Determining Traces of Octamethylpyrophosphoramide (Schradan) in Crops

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The high crop blanks obtained in determining residues of systemic insecticides, such as schradan, made it desirable to develop a new technique, dependent on microdistillation, for separating the insecticide from natural products in crop extracts. The high efficiency of the initial recovery methods, (*a*) maceration of the crop sample with water, followed by chloroform extraction of the macerate, and (*b*) direct solvent extraction by boiling under reflux, is proved. The recovery of schradan added to untreated crops is proved representative of the recovery from a treated crop. The complete analytical technique is described, and blanks and recoveries are listed. This technique should be generally applicable also to determination of residues of other toxic pest control compounds of volatility similar to or greater than that of schradan.

THE SYSTEMIC INSECTICIDE SCHRADAN [octamethylpyrophosphoramide, $(Me_2N)_4P_2O_3$], when applied to a growing crop, is absorbed into the sap stream, which becomes toxic to insects feeding upon it. Schradan is also toxic to animals (*6*, *7*), and foodstuffs which are occasional or minor items of diet are generally regarded as safe only if the concentration of schradan in them is less than 3 p.p.m. (*3*). Work which describes the mode of decomposition of schradan, and of other systemic insecticides, in plants has been prepared for publication (*12*); but it is still necessary to have available a satisfactory analytical technique for estimating residues of schradan in treated crops. This technique should be accurate to at least 0.5 p.p.m.

The separation of schradan from natural compounds has been carried out in two ways (*3*, *9*). In the method worked out in these laboratories (*3*) the natural phosphorus compounds are hydrolyzed in alkaline solution to compounds insoluble in chloroform, and the schradan is extracted by chloroform and estimated as phosphate. The hydrolysis is controlled to leave most of the schradan unhydrolyzed. High blanks are encountered in lemons and peas, because considerable quantities of natural compounds remain unhydrolyzed. Owing to the difficulty of separating completely the emulsions formed, and the consequent holdup of natural compounds in the chloroform, the results are not reliably reproducible to 0.5 p.p.m., even on Brussels sprouts, where the blanks are low. This hydrolysis method is therefore not always workable, and is always tedious. Hall's method (*9*) consists of estimating the schradan in a chloroform extract as dimethylamine. The final estimation is tedious and barely accurate enough. In addition, many plant species contain appreciable quantities of dimethylamino compounds.

Microdistillation has been used successfully for the determination of mipafox (diisopropyl phosphorodiamidic fluoride) residues by one of the authors (I.K.H.O.) and this method of separation (*14*), slightly modified, has proved reasonably successful when applied to schradan in a range of crops. Among these crops are Brussels sprouts, which are economically the most important crop to which schradan is applied, and lemons, which give the highest blanks by the hydrolysis method.

Schradan is volatile enough to be separated from some plant materials by microdistillation at low pressures, if it has been extracted previously by chloroform from the bulk of the plant material. The apparatus used is a modification of that of Klein and Werner (*13*). After the extracting solvent has been evaporated, the residue is left in a small vessel containing a cold finger, the vessel is heated, and a moderate (1 mm. of mercury) vacuum is applied. The convective air stream set up conveys the more volatile part of the residue to the cold finger. In this way schradan (vapor pressure 2.46×10^{-4} mm. at 25° C.) can be separated efficiently at moderate pressures from mixtures in which its vapor pressure must be much lower.

It is usual to estimate the efficiency of recovery of an insecticide in an analytical technique by adding a known amount to an untreated sample of the crop at the earliest possible stage, and determining the insecticide by the usual method. In the method described below, the initial stage is usually a maceration with water, and it is to the water for this maceration that the insecticide can be added. However, the recovery so obtained is not necessarily representative of that obtained from a treated crop, where the systemic residue is within the plant tissue. It will first be proved that the initial methods of extraction are efficient, before the analytical technique is described.

By labeling the insecticide with radioactive phosphorus it is possible to determine, during various operations, the insecticide and any phosphorus compounds derived therefrom, regardless of the large amounts of natural phosphorus compounds present. This makes it possible to account for all the relevant material in a way that is not possible by orthodox analytical technique.

Investigation of Extraction Techniques

Radioactive schradan was prepared from phosphorus oxychloride labeled with phosphorus-32 obtained from the Radiochemical Center, Amersham, by methods published by Heath, Lane, and Park (*12*).

Dilute aqueous solutions of the insecticide containing 0.1% wetter (Lissapol NX), were sprayed onto the foliage of a number of plant species, and at varying times afterwards samples of the foliage were taken for analysis. The sample was cut into convenient pieces and then subjected to the particular extraction technique under test.

Extraction Techniques

Maceration. The plant sample is mixed with 3 to 5 times its own weight of water and reduced to a "soup" in a suitable macerator—e.g., Townson and Mercer top-drive or Waring Blendor. Approximately 3 minutes' macerating is usually adequate. The fibrous plant residues are removed by filtration or centrifugation, and the schradan is extracted from the clear aqueous solution by shaking with an equal volume of chloroform for the subsequent radioassay. Schradan (and all other phos-

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phorus systemic insecticides so far studied) partitions from water in favor of chloroform.

In order to ascertain that the radioactively labeled compounds remaining in the damp residue are similar to those appearing in the aqueous solution and are present in quantities directly proportional to the amount of water remaining in the residue, the residue is subjected to serial washing with further water fractions (conveniently carried out by mixing residue and water for 5 seconds in the macerator) and, after filtering, the activity in the filtrates is assayed. Finally, the radioactivity not removable by this method is determined in the solution obtained after wet-ashing the solid residue.

Radio "counts" of the first clarified macerate of some schradan-treated turnip foliage, two washes of the residue with equal volumes of water, and a final wet ash solution (same volume) are shown in Table I.

Table I. Efficiency of Extraction of Schradan by Maceration from Treated Turnip Foliage

	C.P.M.
1st extract	2382 ± 40
1st wash	236 ± 10
2nd wash	18 ± 4
Wet ash solution	12 ± 4

Errors on counts shown are random errors based on total counts.

The filtrate counts fall approximately in a geometric series, as would be expected if a constant fraction (ca. one-tenth) of the liquid is left in the filter cake. Thus not more than 0.5% of the original radioactively labeled compounds, in this case about 80% unchanged schradan, was found in the first aqueous extract after maceration.

This technique has been proved satisfactory for the leaves of four species and the roots of one; it is reasonable to assume that it will be satisfactory for all soft plant tissues. It can also reasonably be assumed that the very small percentage of the activity which is not brought into solution by maceration is made up of phosphatic degradation products which have become completely combined in the structural materials of the plant.

With very juicy fruit it may be possible to reduce the volume of solvent used, by macerating the pulp directly with chloroform and thus extracting the insecticide from the aqueous juice straight into the organic solvent. (Such a procedure should be adopted only if a top-drive macerator is available.)

Reflux Extraction. The coarsely chopped foliage is mixed with about three times the weight of chloroform, and the solvent is boiled under a reflux condenser for 1 hour. The solid residue is filtered off, using for instance, Monel wire gauze, and the aqueous and chloroform filtrates are separated. The residue is then washed with a further half volume of chloroform. The aqueous filtrate is extracted once with an equal volume of chloroform to remove the insecticide dissolved in it, and the three chloroform extracts are bulked and carried forward for the insecticide assay.

This technique can be elaborated by the use of a Soxhlet apparatus. The chopped vegetable material is introduced into a large thimble—e.g., Whatman 33 × 118 mm.—and extracted in chloroform for 1 hour. There are obvious objections to extracting an undried sample in this way, but such a technique was satisfactory and convenient in some cases.

The efficiency of this second extraction technique was demonstrated by comparative radioassay of the schradan extracted from treated turnip foliage by this method, with that extracted by maceration of a second foliage sample with water, followed by partitioning the clarified aqueous macerate with 2 equal volumes of chloroform. It is shown above that maceration with water is almost completely effective in extracting all of the radioactivity, both as schradan and as its decomposition products, and from the known partition ratio of schradan between water and chloroform it can be calculated that 98% of the insecticide passes into the bulked solvent. By comparison, it was found that 91% of the total schradan present was extracted by the reflux extraction with chloroform.

A similar comparison was carried out using schradan-treated Brussels sprout foliage. The results obtained with two samples are given in Table II.

Table II. Comparative Extraction of Schradan from Treated Brussels Sprout Foliage

	Schradan Extracted, P.P.M.	
Technique	Sample 1	Sample 2
Aqueous maceration and chloroform partitioning	22.1	22.6
Reflux extraction with chloroform	23.6	20.9

Standard deviation ±2.5%.

Macerating vegetable material with water in a high speed macerator renders into aqueous solution between 97 and 99% of all the phosphorus-containing compounds, both insecticide and its breakdown products. By suitably extracting the clarified aqueous macerate with chloroform, essentially all the insecticide can be separated for assay by the appropriate analytical technique.

The results shown above justify the assumption that, when a known amount of insecticide is added to a control plant sample at the earliest stage in an analytical technique (the maceration stage) and the recovery determined, this recovery is truly representative of the recovery obtained for insecticide within the tissues of a plant sample treated with the systemic insecticide in the usual way.

Furthermore, when green foliage is extracted by boiling under reflux with chloroform, 99% or more of the organic-soluble insecticide is removed from the foliage. This method may often be satisfactory and sometimes more convenient than the maceration and partition technique previously discussed. Obviously, when thick tissues are extracted, such as roots or orange peel, preliminary chopping is necessary to allow diffusion to be completed quickly enough.

These results are in accord with previous work on schradan published by Heath, Lane, and Llewellyn (*11*), but certain workers with radioactive schradan (and also dimefox) have extracted a smaller proportion of the radioactivity. Thus Bennett and Thomas (*1*), while generally extracting more than 95%, extracted only about 90% of the total from several plant species several days after spraying. These workers extracted the systemics by boiling plant materials with water. The most important phosphorus-containing decomposition product of schradan is tetramethyl-diamidophosphoric acid, $(Me_2N)_2POOH$, which is hydrolyzed rapidly to phosphoric acid by boiling water (*10*). Much of this phosphoric acid is probably precipitated as insoluble phosphates and is not recovered. This is confirmed by the finding that good recoveries can be obtained by boiling with excess inactive phosphate. David (*4*, *5*) extracted only 60 to 80% of the activity from freshly treated plants by boiling them with dilute sodium hydroxide. These low recoveries are almost certainly explained by the partial alkaline hydrolysis of the insecticide which would occur under the conditions used, followed by precipitation of phosphates as mentioned above.

Satisfactory extraction techniques have been described in some detail and the method of determining the efficiency of recovery has been justified; so it is now possible to describe the complete procedure for carrying out the determination of schradan residues in crops, and to list some typical recoveries and the blanks from untreated crops.

Method

Apparatus and Chemicals A macerator, centrifuge, and the micro-distillation apparatus shown in Figure 1 are used. Other

requirements are a rotary vacuum pump and glass helices.

Chemicals used include 1*N* sodium hydroxide, reagent grade chloroform, 10*N* sulfuric acid, C.P. grade ammonium persulfate, isobutyl alcohol, ethyl alcohol, and concentrated nitric acid. Standard phosphorus solution is prepared from potassium dihydrogen phosphate dried at 100° C. for 4 hours. Ammonium molybdate solution, 50 grams per liter, is kept in a polyethylene or wax bottle. Stannous chloride solution, 10 grams per 25 ml. of hydrochloric acid (specific gravity 1.19), is kept in a brown glass-stoppered bottle, and dilute stannous chloride solution is prepared freshly as required by diluting the stock solution 200-fold with 1*N* sulfuric acid. All reagents should be analytical reagent grade or equivalent.

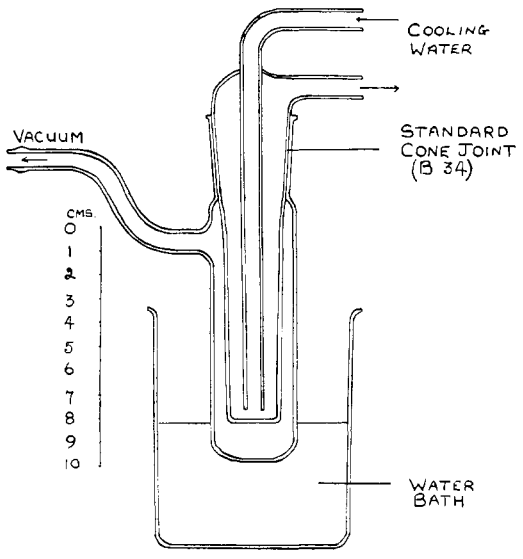

Figure 1. Microdistillation apparatus for schradan

Procedure Macerate 50-gram samples into 50 ml. of water and filter the macerate or clarify by centrifugation, wash the residue with water, and bulk the washings with the aqueous macerate. Add sufficient 1*N* sodium hydroxide to raise the pH to 8 to 10, and extract twice with equal volumes of chloroform each time. Break any emulsion by centrifuging. Clarify the chloroform extracts by filtration and concentrate the bulked chloroform extracts to about 25 ml. Transfer to the microdistillation apparatus, close the top with a stopper, and connect the side arm to a condenser. Distill off the chloroform in order to obtain the schradan-containing residue in the bottom of the vessel. It is helpful to introduce a few wide-turn helices towards the end of the evaporation to reduce splashing of the residue onto the walls of the vessel. Introduce the cooling finger into the microdistillation apparatus and evacuate the vessel to about 1 mm. of mercury pressure by means of the rotary oil pump, connecting through the side arm. Immerse the lower part of the vessel in a water bath and heat to 100° C. for 45 minutes. Release the vacuum and withdraw the cold finger carefully. Wash the schradan residue from the cold finger with a little chloroform, and transfer the washings to a Kjeldahl flask. Add 5 ml. of 10*N* sulfuric acid and 0.3 gram of ammonium persulfate, evaporate until fumes appear, add a little water, and re-evaporate twice. Dilute with a small quantity of water and assay the colorless phosphate solution by the method below, based on that of Berenblum and Chain (*2*).

Cool, transfer to a 25-ml. separating funnel, and dilute (with washings) to about 7 ml. Add 2.5 ml. of ammonium molybdate solution and 8 ml. of isobutyl alcohol. Shake for 30 seconds. Discard the aqueous layer and wash twice with 5 ml. of 1*N* sulfuric acid. It is essential to remove all the molybdate during the two sulfuric acid washes of the phosphomolybdate solution. Transfer quantitatively with 2 ml. of isobutyl alcohol to another separating funnel, add to the alcoholic layer 15 ml. of dilute stannous chloride solution, and shake for 30 seconds. Run off the stannous chloride and transfer the alcoholic layer to a 10-ml. stoppered standard flask. Make up to mark by washing with ethyl alcohol.

Measure the photoabsorption at 7350 A. on a suitable instrument and calculate the recovered schradan from a calibration graph obtained using aqueous solutions of pure potassium dihydrogen phosphate. On the Spekker absorptiometer use a tungsten lamp and H503 and Ilford 608 filters (1-cm. cell or 4-cm. microcell).

A reagent blank should be determined. The blanks and recoveries obtained on the crops so far studied are summarized in Tables III and IV.

The blank determinations on all crops studied are very small and completely satisfactory. In the case of Brussels sprouts and lemon fruit, the two crops on which a comparison with the hydrolysis method (*3*) for determining schradan residues is possible, the com-

Table III. Blanks on Various Crops

Crop	Blanks Estimated as Schradan, P.P.M.
Brussels sprouts	0.07, 0.07, 0.03, 0.0, 0.05
Lemons (fruit)	0.08, 0.09, 0.06, 0.11
Lemons (peel)	0.14, 0.11, 0.13, 0.09
Apples (fruit) at varying	0.01, 0.06, 0.01, 0.04, 0.02, 0.03
Pears (fruit) stages of	0.15, 0.02, 0.14, 0.03, 0.0
Peaches (fruit) ripeness	0.01, 0.02, 0.03, 0.16, 0.02, 0.06, 0.01
Field beans (*Vicia faba*)	0.03, 0.01, 0.02
Potatoes (stored)	0.0, 0.04

Table IV. Recovery of Schradan from Crops[a]

Crop	Schradan, P.P.M. Added	Schradan, P.P.M. Recovered	% Recovery
Brussels sprouts	0.52	0.48, 0.49	93
	0.67	0.60, 0.61	90
	1.83	1.63, 1.67	90
Lemons (fruit)	0.25	0.14, 0.15	58
	0.78	0.60, 0.58	76
Lemons (peel)	0.50	0.25, 0.42, 0.34	67
	1.55	1.23	79
Apples (fruit)	0.11	0.07	64
	0.21	0.16	77
	0.22	0.16	73
	1.01	0.80, 0.79	78
Pears (fruit)	0.11	0.09	82
	0.21	0.13, 0.20, 0.17	80
	0.22	0.20	91
	1.01	0.66, 0.61	63
Peaches (fruit)	0.11	0.07	66
	0.21	0.16	80
	0.22	0.14	68
	1.01	0.77, 0.85	77
Field beans	0.22	0.17	77
	1.10	0.99, 0.91	87
Potatoes (stored)	0.11	0.09	82
	0.21	0.20, 0.14, 0.18	81
	1.01	0.85, 0.78	80

[a] The extraction procedures adopted for some crops were varied slightly from the method published, but these variations are not likely to affect materially the efficiency of extraction. Brussels sprouts were extracted with chloroform in a Soxhlet apparatus, and it was found desirable to raise the pH of the lemon juice to about 5 by adding sodium acetate to the water used for the maceration.

parative blanks are 0.05 as against 0.13 p.p.m. and 0.08 as against about 8.00 p.p.m.

The recoveries from all the crops are satisfactory for the routine determination of residue hazards and replicates fall within ±5% of the mean, where the added concentration of schradan approaches toxicological significance. It would be satisfactory in such routine work to multiply observed results by a factor of 1.5, to get a measure of the schradan residue present in the crop.

The rather low recoveries obtained with lemons are probably explained by the lemon oil's acting as a relatively involatile solvent for schradan, thus preventing the efficient volatilization of the schradan onto the cold finger. Thus, if this method is to be completely satisfactory, plant-derived solvents of schradan must be removed before the distillation stage. This might be achieved by a preliminary extraction of the clarified aqueous macerate with petroleum ether or benzene, in which schradan is not very soluble, and from which the small proportion extracted can easily be removed by a back-wash with water.

The technique is obviously applicable to a number of other compounds of limited volatility, though compounds of much lower volatility than schradan are not likely to distill rapidly enough. It has been used successfully for the determination of mipafox (diisopropyl phosphorodiamidic fluoride) residues the heating bath temperature being 70° C. Compounds much more volatile are not easily separated by this technique, as re-evaporation from the condensing surface will lead to losses. This could be prevented by the use of a cooling system which keeps the surface at a lower temperature—solid carbon dioxide–acetone, for instance—or separation may be performed with a carrier (*8*).

Summary

Both maceration of schradan-treated crops with water, followed by chloroform extraction of the clarified aqueous macerate, and reflux extraction with boiling chloroform are satisfactory methods of recovering the insecticide for residue assay. In a method for the determination of residues in a number of crops, the insecticide is separated from natural products by microdistillation. Recoveries and blanks are satisfactory.

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PESTICIDE RESIDUES

Determining Traces of Tetramethylphosphorodiamidic Fluoride (Dimefox) in Crops

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A satisfactory method for the determination of dimefox residues in food crops must achieve a sensitivity of 0.1 p.p.m. or less. The most general method consists of macerating a 50-gram sample of the crop with water, filtering, extracting with chloroform, evaporating the chloroform to a low bulk, transferring to a microdistillation apparatus, and distilling in the presence of a few drops of glycerol-glycol mixture. The dimefox in the distillate is estimated as phosphate by the method of Berenblum and Chain. An abbreviated version can sometimes be used. The second method, used for oily crops, consists of distilling a macerate in oil and separating the dimefox from interfering compounds in the oily distillate. Satisfactorily low blanks were obtained on 15 crops, and satisfactory recoveries on 10 crops further investigated.

Dimefox, $(Me_2N)_2POF$, commercially available under the trade name Hanane, is a systemic insecticide (*2, 4, 7, 10*) highly toxic to mammals (*3, 5*).

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Work which shows that dimefox is decomposed in plants to harmless products is being prepared for publication. The hazard in consuming treated crops therefore comes from their content of undecomposed dimefox. As tolerable residues are still subject to controversy, a target sensitivity of 0.1 p.p.m. has been adopted

No highly specific group reactions have yet been discovered for any organophosphorus systemic insecticide. Dimefox must therefore be estimated by separating it from natural interfering substances, decomposing it, and de-