 21, 28 and 35 days after treatment and converted to percentage mortalities by Abbott’s formula. Demeton was applied at the rate of 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 pounds of active ingredient per acre in 100 ml. aqueous solutions and in a fertilizer mixture. The fertilizer was applied at the constant rate of 200 pounds of 10-20-0 per acre and was considered to be equivalent to a side dressing. Schradan was applied at the same rates as demeton in 100 ml. aqueous solutions. Each treatment dosage was replicated four times, and four untreated plants served as check replicates. The plants were placed at random on benches in the greenhouse.
Chromatography

Development of chromatographs

Chromatographic separations were attempted on both untreated and treated papers. The treated papers were impregnated with petroleum jelly or with a silicone (Dow Corning 550 or General Electric 9992) by immersing the paper in a 2.5 per cent ether solution of petroleum jelly or a 5 per cent hexane solution of silicone, blotting off the excess solution and allowing the paper to dry. Approximately 5 to 10 µl. of mixture to be separated were applied in acetone solution at 5 cm. intervals along the lower portion of the paper about 5 cm. in from the bottom and side edges. Applications of these quantities produced a spot about 1 cm. in diameter. The solution was allowed to dry in air. Multiple applications were made when necessary in order to have at least the 1 µg. of phosphorus needed for detection. In such cases the spot was air-dried between each application. The paper was then stapled together in the form of a cylinder in such manner that adjacent edges did not come in contact. A soft iron wire was inserted diametrically through the upper end of the paper cylinder. The developing solvent was poured into the bottom of the chromatographic chamber. With a two phase system the phases were separated in a 250 ml. separatory funnel and the more polar phase was poured into the bottom of the chromatographic chamber. The non-polar phase was poured into a 200 ml. beaker and centrally placed in the bottom of the chamber. The paper cylinder was suspended over the solvent by placing a magnet on the top of the glass cover. In placing the cover on the chamber, the paper cylinder was suspended so that it would enclose but not touch
the beaker containing the non-polar phase of the two phase systems. The chromatographic system was allowed to reach equilibrium for 1 hour which was sufficient time for incorporation by the paper of the stationary, non-polar phase of the two phase systems. With a single phase solvent system, the equilibrium period was increased from 2 hours to overnight. The atmosphere of the chamber was kept saturated with solvent by lining the walls of the chamber with filter paper which was immersed in the solvent and extended to within 3 cm. of the top of the chamber. Equilibration and development were conducted at constant 15°C, temperatures with the exception of acid systems which were conducted at 10°C. At the end of equilibration, the paper was allowed to drop into the solvent by removing the magnet. The chromatograph was developed until the solvent front had reached the upper edge of the paper. Usually this required 6 to 12 hours, so that the chromatographs were prepared in the evening and allowed to develop overnight. With some systems, periods up to 36 hours were required for development. After removal from the chamber, the chromatograph was thoroughly dried at room temperatures. The resolved compounds were located by the appropriate method, and \( R_f \) values were calculated by the following formula:

\[
R_f = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent}}
\]

The distance traveled by the compound was determined by measuring to the leading edge of the spot which was usually very well defined.

**Detection of compounds**

Preliminary tests of possible indicators were made by applying 5 to 10 ul. of demeton to a small piece of filter paper. The spot was allowed
to dry and then was sprayed with indicator solution. The concentrated acids, potassium permanganate solution, silver nitrate solution, Feigl's reagent and Hanes-Isherwood's reagent were tested in this manner. The reagent which appeared most useful as an indicator of demeton was then used to spray chromatographs developed with the above solvent systems. The chromatograph was sprayed at the rate of 1 to 2 ml. per 100 cm.² then given whatever additional treatment required for color production.

The methods for hydrolysis of phosphorus compounds with Hanes-Isherwood reagent were conducted as suggested by Hanes and Isherwood (1949) and Bandurski and Axelrod (1951). In addition the period of hydrolysis at 85°C was extended and additional reducing agents were used in attempts to develop the blue color.

Analysis

Preparation of treated plants for analysis

Aphid-free mature bell pepper plants growing in 6 inch pots were treated with demeton in 100 ml. of aqueous solutions at the rates of 0.5, 1.0, 2.0 and 4.0 pounds active ingredient per acre and placed at random on a greenhouse bench. The plants were infested 1 week after treatment with aphids and red spider mites. The insect and mite populations were introduced by placing heavily infested leaves on the fourth and fifth terminal leaf of the test plant. Each treatment was replicated eight times, and eight untreated plants served as check replicates. Survival records were taken 24 hours after the plants were infested to determine whether or not sufficient time had elapsed for incorporation of demeton
into the plant.

The plants were sacrificed and weighed immediately after cutting. Each treatment was divided into two groups of four plants for extraction with different solvents. The four plants, weighing approximately 100 g., from a single treatment were then macerated in a Waring blender with 200 ml. of distilled water. The resulting broth was filtered with suction and washed with an additional 90 ml. of water. The filtrate was extracted with 300 ml. of either petroleum ether or chloroform in a 1000 ml. separatory funnel. The solvent layer was separated and evaporated just to dryness at 50°C in a water bath. Evaporation was aided by directing a stream of warm air over the mouth of the beaker. The residues was taken up in 2 ml. of acetone (total solution). At least 25 ml. of acetone solution were applied in 5 ml. increments to filter paper impregnated with a silicone for chromatography. The size of aliquot applied to the chromatograph was marked on the edge of the paper. If several chromatographs were used for residues analysis, these values were pooled to determine the aliquot size for the calculations.

Preparation of oxidized derivatives of demeton

These syntheses were performed in similar manner to those described by Hinsberg (1910) and Pummerer (1910).

The sulfoxide derivatives of demeton were prepared by reacting 2.38 g. (equimolar) of 30 per cent hydrogen peroxide with 5.35 g. of demeton dissolved in 5 ml. of glacial acetic acid. The reaction was allowed to continue for 1 week at room temperatures. The sulfone derivatives were
prepared by adding 5.55 g. of peroxide (30 per cent molar excess) to 5.435 g. demeton dissolved in 5 ml. glacial acetic acid. This preparation was mixed in an ice-salt bath at approximately 0° C. The more highly oxidized derivatives were prepared in similar manner by adding 7.93 and 10.31 g. of peroxide (30 per cent molar excesses) to samples of 5.435 g. demeton in 5 ml. glacial acetic acid. These reactions were also started in the ice-salt bath at 0° C. The cooled preparations were allowed to rise to room temperature gradually as the ice-salt bath melted and were permitted to react at room temperatures for 1 week. At the end of the reaction period, 10 ml. water and 50 ml. hot ether were added to the preparations. The solutions were filtered into 250 ml. separatory funnels and separated. The residue remaining on the filter paper was dissolved in carbon disulfide. The aqueous extract was re-extracted with 20 ml. carbon disulfide and discarded. The two extracts and the residue from the filter paper were combined and allowed to concentrate by partial evaporation. Five to 10 ml. of the concentrated carbon disulfide solutions were applied to the same chromatographs as the plant extracts. Purification of the mixed products was not attempted.

Chromatography of plant extracts, demeton and oxidized derivatives of demeton

Chromatographs containing plant extract, demeton and oxidized derivatives of demeton were developed in a solvent system of benzene-methyl ethyl ketone-water in similar manner to that already described. After development the chromatographs were dried and sprayed with Hanes-Isherwood’s reagent. The color of reduced molybdenum blue was developed by hydrolysis at 85° C. for 2 to 4 hours in moist air.
Proof of structure of demeton components and compositional analyses of the mixture

These tests were a modification of the usual quantitative methods for analysis of mercaptans as described by Kimball et al. (1921). To determine the chromatographic position of \(0_2\hspace{0.5em}S\)-diethyl \(O\)-2(ethylmercapto)-ethyl thiophosphate (III) and \(0,0\)-diethyl \(S\)-2(ethylmercapto) ethyl thiophosphate (II), the ester linkage between phosphorus and sulfur was hydrolyzed. The liberated mercaptans were determined by spraying with a solution of iodine. This test established the structure of two of the components in demeton and indicated the position of all three resolved isomers on developed chromatographs.

Chromatographs of demeton mixtures and plant extract were developed with the benzene-methyl ethyl ketone-water solvent system. The dried chromatograph was sprayed with \(3\) \(N\) sodium hydroxide and allowed to hydrolyze for \(1\) hour at room temperatures. After hydrolysis the liberated mercaptides were converted to mercaptans by spraying with \(3\) \(N\) hydrochloric acid. The position of the isomers on the paper was then determined by spraying with \(0.1\) \(N\) iodine solution. The last two steps could be combined by making the iodine solution with \(3\) \(N\) hydrochloric acid. The presence of mercaptan was indicated by bleaching of the iodine solution against a deep reddish-brown background. To determine which isomer liberated 2-mercaptoethylthioethane, a second series of chromatographs was treated with sodium hydroxide, sprayed with hydrochloric acid and heated at \(50^\circ\) C. for several minutes. This treatment drove off the more volatile ethyl mercaptan (B.P. 34.5-35.5° C.) leaving only 2-mercaptoethylthioethane (B.P. 187.5-188.5° C.) which produced the color reaction when
sprayed with the aqueous iodine or the acid iodine solutions. After heating the position of 0,0-diethyl O-2(ethylmercapto)ethyl thionophosphate (I) was noted as a yellow spot.

Analysis for isomeric percentage composition of the mixture was made by phosphorus estimation of resolved compounds from chromatographs on which the applied mixture was accurately weighed. In addition the percentage composition of the mixture was analyzed for mercaptans by a method similar to that described by Kimball et al. (1921) and Siggia (1949) and for thionosulfur in similar manner to that described in Furman (1939).

A precisely weighed sample of demeton was hydrolyzed by 50 ml. 3 N methanolic potassium hydroxide for 1 hour in a 250 ml. distillation flask. The flask was fitted with a ground glass stopper carrying a separatory funnel and was connected by air-tight fittings to an adsorption vessel containing 50 ml. standard 0.1 N iodine solution. After hydrolysis, 15 ml. concentrated hydrochloric acid was slowly added from the separatory funnel, and the contents of the flask were boiled at moderate temperatures for 30 minutes. The low boiling ethyl mercaptan was absorbed by the iodine solution and estimated by titration with standard 0.1 N sodium thiosulfate solution using a starch indicator. The high boiling 2-mercaptothioethane remaining in the distillation flask was estimated by adding 50 ml. standard iodine solution and titrating the excess with standard 0.1 N sodium thiosulfate. The thionosulfur was estimated by refluxing a precisely weighed sample of demeton in 5 ml. of 55 per cent nitric acid for 3 to 4 hours. The excess nitric acid was volatilized by
heating. The sulfuric acid formed during the reaction was precipitated with bensidine. The precipitate was separated quantitatively by filtration, washed with distilled water and dried. The filter paper with precipitate was transferred to an Erlemeyer flask. Twenty-five ml. water were added and the flask was shaken vigorously until the solution was homogeneous. Phenolphthalein indicator was added and the flask was heated to 50° C. The contents of the flask were titrated with standard 0.1 N sodium hydroxide. Just before the end point was reached, the mixture was boiled for 5 minutes and the titration was completed.

**Estimation of phosphorus**

The area covered by the phosphorus compound on the chromatograph was clipped out and digested in a micro-Kjeldahl digestion apparatus with perchloric acid. Two glass beads were added to prevent bumping of liquid when heated. If necessary, several drops of hydrogen peroxide were added to complete combustion. After cooling, the contents of the micro-Kjeldahl flask were quantitatively rinsed into a 25 ml. volumetric flask. Two ml. of amidol reagent and 1 ml. of ammonium molybdate solution were added, and the flask was filled to the mark with distilled water. The colored solution was mixed, transferred to colorimeter tubes, allowed to stand for 15 minutes and read at 640 to 680 mm. in a photometer set at zero density. The photometer was set at zero density by correcting for the reading obtained when a filter paper blank of identical area was cut from the same chromatograph as the unknown and carried through the same procedure.

The amount of phosphorus in the sample was determined by comparison
with a phosphorus standard according to the following formula:

\[
\text{ug. P in unknown} = \frac{\text{Density of Unknown}}{\text{Density of Standard}} \times \text{ug. P in standard}
\]

The density of standard phosphate was established for each set of unknowns by transferring an appropriate dilution of stock phosphate solution containing 50 ug. phosphorus to a 25 ml. volumetric flask. Two ml. perchloric acid, 2 ml. amidol reagent and 1 ml. of ammonium molybdate solution were added, and the flask was filled to the mark with distilled water. This solution was mixed, transferred to colorimeter tubes, allowed to stand for 15 minutes and read as above in a photometer set at zero density for a reagent blank of distilled water prepared in similar manner.

The amount of active ingredient present was then obtained from a calibration curve of ug. of phosphorus plotted against ug. demeton. This curve was obtained by performing the same procedure on samples of 20, 40, 80, 125, 160, 200, 250, 300, 350, 400 and 450 ug. of demeton which were compared with a standard phosphate curve.

All analyses were made on duplicate samples of solution and the readings were averaged.

To determine the total amount of demeton present in the extract, the following formula was used:

\[
\text{ug. demeton} = \frac{\text{ug. demeton in aliquot} \times 2000}{\text{ul, in aliquot}}
\]

The figure (2000) in the formula was the amount of solution from which the aliquot was taken. The amount of demeton present per g. of sample was found by dividing the amount in the extract by the green weight of the sample. This value as parts per million was obtained when the sample weight in ug. was used.
contact toxicity tests

The method of Litchfield and Wilcoxon (1949) was used for the analysis of these tests to calculate the dose-effect curves shown in Figure 1. The ED50 and slope values with their respective limits at the 0.05 level for significance are presented for each insecticide in Table 1. The ED50 values are given as pounds active ingredient applied per acre.

Table 1. The Median Effective Dosage and Slope Function with Limits for 19/20 Probability of Demeton, Parathion and Schradan in Contact Toxicity Tests on the Green Peach Aphid.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>ED50 (Limits)</th>
<th>Slope (Limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demeton</td>
<td>0.007 (0.006-0.010)</td>
<td>2.6 (2.0-3.4)</td>
</tr>
<tr>
<td>Parathion</td>
<td>0.028 (0.019-0.034)</td>
<td>2.2 (1.4-3.3)</td>
</tr>
<tr>
<td>Schradan</td>
<td>0.170 (0.123-0.234)</td>
<td>2.8 (2.0-3.8)</td>
</tr>
</tbody>
</table>

Tests for parallelism indicated that the slope ratios of the curves in Figure 1 were not significantly different and permitted the comparison of insecticides by tests of relative potency. Table 2 shows the slope and potency ratios of each insecticidal comparison with their respective limits at the 0.05 level of significance.
FIG. 1. DOSAGE-MORTALITY CURVES COMPARING THE CONTACT TOXICITY OF DEMETON, PARATHION AND SCHRADAN SPRAYS TO THE GREEN PEACH APHID, MYZUS PERSICAE (SULZ.)
Table 2. The Slope and Potency Ratios with Limits for 19/20 Probability for Comparison of Demeton, Parathion and Schradan in Contact Toxicity Tests on the Green Peach Aphid.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Slope ratio (Limits)</th>
<th>Potency ratio (Limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demeton vs. Parathion</td>
<td>1.2 (0.7-1.9)</td>
<td>3.8 (2.3-6.4)</td>
</tr>
<tr>
<td>Demeton vs. Schradan</td>
<td>1.1 (0.7-1.6)</td>
<td>23.0 (15.1-34.9)</td>
</tr>
<tr>
<td>Parathion vs. Schradan</td>
<td>1.3 (0.8-2.1)</td>
<td>6.0 (3.5-11.1)</td>
</tr>
</tbody>
</table>

These results indicate that demeton is a very effective contact insecticide in controlling the green peach aphid. The contact toxicity of demeton in these tests was 2.3 to 6.4 times that of parathion and 15.1 to 34.9 times that of schradan. Schradan was the poorest contact insecticide used in these tests.

Residual toxicity tests with insecticides applied to plant foliage

Since contact toxicity tests indicated that demeton sprays were highly toxic to the green peach aphid, tests were conducted to compare the residual toxicities of demeton, parathion and schradan sprays. The results of these tests are presented in Table 3. The percent mortalities were calculated by Abbott's formula, and the dosages are given as pounds active ingredient per acre. Infestation inspections were also made 21 days after treatment. Records were not taken at that time because the check plants had lost most of their leaves. The loss of tagged leaves in the checks prevented the calculation of percent mortalities after the 14 day record. The test was considered terminated as of that date.
Table 3. Infestations of the Green Peach Aphid on Bell Pepper Plants Treated with the Indicated Insecticides as Sprays.

<table>
<thead>
<tr>
<th>Treatment (lbs. active ingredient per acre)</th>
<th>No. reps.</th>
<th>No. live aphids</th>
<th>days indicated after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Schradan 0.06 % Mortality</td>
<td>4</td>
<td>513</td>
<td>480</td>
</tr>
<tr>
<td>Schradan 0.15 % Mortality</td>
<td>4</td>
<td>1041</td>
<td>739</td>
</tr>
<tr>
<td>Schradan 0.25 % Mortality</td>
<td>4</td>
<td>752</td>
<td>360</td>
</tr>
<tr>
<td>Schradan 0.5 % Mortality</td>
<td>4</td>
<td>904</td>
<td>125</td>
</tr>
<tr>
<td>Schradan 1.0 % Mortality</td>
<td>4</td>
<td>956</td>
<td>21</td>
</tr>
<tr>
<td>Parathion 0.03 % Mortality</td>
<td>4</td>
<td>683</td>
<td>13</td>
</tr>
<tr>
<td>Parathion 0.04 % Mortality</td>
<td>4</td>
<td>483</td>
<td>5</td>
</tr>
<tr>
<td>Parathion 0.05 % Mortality</td>
<td>4</td>
<td>477</td>
<td>13</td>
</tr>
<tr>
<td>Parathion 0.08 % Mortality</td>
<td>4</td>
<td>799</td>
<td>27</td>
</tr>
<tr>
<td>Parathion 0.16 % Mortality</td>
<td>4</td>
<td>765</td>
<td>6</td>
</tr>
<tr>
<td>Demeton 0.015 % Mortality</td>
<td>4</td>
<td>749</td>
<td>13</td>
</tr>
<tr>
<td>Demeton 0.025 % Mortality</td>
<td>4</td>
<td>356</td>
<td>2</td>
</tr>
<tr>
<td>Demeton 0.04 % Mortality</td>
<td>4</td>
<td>284</td>
<td>2</td>
</tr>
<tr>
<td>Demeton 0.08 % Mortality</td>
<td>4</td>
<td>481</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 3. (Continued)

<table>
<thead>
<tr>
<th>Treatment (lbs. active ingredient per acre)</th>
<th>No. reps.</th>
<th>No. live aphids days indicated after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demeton 0.16</td>
<td>4</td>
<td>248 0 1 16</td>
</tr>
<tr>
<td>% Mortality</td>
<td>4</td>
<td>100.0 99.6 94.4</td>
</tr>
<tr>
<td>Check</td>
<td>4</td>
<td>537 614 727 612</td>
</tr>
</tbody>
</table>

These results indicate that demeton and chradan sprays have longer residual action than parathion sprays. In these tests the toxicity of parathion to the green peach aphid started to decrease sometime during the second week after treatment. Apparently demeton and parathion sprays produced their effect on the green peach aphid much sooner and at much lower dosages than chradan.

Residual toxicity tests with insecticides applied to plant roots

Tests were conducted which showed that demeton and chradan were translocated in bell pepper plants in sufficient quantity to render treated plants toxic for long periods to the green peach aphid. The results of these tests are shown in Table 4. The percent mortalities were calculated by Abbott's formula. Dosage levels are given as pounds active ingredient per acre.

These results indicate that both demeton and chradan are translocated in bell pepper plants in sufficient quantities to control the green peach aphid for long periods. Generally demeton appeared to be more
Table 4. Infestations of the Green Peach Aphid on Bell Pepper Plants Treated with Demeton and Schradan Applications to Roots.

<table>
<thead>
<tr>
<th>Treatment (lbs. active ingredient per acre)</th>
<th>No. reps.</th>
<th>No. live aphids</th>
<th>days indicated after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td><strong>Demeton-Fertilizer</strong></td>
<td>4</td>
<td></td>
<td>531</td>
</tr>
<tr>
<td>% Mortality</td>
<td>4</td>
<td></td>
<td>27.3</td>
</tr>
<tr>
<td><strong>Demeton-Solution</strong></td>
<td>4</td>
<td></td>
<td>518</td>
</tr>
<tr>
<td>% Mortality</td>
<td>4</td>
<td></td>
<td>73.6</td>
</tr>
<tr>
<td><strong>Demeton</strong></td>
<td>4</td>
<td></td>
<td>997</td>
</tr>
<tr>
<td>% Mortality</td>
<td>4</td>
<td></td>
<td>97.9</td>
</tr>
</tbody>
</table>

<sup>a</sup>One leaf missing; initial count of 596 used to calculate % mortality.

<sup>b</sup>One leaf missing; initial count of 637 used to calculate % mortality.

<sup>c</sup>Two leaves missing; initial count of 561 used to calculate % mortality.
Table 4. (Continued)

<table>
<thead>
<tr>
<th>Treatment (lbs. active ingredient per acre)</th>
<th>No. reps.</th>
<th>No. live aphids days indicated after treatment</th>
<th>0</th>
<th>2</th>
<th>7</th>
<th>11</th>
<th>21</th>
<th>28</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>l.0</td>
<td>4</td>
<td></td>
<td>502</td>
<td>61</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>% Mortality</td>
<td>89.4</td>
<td>99.9</td>
<td>99.7</td>
<td>98.6</td>
<td>98.9</td>
<td>98.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.0</td>
<td>4</td>
<td></td>
<td>625</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% Mortality</td>
<td>96.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.0</td>
<td>4</td>
<td></td>
<td>694</td>
<td>30</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>% Mortality</td>
<td>96.2</td>
<td>100.0</td>
<td>99.9</td>
<td>100.0</td>
<td>99.6</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Schradan-Solution

| 0.5 | 4 | 912 | 687 | 1284 | 1122 | 660 | 768 | 646 |
|% Mortality | 34.4 | 8.2 | 8.6 | 6.2 | 19.7 | 27.3 |
| 1.0 | 4 | 658 | 555 | 816 | 705 | 598 | 669 | 489 |
|% Mortality | 26.6 | 19.2 | 20.4 | 4.8 | 19.6 | 25.5 |
| 2.0 | 4 | 687 | 802 | 1062 | 899 | 620 | 788 | 690 |
|% Mortality | 0.0 | 1.1 | 2.7 | 5.6 | 9.1 | 14.3 |
| 4.0 | 4 | 581 | 640 | 1072 | 973 | 1104 | 669 | 516 |
|% Mortality | 4.0 | 0.0 | 0.0 | 0.0 | 8.9 | 24.2 |
| 8.0 | 4 | 801 | 966 | 1107 | 862 | 426 | 585 | 415 |
|% Mortality | 0.0 | 9.9 | 20.0 | 41.3 | 42.1 | 55.7 |
| 16.0 | 4 | 646 | 657 | 221 | 142 | 53 | 56 | 23 |
|% Mortality | 11.4 | 77.7 | 82.8 | 91.7 | 91.0 | 97.1 |

Check

Untreated | 4 | 767 | 880 | 1177 | 1032 | 733 | 968 | 899 |

\[^d\] One leaf missing; initial count of 758 used to calculate % mortality.

\[^e\] One leaf missing; initial count of 560 used to calculate % mortality.
toxic and faster acting than schradan. There was little apparent difference in the effectiveness of aqueous solutions and fertilizer mixtures of demeton. The aqueous solutions of demeton appeared to be incorporated more rapidly by the bell pepper plants in this test.

Chromatography

Chromatography of demeton

The above mentioned biological tests indicated that bioassay of bell pepper plants with the green peach aphid would serve to show when demeton was present in sufficient quantities for making analyses of residues. It was necessary to find a solvent system which would separate mixtures of the probable components of demeton. The results of tests with various solvent systems are given in Table 5.

The use of non-impregnated paper in the early phases of the study coupled with poor solvent systems indicated that a revision of technique was necessary. The reversed phase systems of Winteringham (1950) and Kritchevsky and Tiselius (1951) suggested a possible direction for exploration. Impregnation with petroleum jelly, as indicated in Table 5, was not successful and was discarded in favor of silicone impregnated paper. Several solvent systems were found to resolve demeton into only two components. Apparently two of the isomers had identical mobilities in these systems. It was also possible that isomerization to a single isomer was favored by some solvent systems. Gross visual comparisons of size and color intensity of the molybdenum blue spots obtained with the benzene-methanol-water system indicated that this system favored the
<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Paper treat.</th>
<th>Temp. (°C.)</th>
<th>0,0-diethyl 0-2- (ethylmercapto)ethyl thionophosphate</th>
<th>0,0-diethyl 0-2- (ethylmercapto)ethyl thio phosphate</th>
<th>0,0-diethyl S-2- (ethylmercapto)ethyl thio phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid-n-Butanol-HOH</td>
<td>None</td>
<td>Room</td>
<td>Indeterminate due to trailing.</td>
<td>Indeterminate due to trailing.</td>
<td></td>
</tr>
<tr>
<td>Acetic acid-Ethyl acetate-HOH</td>
<td>None</td>
<td>Room</td>
<td>Indeterminate due to trailing.</td>
<td>Indeterminate due to trailing.</td>
<td></td>
</tr>
<tr>
<td>GE9992</td>
<td>4</td>
<td>15</td>
<td>0.05 ± 0.006</td>
<td>0.62 ± 0.02</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>Benzene-Dioxane-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.08 ± 0.006</td>
<td>0.72 ± 0.01</td>
<td>0.91 ± 0.007</td>
</tr>
<tr>
<td>Benzene-Methanol-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.03 ± 0.006</td>
<td>0.41 ± 0.006</td>
<td>1.00 ± 0.02</td>
</tr>
<tr>
<td>Benzene-Methyl ethyl ketone-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.26 ± 0.02</td>
<td>No resolution</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>n-Butanol-Urea</td>
<td>None</td>
<td>Room</td>
<td>Indeterminate due to trailing.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform-Ethanol-HOH</td>
<td>Petrol. jelly</td>
<td>4</td>
<td>Poor separation. Trailing. Dropped in favor of Chloroform-Methanol-HOH.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------</td>
<td>-------------</td>
<td>--------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform-Dioxane-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.26 ± 0.01, No resolution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform-Methanol-HOH</td>
<td>GE9992</td>
<td>4</td>
<td>Poor separation, not calculated.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform-Methanol-Formamide</td>
<td>GE9992</td>
<td>15</td>
<td>0.20 ± 0.03, No resolution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform-Methyl cello-solve-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.08 ± 0.02, No resolution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform-Methyl ethyl ketone-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.06 ± 0.03, No resolution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol-Ammonium hydroxide-HOH</td>
<td>None, Petrol. jelly</td>
<td>Room, 4</td>
<td>Indeterminate due to trailing. No resolution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate-Formamide-Pyridine</td>
<td>None, Petrol. jelly</td>
<td>Room, 4</td>
<td>Indeterminate due to trailing. No resolution</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5. (Continued)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate-Formamide-Triethanolamine</td>
<td>Petrol. jelly</td>
<td>4</td>
<td>Indeterminate due to trailing.</td>
</tr>
<tr>
<td>Formic acid-Methanol-HOH</td>
<td>None</td>
<td>4</td>
<td>0.92 No resolution.</td>
</tr>
<tr>
<td>n-Hexane-Dioxane-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.87 ± 0.07 No resolution</td>
</tr>
<tr>
<td>n-Hexane-Methanol-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.86 ± 0.03 No resolution</td>
</tr>
<tr>
<td>n-Hexane-Methyl cellosolve-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.78 ± 0.05 No resolution</td>
</tr>
<tr>
<td>n-Hexane-Methyl ethyl ketone-HOH</td>
<td></td>
<td></td>
<td>0.94 ± 0.004k</td>
</tr>
<tr>
<td>Methanol-None</td>
<td>4</td>
<td>45-5-50 mixture. Indeterminate.</td>
<td></td>
</tr>
<tr>
<td>Ammonium-hydroxide-HOH</td>
<td>None</td>
<td>4</td>
<td>0.86 No resolution. No resolution</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.84 ± 0.005</td>
</tr>
</tbody>
</table>
Table 5. (Continued)

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Paper treat.</th>
<th>Temp. (°C.)</th>
<th>( R_f ) ± Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl cellosolve-Methyl ethyl ketone-Ammonium hydroxide</td>
<td>None</td>
<td>4</td>
<td>No resolution. Not calculated.</td>
</tr>
<tr>
<td>iso-Octane-Dioxane-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td>iso-Octane-Methanol-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.82 ± 0.02</td>
</tr>
<tr>
<td>iso-Octane-Methyl cellosolve-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.81 ± 0.01</td>
</tr>
<tr>
<td>iso-Octane-Methyl ethyl ketone-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.04 ± 0.006</td>
</tr>
<tr>
<td>Petroleum ether-Methanol-HOH</td>
<td>GE9992</td>
<td>15</td>
<td>0.70 ± 0.01</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------</td>
<td>-------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Petrolatum etha-Methanol-Formamide</td>
<td>H9992</td>
<td>15</td>
<td>No resolution. Moved with solvent front.</td>
</tr>
<tr>
<td>Phenol-HOH</td>
<td>None</td>
<td>Room</td>
<td>Discarded. Color failure with KMnO$_4$.</td>
</tr>
</tbody>
</table>
isomerization of the thionophosphate isomer (I) to the thiophosphate iso-
momers (II and III). It was believed that this might be caused by the
methanol in the mixture, since other systems containing benzene, water and
dioxane or methyl ethyl ketone produced no indication of increased isomer-
ization. The benzene-methyl cellosolve-water system apparently resolved
only two components of demeton mixtures. Attempts to substitute formamide
for the water in several solvent systems were unsuccessful. Apparently
the mixture to be chromatographed was diffusely spread along the moving
solvent front and no resolution was obtained.

Adequate resolution of the components of demeton, as indicated in
Table 5, was obtained with solvent systems composed of benzene-dioxane-
water, benzene-methyl ethyl ketone-water, iso-octane-methyl ethyl ketone-
water and hexane-methyl ethyl ketone-water.

A two phase solution of benzene-methyl ethyl ketone-water was
selected as the solvent system for chromatographing plant extracts,
because its ingredients were available in sufficient quantity to complete
the study. It was felt that the benzene-dioxane-water system was superior,
because it produced more compact definition of spots, and the less mobile
isomers were more widely separated. Unfortunately the supply of dioxane
was diminished before the extracts were chromatographed. The writer did
not wish to delay the study for delivery of new solvent. Specimen chromato-
graphs of these two systems are reproduced in Figure 2. Unfortunately the
benzene-methyl ethyl ketone-water gave poor contrast and did not repro-
duce well.
FIG. 2. REVERSED PHASE PAPER PARTITION CHROMATOGRAPHS OF TECHNICAL DEMETON USING (A) BENZENE-METHYL ETHYL KETONE WATER AND (B) BENZENE-DIOXANE-WATER AS SOLVENT SYSTEMS ON WHATMAN NO.1 PAPER IMPREGNATED WITH GE 9992 SILICONE.
Detection of demeton

The results of these studies are shown in Table 6. Preliminary tests with possible indicator reagents showed that concentrated acids, and Feigl's reagent for phosphate were unsatisfactory for location of demeton. These tests also indicated that ammonical silver nitrate, potassium permanganate and Hanes-Is herwood reagent might be used to locate demeton on filter paper. Chromatographs sprayed with the silver nitrate and potassium permanganate solutions gave varying degrees of success. The permanganate reagent did not produce a color reaction on chromatographs developed with solvent systems containing phenol.

The hydrolysis and reduction methods of Hanes and Is herwood (1949) and Bandurski and Axelrod (1951) produced variable and generally poor results. It was necessary to extend the hydrolysis period with Hanes-Is herwood reagent 2 or more hours. Usually the reduced phosphomolybdate showed without additional treatment when the hydrolysis period was lengthened. Excessive background color and charring of the paper occasionally occurred which obscured the molybdenum blue color production. Charring was reduced when the atmosphere of the oven was kept saturated with water vapor. The interference from charring was minimized and that of background was selectively bleached with a spray containing equal parts of ammonium hydroxide and distilled water.

The Hanes-Is herwood reagent with an extended hydrolysis period and, if necessary, stannous chloride reduction or autoclaving was used for the later phases of study. The phosphate test was more specific than the silver nitrate and potassium permanganate tests. Almost any reducing substance will give a positive test with the latter reagents.
<table>
<thead>
<tr>
<th>Reagent</th>
<th>Hydrolysis period</th>
<th>Reducing agent</th>
<th>Color Spot</th>
<th>Background</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>None</td>
<td>None</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂SO₄</td>
<td>None</td>
<td>None</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HNO₃</td>
<td>None</td>
<td>None</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feigl's</td>
<td>Dry at 700 °C.</td>
<td>Benzidine HCl</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonical AgNO₃</td>
<td>10 min. at 1000 °C</td>
<td>None</td>
<td>Brown</td>
<td>Buff</td>
<td>Results variable.</td>
</tr>
<tr>
<td>KMnO₄–Na₂CO₃</td>
<td>None</td>
<td>None</td>
<td>Yellow</td>
<td>Wine</td>
<td>Results variable. Fades with drying to uniform color. Negative with phenol-water solvent system.</td>
</tr>
<tr>
<td>Hanus–Isherwood</td>
<td>7 min.</td>
<td>H₂S</td>
<td>Negative</td>
<td>Blue</td>
<td>Results variable and often negative. Results same as with ultra-violet autoclave Blue background with NH₄OH.</td>
</tr>
<tr>
<td></td>
<td>1 min.</td>
<td>Ultra-violet</td>
<td>Blue</td>
<td>Blue</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 min.</td>
<td>Autoclave</td>
<td>Blue</td>
<td>Blue</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 min.</td>
<td>H₂S</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 hour</td>
<td>None</td>
<td>Blue</td>
<td>0-Blue</td>
<td>Moist atmosphere necessary in chamber to reduce charring. Bleach background with NH₄OH spray. Intensity of spot often increased with SnCl₂ spray or autoclaving at 8-10 lb. and bleaching background.</td>
</tr>
<tr>
<td></td>
<td>2 hour</td>
<td>None</td>
<td>Blue</td>
<td>0-Blue</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 hour</td>
<td>None</td>
<td>Blue</td>
<td>0-Blue</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 hour</td>
<td>None</td>
<td>Blue</td>
<td>0-Blue</td>
<td></td>
</tr>
</tbody>
</table>
Analysis

Chromatography of plant extracts

Results of bioassay of bell pepper plants infested with the green peach aphid and with red spider mites, Table 7, indicated that sufficient time had elapsed for incorporation of demeton into the treated plants. The plants were sacrificed and extracted. The extracts were chromatographed on silicone impregnated filter paper with the benzene-methyl ethyl ketone-water solvent system. Demeton and its oxidized derivatives were simultaneously developed on the same chromatograph. The results of these tests are presented in Table 8.

Table 7. Infestations of the Green Peach Aphid and Red Spider Mites on Bell Pepper Plants Treated with the Indicated Dosages of Demeton.

<table>
<thead>
<tr>
<th>Demeton (lbs. active ingredient per acre)</th>
<th>No. reps.</th>
<th>No. live aphids 2h hrs. after infested</th>
<th>No. red spider mites 2h hrs. after infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>8</td>
<td>16</td>
<td>99</td>
</tr>
<tr>
<td>1.0</td>
<td>8</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>2.0</td>
<td>8</td>
<td>38</td>
<td>29</td>
</tr>
<tr>
<td>4.0</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Check</td>
<td>8</td>
<td>163</td>
<td>211</td>
</tr>
</tbody>
</table>

The Rf values, Table 8, indicate that only the O,O-diethyl S-2-(ethylmercapto)ethyl thiophosphate isomer (II) in demeton mixtures entered and was translocated in mature bell pepper plants during the first 8 days after the insecticide was applied to the roots in aqueous solutions. The derivatives, formed in reactions of demeton with three
Table 8. Rf Values and Their Standard Deviations (s) of Demeton Isomers, Oxidized Demeton Derivatives and Chloroform and Petroleum Ether Extracts of Bell Pepper Plants Obtained with a Solvent System of Benzene-Methyl Ethyl Ketone-Water on Whatman No. 1 Filter Paper Impregnated with DC-550 and GE-9992 Silicons.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Extracting solvent</th>
<th>Silicone</th>
<th>Known</th>
<th>Unknown indicated as lbs. active ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>0,0-diethyl O-2-</td>
<td>CHCl₃</td>
<td>DC-550</td>
<td>0.15 ± 0.03</td>
<td>---</td>
</tr>
<tr>
<td>(ethylmercapto)-</td>
<td>CHCl₃</td>
<td>GE-9992</td>
<td>0.03 ± 0.004</td>
<td>---</td>
</tr>
<tr>
<td>ethyl thiono-</td>
<td>Pet. ether</td>
<td>DC-550</td>
<td>0.15 ± 0.03</td>
<td>---</td>
</tr>
<tr>
<td>phosphate</td>
<td></td>
<td></td>
<td></td>
<td>0.30 ± 0.08</td>
</tr>
<tr>
<td>0,8-diethyl O-2-</td>
<td>CHCl₃</td>
<td>DC-550</td>
<td>0.30 ± 0.08</td>
<td>---</td>
</tr>
<tr>
<td>(ethylmercapto)-</td>
<td>CHCl₃</td>
<td>GE-9992</td>
<td>0.41 ± 0.006</td>
<td>---</td>
</tr>
<tr>
<td>ethyl thio-</td>
<td>Pet. ether</td>
<td>DC-550</td>
<td>0.30 ± 0.08</td>
<td>---</td>
</tr>
<tr>
<td>Compound</td>
<td>Extracting solvent</td>
<td>Silicone Known</td>
<td>( R_f + a )</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>--------------------</td>
<td>----------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>0.4-Dimethyl-1,2,3- (ethylthio)triazole</td>
<td>CEH13</td>
<td>0.59 ( \pm 0.02 )</td>
<td>1.0 ( \pm 0.02 )</td>
<td></td>
</tr>
<tr>
<td>0.4-Dimethyl-1,2,3- (ethylthio)triazole plus (0)</td>
<td>CEH13</td>
<td>0.86 ( \pm 0.02 )</td>
<td>1.0 ( \pm 0.02 )</td>
<td></td>
</tr>
<tr>
<td>0.4-Dimethyl-1,2,3- (ethylthio)triazole plus 2 (0)</td>
<td>CEH13</td>
<td>0.72 ( \pm 0.02 )</td>
<td>1.0 ( \pm 0.02 )</td>
<td></td>
</tr>
<tr>
<td>0.4-Dimethyl-1,2,3- (ethylthio)triazole plus 3 (0)</td>
<td>CEH13</td>
<td>Same as + 2 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4-Dimethyl-1,2,3- (ethylthio)triazole plus 4 (0)</td>
<td>CEH13</td>
<td>Same as + 2 (0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Parent compounds also present.*

\( a \cdot t = 1.20; P > 20\%
\( b \cdot t = 0.00; P > 20\%
\( c \cdot t = 1.20; P > 20\%
\( d \cdot t = 1.20; P > 20\%
molar and four molar quantities of hydrogen peroxide, gave identical \( R_f \) values to the derivatives from reactions of demeton with equimolar and two molar quantities of peroxide. In addition considerable quantities of free sulfur were formed in the reaction of demeton with the three molar and four molar quantities of peroxide. These findings were taken as evidence that attempts to oxidize the P-S-C bonds resulted in some decomposition of the demeton molecule. There was also some free sulfur formation in the reaction of demeton with two molar peroxide indicating the replacement of thiono-sulfur in \( \text{O}_2\text{O-diethyl O-2(ethylmercapto)ethyl thionophosphate} \) by oxygen. Oxidative replacement of thiono-sulfur probably accounts for some of the free sulfur formed in the attempts to produce the more highly oxidized demeton derivatives. Further characterization of oxidized demeton derivatives was not attempted because the plant extracts had very similar \( R_f \) values to those of unchanged \( \text{O}_2\text{O-diethyl S-2(ethylmercapto)ethyl thiophosphate} \).

Proof of structure of demeton components and compositional analyses of the mixture

Results of these tests, Table 9, showed that the mixture of technical demeton used in this investigation contained about 24.1 per cent \( \text{O}_2\text{O-diethyl S-2(ethylmercapto)ethyl thiophosphate (II)} \), 20.2 per cent \( \text{O}_2\text{O-diethyl O-2(ethylmercapto)ethyl thionophosphate (I)} \) and 54.9 per cent \( \text{O}_2\text{S-diethyl O-2(ethylmercapto)ethyl thiophosphate (III)} \). The tests for liberation of mercaptans definitely proved the presence of the two thionophosphate isomers (II and III) in addition to the thionophosphate isomer (I) and indicated the position of all three isomers on the chromatographs.
### Table 9. Analyses of Percentage Isomeric Composition of Demeton and Tests for Liberation of Mercaptans by Demeton Isomers and Bell Pepper Plant Extracts.

<table>
<thead>
<tr>
<th>Compound</th>
<th>E&lt;sub&gt;2&lt;/sub&gt;</th>
<th>ug. P</th>
<th>ug. Demeton</th>
<th>%age in mixture</th>
<th>Color test for mercaptans</th>
<th>Room temperatures</th>
<th>After heating (50°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0,0-diethyl</td>
<td>0.03</td>
<td>32.2</td>
<td>2h8</td>
<td>20.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Red-Brown</td>
<td>Red-Brown</td>
<td>Yellow</td>
</tr>
<tr>
<td>0-2(ethyl-mercaptopo)ethyl thionophosphate</td>
<td>0.15</td>
<td></td>
<td></td>
<td>20.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Red-Brown</td>
<td>Red-Brown</td>
<td>Yellow</td>
</tr>
<tr>
<td>0,3-diethyl</td>
<td>0.41</td>
<td>87.7</td>
<td>675</td>
<td>54.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>White</td>
<td>Red-Brown</td>
<td>Red-Brown</td>
</tr>
<tr>
<td>0-2(ethyl-mercaptopo)ethyl thionophosphate</td>
<td>0.30</td>
<td></td>
<td></td>
<td>51.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>White</td>
<td>Red-Brown</td>
<td>Red-Brown</td>
</tr>
<tr>
<td>0,0-diethyl</td>
<td>1.00</td>
<td>38.6</td>
<td>297</td>
<td>24.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>White</td>
<td>Red-Brown</td>
<td>White</td>
</tr>
<tr>
<td>S-2(ethyl-mercaptopo)ethyl thionophosphate</td>
<td>0.99</td>
<td></td>
<td></td>
<td>23.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>White</td>
<td>Red-Brown</td>
<td>White</td>
</tr>
</tbody>
</table>

**Plant Extract (Indicated as lbs. active ingredient per acre)**

- **Check**
  - ug. P: 1.00
  - Color: Red-Brown

- **0.5**
  - ug. P: 1.00
  - Color: Red-Brown

- **1.0**
  - ug. P: 1.00
  - Color: Red-Brown

- **2.0**
  - ug. P: 1.00
  - Color: Red-Brown

- **4.0**
  - ug. P: 1.00
  - Color: Red-Brown

---

<sup>a</sup>Phosphate analyses: 1230 ug. demeton applied.

<sup>b</sup>Thione-sulfur analyses: 129.10 ml. 0.1 N NaOH = 0.2069 g. thionosulfur in 1.0222 g. demeton.

<sup>c</sup>Mercaptan analyses: 0.5025 g. demeton hydrolyzed. 41.26 ml. 0.1 N thiosulfate = ethyl mercaptan.

9.62 ml. 0.1 N thiosulfate = mercaptoethylthioethane.
The positive reaction by the treated plant extracts to liberation of 2-mercaptoethyl-thioethane, Table 9, and the statistically identical \( R_f \) values, Table 8, of the extract and known compound are strong evidence that the 0,0-diethyl S-2(ethylmercapto)ethyl thiophosphate isomer (II) was incorporated and translocated without conversion in these tests. No interference of color reactions by extracted plant pigments was noted.

The test for liberated mercaptans could be employed as a chromatographic indicator spray reagent. It was found to be especially useful in cases where 0,0-diethyl 0-2(ethylmercapto)ethyl thionophosphate (I) and 0,5-diethyl 0-2(ethylmercapto)ethyl thiophosphate (III) failed to resolve completely due to poor impregnation of the filter paper with silicone. Apparently the physical properties of 0,3-diethyl 0-2(ethylmercapto)-ethyl thiophosphate (III) are more similar to those of the thionophosphate isomer (I) than to 0,0-diethyl S-2(ethylmercapto)ethyl thiophosphate (II) as was assumed by Hartley (1952). The \( R_f \) values obtained with the procedure herein described were difficult to calculate due to shrinkage and subsequent curling of the filter paper when the strong caustic solution was applied. To calculate these values it was necessary to exercise extreme care when flattening and stretching the filter paper to its original size. These faults might be reduced with proper adjustment of reagents.

**Estimation of phosphate**

Areas, containing phosphate compounds having identical \( R_f \) values to the introduced materials, were clipped, pooled, digested and colorimetrically analyzed for phosphate. The amount of active material
<table>
<thead>
<tr>
<th>Treatment (lbs. active ingredient per acre)</th>
<th>Extracting solvent</th>
<th>Silicic acid weight (g.)</th>
<th>Green aliquot (ul.)</th>
<th>ug. P (Aliquot)</th>
<th>ug. Demeton (Aliquot)</th>
<th>Total demeton (ug.)</th>
<th>p.p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>Pet. ether</td>
<td>DC-550</td>
<td>100.35</td>
<td>Blank</td>
<td>Blank</td>
<td>0.0</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>DC-550</td>
<td>87.43</td>
<td>75</td>
<td>Blank</td>
<td>13.8</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>GE-9992</td>
<td>87.43</td>
<td>100</td>
<td>Blank</td>
<td>3.0</td>
<td>3.8</td>
</tr>
<tr>
<td>0.5</td>
<td>Pet. ether</td>
<td>DC-550</td>
<td>80.50</td>
<td>25</td>
<td>0.1</td>
<td>0.3</td>
<td>64.0</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>DC-550</td>
<td>90.05</td>
<td>100</td>
<td>1.8</td>
<td>13.8</td>
<td>276.0</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>GE-9992</td>
<td>90.05</td>
<td>50</td>
<td>1.1</td>
<td>8.5</td>
<td>340.0</td>
</tr>
<tr>
<td>1.0</td>
<td>Pet. ether</td>
<td>DC-550</td>
<td>65.60</td>
<td>25</td>
<td>1.7</td>
<td>13.0</td>
<td>1040.0</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>DC-550</td>
<td>82.13</td>
<td>75</td>
<td>3.2</td>
<td>24.6</td>
<td>656.1</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>GE-9992</td>
<td>82.13</td>
<td>50</td>
<td>2.2</td>
<td>16.9</td>
<td>676.0</td>
</tr>
<tr>
<td>2.0</td>
<td>Pet. ether</td>
<td>DC-550</td>
<td>128.55</td>
<td>25</td>
<td>1.8</td>
<td>13.8</td>
<td>1104.0</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>DC-550</td>
<td>71.25</td>
<td>100</td>
<td>5.1</td>
<td>39.2</td>
<td>784.0</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>GE-9992</td>
<td>71.25</td>
<td>125</td>
<td>9.0</td>
<td>69.2</td>
<td>1107.2</td>
</tr>
<tr>
<td>4.0</td>
<td>Pet. ether</td>
<td>DC-550</td>
<td>101.80</td>
<td>25</td>
<td>5.3</td>
<td>40.7</td>
<td>3256.0</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>DC-550</td>
<td>75.00</td>
<td>225</td>
<td>75.3</td>
<td>579.2</td>
<td>5199.1</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>GE-9992</td>
<td>75.00</td>
<td>50</td>
<td>8.7</td>
<td>66.9</td>
<td>5352.0</td>
</tr>
</tbody>
</table>

*Only 0,0-diethyl S-2(ethylmercapto)ethyl thiophosphate involved as indicated in Table 9.
expressed as ug. demeton present in the pooled chromatographic aliquots, was obtained from Figure 3. Figure 3 was established from phosphate analyses of demeton samples. The results of the residue analyses are presented in Table 10. These results indicate that petroleum ether might not be as satisfactory a solvent for extraction as chloroform. The residue analyses, Table 10, agreed quite well with the bioassay for demeton incorporation, Table 7, with the possible exception of treatments of demeton at the rate of 2 pounds active ingredient per acre. This apparent disparity was probably due to large biological variations in experimental material which could not be isolated. Differences in insecticidal susceptibility within the aphid and mite populations as well as differences in the rate of incorporation of demeton within the treated plants might account for the disagreement between Tables 7 and 10.
FIG. 3. PHOSPHORUS CONTENT OF THE TECHNICAL DEMETON SAMPLE STUDIED.
DISCUSSION

Bioassay of demeton showed that spray applications of the insecticide were highly effective as a contact poison and had good residual action on the green peach aphid. The results of spray tests for residual action did not specifically indicate that demeton was translocated in bell pepper plants after treatment. This study would have been strengthened if these tests had been designed to show that demeton might be translocated following spray applications. It would have been necessary to spray the top halves of one group of plants and the bottom halves of a second group to secure this information. Infestation records of aphids before and after treatment on the sprayed and unsprayed portions of the plants would indicate translocation of insecticide. In addition such a test would indicate the direction of movement of insecticide in the plant. Mortality records of the green peach aphid on bell pepper plants treated with root applications of demeton indicated that the insecticide was taken up by the roots and translocated upward to the aerial portions of the treated plants. For the purposes of this study, the translocation of the insecticide following its application to the roots was considered sufficient evidence that bell pepper plants infested with the green peach aphid could be used to indicate the incorporation of demeton into the plant prior to analysis of residues.

The determination of a satisfactory solvent system for chromatographic separations was a most difficult and perplexing problem. None of the systems used in previous studies of metabolically important
phosphate esters were found to be satisfactory. The reversed phase methods of chromatographic separation were found to be most satisfactory for separating the isomers of demeton. Careful impregnation of the paper was very important. It was noted that impregnation from a solution whose percentage silicone content was based on weight/volume instead of volume/volume proportions resulted in almost complete failure of the 0,5-diethyl O-2(ethylmercapto)ethyl thiophosphate ester (III) to separate from 0,0-diethyl O-2(ethylmercapto)ethyl thionophosphate (I). The spray reagents for liberation of mercaptans were useful for determining Rf values of isomeric components which failed to separate completely. Incomplete separation usually produced a more intense blue spot where the compounds overlapped when the production of molybdenum blue was used to locate the resolved compounds on chromatographs. Slight variations of the solvent system used in the final phases of the study did not greatly affect the separation of the isomers. Apparently it was necessary only to keep the two phases of the system saturated with respect to the components of the opposite phase.

The methods of preparing the treated plants could probably be improved by recording the green weight, drying at 50° C. temperatures in an oven before maceration and extracting the macerate before filtering.

With the instruments used for phosphate estimation in this investigation, the most accurate determinations were made when a reading in the range of 100 to 150 Klett units was obtained. Analyses of phosphate in samples containing between 250 and 400 ug. demeton produced readings in
this range with the method of Allen (1940). The low reading corresponds to about 2.5 p.p.m. demeton in a 100 g. sample of plant material if all of the insecticide was measured. Readings corresponding to as little as 0.2 p.p.m. demeton in 100 g. plant material could be determined with less accuracy. In order to get this accuracy, larger aliquots than those used in this investigation would have to be applied to filter paper chromatographs. Greater precision at lower concentrations or with less plant material could probably be obtained with one of the analytical procedures which used stannous chloride as a reducing agent. Analyses based on sulfur content might be more precise under these conditions, since the atomic ratio of sulfur is about twice that of phosphorus in the demeton molecule. Recovery experiments would have strengthened this investigation and would have indicated where revision or modification of the extraction technique was needed.

Chemical analyses of demeton and resolved demeton components showed that the sample used in this study was a mixture of 24.1 per cent 0,0-diethyl S-2(ethylmercapto)ethyl thiophosphate (II), 54.9 per cent 0,3-diethyl O-2(ethylmercapto)ethyl thiophosphate (III) and 20.2 per cent 0,0-diethyl O-2(ethylmercapto)ethyl thiophosphate (I). Chromatographic separation of mature bell pepper plant extracts strongly indicate that only the unconverted 0,0-diethyl S-2(ethylmercapto)ethyl thiophosphate isomer (II) from the demeton mixture was incorporated and translocated in this study. Rf values for the plant extract and this isomer were almost identical and were not statistically different. In addition the plant extract produced a positive reaction for the liberation of 2-mercaptethylichthioethane. These findings were taken as strong indications that
conversion did not occur in these investigations. Interference of color production by extracted plant pigments was not noted.

The results of this investigation do not completely exclude the possibility that the active compound in extracts of mature bell pepper plants was a derivative of 0,0-diethyl S-2(ethylmercapto)ethyl thio-phosphate (II) or of the other isomers in demeton. The behavior of the parent isomer ($R_f$ of 1.00 ± 0.02) on silicone impregnated filter paper with the benzene-methyl ethyl ketone-water solvent system failed to differentiate any derivative which might be more mobile in the system. The extracts of treated plants and the resolved 0,0-diethyl S-2(ethylmercapto)ethyl thio-phosphate isomer (II) produced identical positive reactions to liberation of 2-mercaptoethylthioethane. However, the corresponding sulfoxide (VI) and sulfone (VII) derivatives or any other derivative (IV and V) with a high boiling point and a hydrolytically freed -SH group would give a positive reaction. Through an oversight undeveloped chromatographs of plant extract were not reserved for testing the presence of the $-\text{CH}_2\text{SCH}_2-$, $-\text{CH}_2\text{S(O)CH}_2-$ and $-\text{CH}_2\text{S(O)CH}_2-$ groups. As near as could be determined in this study, the active material in extracts of mature bell pepper plants was not an oxidized derivative of the parent compounds.

The investigation, described herein, supported the contention of Hartley (1952) that the bulk of the insecticidal activity of demeton was principally due to the 0,0-diethyl S-2(ethylmercapto)ethyl thio-phosphate (II) content of the mixture. For this reason it is likely that considerable error exists in much of the early experimental work with demeton due to variations in the content of 0,0-diethyl S-2(ethylmercapto)ethyl thio-phosphate (II) among different samples. The labeling of the active
ingredient as O₂O-diethyl O-2(ethylmercapto)ethyl thionophosphate (I) is also a poor practice.

In previous paragraphs it has been indicated that insecticidal conversion was not apparent in this investigation. Hartley (1952) and more recently Metcalf (1954) have stated in popular articles that both O₂O-diethyl S-2(ethylmercapto)ethyl thio phosphate (II) and O₂O-diethyl O-2(ethylmercapto)ethyl thionophosphate (I) are converted to more toxic derivatives by plant metabolic activities. Detailed results were not given by either author nor was the presence of the O₂S-diethyl O-2-(ethylmercapto)ethyl thio phosphate isomer (III) noted. In both of these investigations, tracer labeled insecticides were applied to seedling plants or young trees. Consequently it was likely that conversion would occur in a young plant or early in the season in the case of a perennial. In these instances the plant would be in a state of high metabolic activity. The failure to get conversion of demeton by the fruiting bell pepper plants in the experiments of the present investigation might be due to the use of older plants whose metabolic activities were somewhat reduced.

The investigator hopes to continue this investigation as the demands and obligations of his present position permit the time for additional work. It is felt that the extraction procedures, chromatographic solvent systems and analytical methods can be improved. In addition, a wider variety of plants should be surveyed over various developmental stages, and the periods between treatment and sacrifice should be varied. It would also be of interest to study any changes in demeton which might occur in the soil before plant incorporation and to explore the problems of selective absorption of certain isomers of demeton mixtures.
SUMMARY

Preliminary bioassay indicated that demeton was translocated in bell pepper plants in amounts toxic to the green peach aphid. This insect and red spider mites were used to indicate the incorporation of demeton by mature bell pepper plants.

Reversed phase partition chromatographic separations of demeton on Whatman No. 1 filter paper impregnated with silicones (Dow Corning 550 or General Electric 9992) by a two phase solvent mixture of benzene-methyl ethyl ketone-water indicated that the insecticide sample used was composed of three isomeric compounds. Phosphate analyses of resolved compounds on chromatographs showed this mixture to be composed of 24.1 per cent $O,O$-diethyl $S$-$2(\text{ethylmercapto})$ethyl thiophosphate (II), 54.9 per cent $O,S$-diethyl $O$-$2(\text{ethylmercapto})$ethyl thiophosphate (III) and 20.2 per cent $O,O$-diethyl $O$-$2(\text{ethylmercapto})$ethyl thionophosphate (I). The compounds were located by production of molybdenum blue complex. The chromatographic locations and structures of the thiophosphate esters (II and III) were proved by iodine oxidation of liberated mercaptans. The presence of the thiono-isomer (I) was also indicated by iodine oxidation and proved by analysis of thiono-sulfur. Values similar to those given by the phosphate analyses for the thionophosphate esters (II and III) were obtained by iodometric analyses of the mercaptans liberated by alkaline hydrolysis. Analysis of thionosulfur gave a value for the thionophosphate ester (I) similar to that obtained by phosphate analyses.
Chromatographic separations of demeton treated bell pepper plant extracts indicated that the unchanged O,O-diethyl S-2(ethylmercapto)-ethyl thiocephosphate isomer (II) was probably the active toxicant in mature plants during the first week after application to roots. Phosphate analyses of chromatographically resolved compounds in bell pepper plant extracts appeared to be an adequate method for routine determination of demeton residues.

It is suggested that additional studies of this type be made on younger plants over longer experimental periods. These methods should be also extended to investigations on a wide variety of plants which are of economic importance.
CONCLUSIONS

1. The contact toxicity of demeton to the green peach aphid is very high.

2. Sufficient quantities of demeton are incorporated and translocated by bell pepper plants to render these plants toxic to the green peach aphid and red spider mites.

3. The sample of demeton used in this investigation was a mixture of three isomers. These isomers were 24.1 per cent 0,0-diethyl S-2-(ethylmercapto)ethyl thiophosphate (II), 54.9 per cent 0,3-diethyl O-2(ethylmercapto)ethyl thiophosphate (III) and 20.2 per cent 0,0-diethyl O-2(ethylmercapto)ethyl thionophosphate (I).

4. The translocated insecticidal activity in mature bell pepper plants during the first week after application of demeton is apparently due only to adsorption of unchanged 0,0-diethyl S-2(ethylmercapto)ethyl thiophosphate (II).


6. Additional investigation is needed.
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