Paper Chromatography of the Systemic Insecticides, Demeton and Schradan

R. B. MARCH, R. L. METCALF, and T. R. FUKUTO
Department of Entomology, University of California, Citrus Experiment Station, Riverside, Calif.

In order to understand the complex biochemistry of the systemic insecticides, demeton and schradan, it is essential to separate and identify the components of the technical insecticides and their metabolites and degradation products in plants, insects, and mammals. Two paper-chromatographic techniques have been applied to the separation and identification of the components of technical demeton and related compounds and a third has been developed for technical schradan and related phosphoramide esters.

In studies of the mode of action of the systemic insecticides, *O,O*-diethyl *O*-[2-(ethylmercapto)ethyl] thionophosphate demeton (common name suggested by Chemagro Corp., N. Y.), and octamethylpyrophosphoramide schradan, an understanding of their complex biochemistry required separation and identification of the components of the technical insecticides and their metabolites and degradation products in plants, insects, and mammals. During investigations on the mode of action of parathion and related organophosphorus esters (*8*, *9*), a method was developed for the reversed phase paper chromatography of such compounds (*10*). This method has now been successfully applied for use with demeton but is completely ineffective for use with schradan.

Gage (*3*) has reported a paper-chromatographic method for parathion and related esters employing propylene glycol–impregnated paper and petroleum ether–benzene (4 to 1) as the mobile solvent. This method provides a progression of spots for parathion and related compounds in the exact reverse order of the technique noted above (*10*). It has been possible to apply this technique for use with demeton. A paper-chromatographic method for the separation of the components of technical demeton reported by Koike (*7*) employs untreated paper and phenol-water (4 to 1) as the mobile solvent.

Gardner and Heath (*4*) have demonstrated by column chromatography that technical demeton is a mixture of two active isomers, *O,O*-diethyl *O*-[2-(ethylmercapto)ethyl] thionophosphate (thiono isomer) and *O,O*-diethyl *S*-[2-(ethylmercapto)ethyl] thiophosphate (thiol isomer), by partitioning technical demeton with methanol-iso-octane on a kieselguhr column. This method should be a valuable tool in addition to the paper techniques, especially for preparative chromatography.

The development of a paper-chromatographic method for schradan presented a considerably different situation than that for parathion or demeton, because schraden is miscible with both water and most organic solvents. Because of success with impregnated-paper techniques, this line of attack was continued. A great variety of polar solvents as the stationary phase, nonpolar solvents as the mobile phase, and vice versa were investigated with limited success. Gage's method (*3*), which is not effective for use with schradan, was helpful in directing attention to the employment of concentrations of glycol as high as 50% in the impregnating solution and thus in the final development of the technique for schradan.

Experimental

Chromatography The ascending technique of paper chromatography is employed using tapered strips of Whatman No. 1 filter paper, 205 mm. in length, 24 mm. in width at the top, and 18 mm. at the bottom. The solution of the compounds to be separated is applied 1.5 to 2 cm. from the bottom of the strip in 5 to 10 μl. of acetone or other volatile solvent. The area of the applied spot is held at a minimum by the multiple application of 1-μl. drops. For development of the chromatogram, the papers are placed in stoppered borosilicate glass test tubes (standard 200 × 25 mm. size) containing 2 ml. of the mobile solvent. The papers are folded at the top to give a final length of 190 mm. and to provide a snug fit in the tubes in order to prevent collapse of the papers and contact of their lower edges with the sides of the tubes. The mobile solvent is permitted to ascend by capillarity to about 13 to 15 cm. above the applied spot. The papers are then removed and dried in the air, and the location of the separated constituents is determined by means of the detection techniques.

Demeton The method for demeton, adapted from the method for parathion (*10*), consists in impregnating Whatman No. 1 filter paper with Silicone 550, Dow Corning Corp., Midland, Mich., from a 5% (weight by volume) petroleum ether (Skellysolve B) solution, allowing the papers to dry on paper toweling in the air, applying the phosphate esters or evaporated extracts of demeton-treated plant or animal tissues, and employing the upper phase from a mixture by volume of 10 parts of chloroform, 10 parts of absolute ethyl alcohol, and 6 parts of water as the mobile solvent. R_f values for the thiono and thiol isomers present in technical demeton and related compounds are given in Table I. The values reported are average values for compounds prepared in these laboratories (*2*) or received from other sources.

Table I. R_f Values for Thiono and Thiol Isomers of Technical Demeton and Related Compounds

Compound	R_f, 27° ± 1° C.
O,O-Diethyl *O*-[2-(ethylmercapto)ethyl]thionophosphate (thiono isomer)	0.05 ± 0.01[a]
O,O-Diethyl *S*-[2-(ethylmercapto)ethyl]thiophosphate (thiol isomer)	0.69 ± 0.04
O,O-Diethyl *O*-[2-(ethylmercapto)ethyl] phosphate	0.96 ± 0.01
Principal plant metabolite, thiono isomer	0.89 ± 0.08
Principal plant metabolite, thiol isomer	0.95 ± 0.02

[a] Standard deviation.

In addition, Gage's technique (*3*) has been modified in minor detail and applied to the chromatography of demeton. Whatman No. 1 filter paper

is impregnated with propylene glycol from a 50 volume % absolute ethyl alcohol solution. A mixture by volume of 4 parts of petroleum ether (Skellysolve B) and 1 part of toluene saturated with propylene glycol is employed as the mobile solvent. Using this technique, satisfactory separations of demeton and related esters have been achieved and the following R_f values were obtained for—thiono isomer, 0.99; thiol isomer, 0.80; and their principal plant metabolites, 0.01 to 0.03.

Schradan In the method for schradan, Whatman No. 1 filter paper is impregnated with ethylene glycol from a 50 volume % absolute ethyl alcohol solution and the papers are separated on paper toweling and dried in an oven at 80° C. for 12 minutes. The phosphoramide esters or evaporated extracts of schradan-treated plant and animal tissues are applied to the papers and ascending chromatography is employed using as mobile solvent a mixture of equal parts by volume of α,α,α-trichlorotoluene, carbon tetrachloride, and toluene, saturated with ethylene glycol. Because the concentration of impregnated ethylene glycol is very critical for reproducible R_f values and uniform and clean-cut spots, it was necessary to store the impregnated papers in an ethylene glycol-saturated atmosphere if they were not used immediately following preparation. Propylene glycol-impregnated papers, as well as different combinations of the above solvents and others including dimethyl formamide, will achieve distinct separation of schradan and related compounds. The above combination, however, effected the greatest separation and most uniform and clean-cut spots of all those investigated. R_f values for technical schradan, pure schradan, its plant or animal tissue activated metabolite, and related phosphoramide esters are given in Table II. These compounds have been prepared in these laboratories or received from other sources.

Table II. R_f Values for Schradan and Related Phosphoramide Esters

Compound	Component No.	R_f, 27° ± 1° C.
Technical schradan	1	0
	2[a]	0.31 ± 0.05[b]
	3[a]	0.43 ± 0.05
	4	0.57 ± 0.05
	5	0.79 ± 0.01
Pure schradan	1[a]	0.32 ± 0.03
Activated schradan	1	0.16 ± 0.02
Hexaethylphosphoramide	1	0
	2[a]	0.94 ± 0.02
Tetramethyldiamidophosphoryl chloride	1	0
	2[a]	0.72 ± 0.04
	3	0.82 ± 0.04
Decamethyltriphosphoramide	1	0
	2[a]	0.16 ± 0.03
	3[a]	0.23 ± 0.03
	4	0.36 ± 0.04
O-Ethyl octamethyltriphosphoramide	1	0
	2[a]	0.33 ± 0.05
	3	0.44 ± 0.03
Unsymmetrical *O,O*-diethyl tetramethylpyrophosphoramide	1	0
	2[a]	0.48 ± 0.05
	3[a]	0.77 ± 0.04
Symmetrical *O,O*-diethyl tetramethylpyrophosphoramide	1	0
	2	0.35 ± 0.08
	3[a]	0.65 ± 0.08

[a] Major components.
[b] Standard deviation.

Detection Techniques A colorimetric technique for the location of the separated constituents was successfully adapted for technical schradan from that reported by Hanes and Isherwood (*5*) and Bandurski and Axelrod (*1*) for some naturally occurring phosphate esters. Following the development of the chromatogram, the papers are allowed to dry in the air, and sprayed with a reagent composed of 5 ml. of 72 weight % perchloric acid, 25 ml. of 4 (weight by volume) % ammonium molybdate, 10 ml. of 1*N* hydrochloric acid and 60 ml. of water. They are then heated in an oven for 2 minutes at 80° C. and exposed for 15 to 30 minutes under an ultraviolet source filtered to produce radiation at 3650 A. The phosphomolybdenum blue spots of the more easily hydrolyzable compounds appear within a short time and those for the more difficultly hydrolyzable compounds within 24 hours. As little as 1 γ of schradan can be detected in this way and quantitative estimations may be accomplished with a densitometer. The spray reagent is most effective when freshly prepared, but it may be stored under refrigeration for a week or more. In some cases it may be necessary to spray the papers a second time to produce satisfactory color development.

A completely satisfactory colorimetric technique for location of the spots of the separated constituents for technical demeton remains to be developed, although the phosphomolybdate technique, discussed in detail for schradan, provides a partially satisfactory method. It is difficult with this method to hydrolyze demeton and related esters without employing conditions which also destroy the paper chromatogram. Under the milder conditions, which are satisfactory for schradan, qualitative phosphomolybdenum blue spots will develop in 24 to 72 hours, but hydrolysis apparently is not complete enough to be sensitive for less than 25 to 50 γ.

Satisfactory location of the separated constituents may be achieved by using phosphorus 32- or sulfur 35-labeled compounds, and attaching the developed chromatogram to a flat stainless steel slide, designed to move through a manual sample changer under a Geiger-Müller tube. A 0.5 × 3.0 cm. collimating slit in a 1.5-mm. thick aluminum plate is placed between the tube and the chromatogram, and the strip is manually shifted in 0.5-cm. increments under the tube while activity is determined with standard scaling equipment. Quantitative information may be determined from summation of the areas under the activity–R_f curves (Figures 1 and 2).

Additional information may be obtained by using autoradiographic techniques. The developed chromatogram, following determination of radioactivity, is placed on a piece of Eastman No-Screen x-ray film and exposed for 1 to 2 weeks, after which the film is developed. Location of separated constituents may be determined visually and quantitative estimations may be accomplished with a densitometer. This method is especially useful in locating and estimating those constituents which occur in such small amounts that it is uneconomical to count them for length of time required to obtain necessary statistical accuracy.

In the case of demeton and those related compounds which show marked anticholinesterase activity, additional information on the location of the chromatographed compounds may be determined by cutting the chromatogram into consecutive segments, dicing the segments directly into Warburg flasks containing the cholinesterase preparation, and determining anticholinesterase activity by standard manometric techniques (*8*).

Because of the lack of pure reference standards it has not been possible to establish the identity of all spots separated from technical schradan with certainty, for nearly all of the compounds listed in Table II contain two or more components. This has resulted from the difficulty in purifying the compounds by the normally utilized techniques, and in part, from their instability in storage. Hartley *et al.* (*6*) have shown by chemical analysis that technical schradan

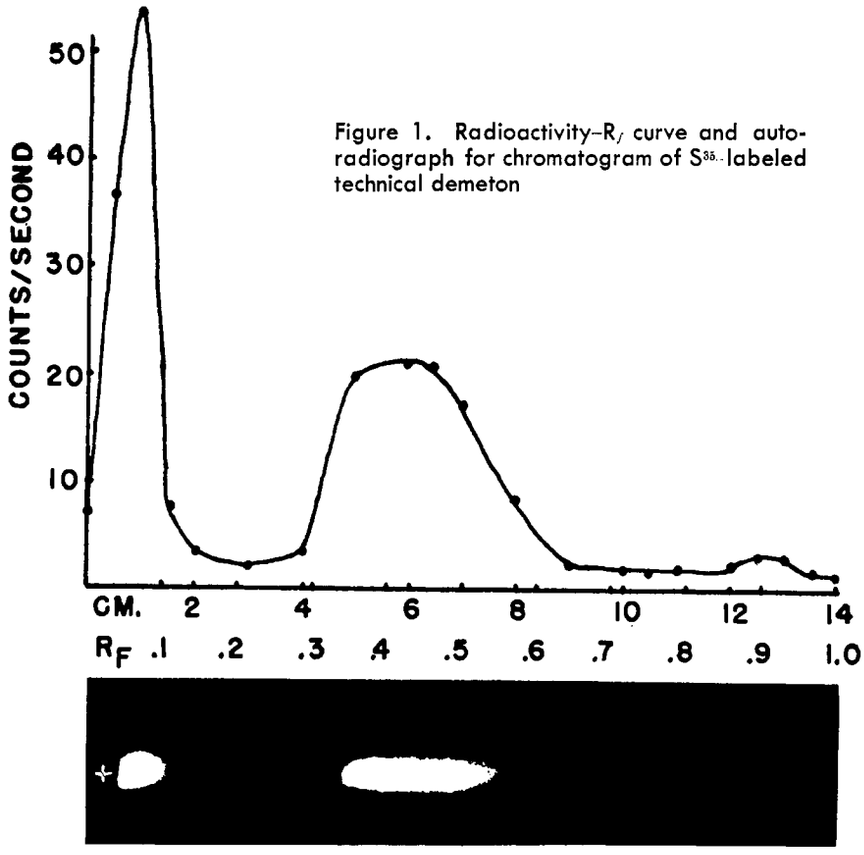

Figure 1. Radioactivity-R_f curve and autoradiograph for chromatogram of S^{35}-labeled technical demeton

contains about 85% phosphorus compounds, extractable by chloroform from aqueous alkali. Expressed by weight they consisted of R_3PO, 17.3%; $R_4P_2O_3$, 40.4%; $R_5P_3O_5$, 39.1%; and $R_8P_3O_6$, 3.2%, where R denotes the dimethylamino group. The relative amounts of the separated components of technical schradan (Table II) estimated with a densitometer following color development with the phosphomolybdate reagent were found to be: spot 1, 12%; spot 2, 44%; spot 3, 28%; spot 4, 5%; and spot 5, 11%. Spots 1, 2, and 3 appeared in all samples of technical schradan examined but spots 4 and 5 were missing in chromatograms of a number of technical products even when using the highest concentration possible without overloading the paper.

On the basis of these data and other observations, the identity of the components may be in part as follows:

1. This spot contains those compounds which do not move from the origin and it appears almost immediately after spraying with the phosphomolybdate reagent as a yellow spot which rapidly becomes blue upon exposure to ultraviolet light. Thus it may contain bisdimethylamino phosphate and other hydrolyzed or unreacted compounds.

2. Based on the appearance of a single spot at this R_f for all dilutions of technical and pure schradan, this spot must be octamethylpyrophosphoramide.

3. The detection of a major component at this R_f for all samples of technical schradan examined suggests that it may be decamethyltriphosphoramide. This is not substantiated, however, by the major components separated from a sample submitted as this compound. This sample was separated into two major components, 2 and 3, with R_f values of 0.16 and 0.23, respectively, and another component, 4, with an R_f of 0.36. The R_f values for components 3 and 4 correspond within the limits of the standard deviations with components 2 and 3 of technical schradan. Component 2 with R_f of 0.16 does not have its counterpart in technical schradan and its presence appeared to delay the movement of the other components along the chromatogram. It would seem most likely that the submitted sample of decamethyltriphosphoramide had deteriorated in storage, since the analysis showed its purity as correct when prepared (*12*) and has been shown to be decomposed by heat essentially to octamethylpyrophosphoramide (*13*). Thus very little emphasis can be placed on the lack of a major component in this sample corresponding to component 3 of technical schradan, but its presence even as a minor component supports the identification of component 3 of technical schradan as decamethyltriphosphoramide. A very similar situation exists for the closely related *O*-ethyloctamethyltriphosphoramide.

4. The identity of this spot is undetermined, although by elimination and on the basis of the chemical analysis of technical schradan (*6*) it may be the trimeric dimethylamidopolymetaphosphate.

5. Because this spot has the highest R_f value and closely resembles that of hexaethylphosphoramide, it may be hexamethylphosphoramide. This is further suggested by the relative amount of this component present in comparison with the value for hexamethylphosphoramide by chemical analysis (*6*).

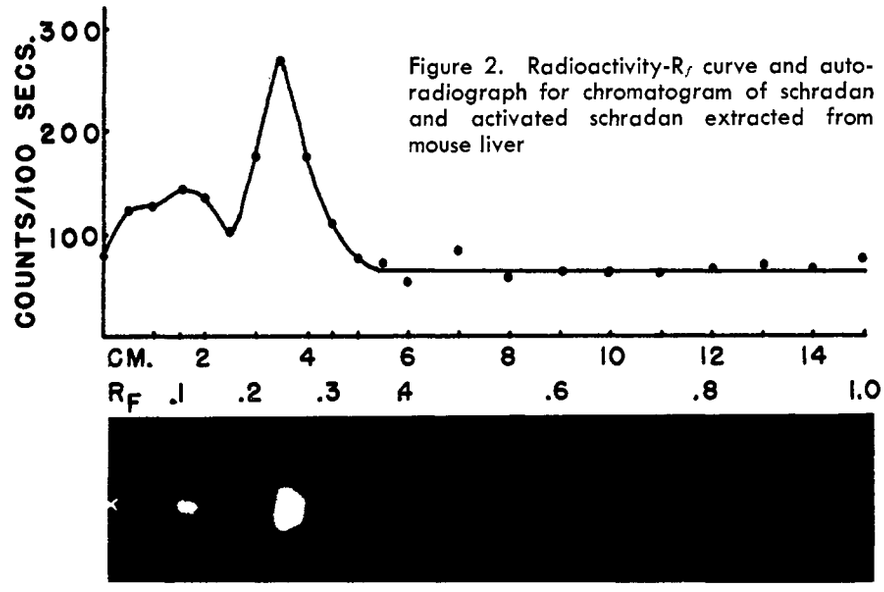

Figure 2. Radioactivity-R_f curve and autoradiograph for chromatogram of schradan and activated schradan extracted from mouse liver

Summary

The components of technical demeton and related compounds have been separated by means of two paper-chromatographic techniques and of technical schradan and related phosphoramide esters by means of a third paper-chromatographic technique.

These techniques have materially aided in investigations on the systemic insecticides for the preparation and determination of purity of phosphorus-

32–labeled demeton and its isomerization to the thiol isomer (*2*); the translocation and biochemistry of Systox (demeton) in plants; the determination and characterization of residues of Systox in citrus, walnuts, apples, pears, potatoes, and sugar beets; the preparation and determination of purity of phosphorus-32–labeled schradan; the determination of schradan residues in cotton; and the comparative biochemistry of schradan in the white mouse, squash bug, and American roach (*11*).

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R. M. Chatters, Department of Botany, Oklahoma A & M College, Stillwater, Okla.	Sulfur-35-labeled sample of technical demeton
Farbenfabriken Bayer, Leverkusen, Germany	Phosphorus-32–labeled sample of demeton
Pest Control Limited, England	Technical schradan
Carbide and Carbon Chemicals Corp., New York	Hexaethylphosphoramide
Dow Chemical Co., Midland, Mich.	Decamethyltriphosphoramide
Monsanto Chemical Co., St. Louis, Mo.	*O*-Ethyl octamethyltriphosphoramide
	Unsymmetrical *O,O*-diethyl tetramethylpyrophosphoramide
Victor Chemical Works, Chicago, Ill.	Symmetrical *O,O*-diethyl tetramethylpyrophosphoramide

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PESTICIDE FORMULATIONS

A Selected Aromatic Fraction Naturally Occurring In Petroleum as a Pesticide Solvent

FRANKLIN C. NELSON and GEORGE W. FIERO
Esso Standard Oil Co., New York 19, N. Y.

Heavy aromatic naphtha, a selected fraction naturally occurring in petroleum, is an excellent solvent for pesticide formulations even at low temperatures. Its lower boiling range results in more rapid evaporation, an advantage when used for household or agricultural applications. Used in normal concentrations, it appears to be nonphytotoxic to pears, tomatoes, apples, celery, or citrus fruits and no more phytotoxic to corn than other commercial aromatic solvents. Animal toxicity studies indicate that there is no health hazard in connection with its use as a pesticide solvent.

EMULSIVE CONCENTRATES of pesticide formulations have increased greatly in use in the past few years. Emulsion prepared from these concentrates have less tendency to drift over adjacent fields than dusts, and they adhere better to plants. They are easier to use than wettable powders because there is no danger of orifice clogging. On the other hand, emulsive concentrates possess the disadvantage that when subjected to severe cold temperatures, certain pesticides may settle out. Also, in certain cases the solvent employed may possess herbicidal properties.

Aromatic products associated with petroleum may be obtained by thermal decomposition or cracking (whereby side chains are removed from aromatic nuclei and some cyclization takes place), catalytic reforming (whereby naphthenes are converted to aromatics), and physical separation of naturally occurring aromatics from an appropriate petroleum distillate. A study of aromatics from these sources indicates that the latter two possess similarity in solvent and stability properties, but commercial catalytic reforming processes tend to produce aromatics of undesirably low molecular weight. Efforts have therefore been concentrated on physical separation of aromatics. The molecular weight of the aromatic can be adjusted by selection of the proper distillate. The culmination of this research resulted in the production of heavy aromatic naphtha which, by reason of its physical properties as well as inherent solvent and stability characteristics, is an excellent and economical solvent for pesticides.

The following table is a usual inspection of heavy aromatic naphtha.

Initial boiling point, ° F.	328
10%	395
50%	448
90%	495
Final boiling point, ° F.	540
Specific gravity	0.9267
API gravity	20.9
Kauri butanol value	96
Per cent aromatics	85.5
Mixed aniline point, ° C.	25
Color, Tag Robinson	17
Flash (P.M.C.C.), ° F.	150

The boiling range, with 80% between 395° and 495° F., is considerably below that of many commercial petroleum pesticide solvents obtained from catalytically cracked distillates. This is of