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Received for review May 7, 1959. Accepted September 25, 1959. Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. Investigation supported in part by grants from the Public Health Service, National Institutes of Health, and the U. S. Atomic Energy Commission, Contract No. AT(11-1)-64, Project No. 14.

INSECTICIDE METABOLISM

Prechromatographic Purification of Insecticides from Insect Tissue Extracts

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Paper chromatograms of acetone extracts of insecticides and their metabolites from insect tissues often are smeared and unrecognizable. When a crude extract is spotted on filter paper and eluted with acetonitrile, the solvent selectively extracts insecticides, metabolites, and 20 to 30% of the insect lipide material, but not the interfering lipides. The purified extracts give clear chromatograms, on which insecticides and metabolites can be identified. This rapid and simple purification is very useful in studies of insecticide metabolism.

PAPER CHROMATOGRAPHY is used in the study of insecticide metabolism in plants, insects, and higher animals. In the course of studies on the metabolism of DDT, Systox, Thimet, and Sevin in houseflies, mosquitoes, German roaches, and milkweed bugs, it became evident that acetone extracts of insect tissues contain large amounts of lipoid material which causes smearing of paper chromatograms. This results in poorly defined spots, incomplete separation of spots, and shifting of R_f values. Therefore a method had to be devised for removing interfering materials from the extract before running a paper chromatogram. The acetonitrile method described in this paper has been successful with all the insecticides and insects so far studied in this laboratory, but only a few typical examples are presented here.

Procedure

Insects amounting to several hundred milligrams wet weight are homogenized in 5 ml. of acetone. A pinch of anhydrous sodium sulfate is added to remove water present in the homogenate, which is then centrifuged at 2000 r.p.m. for 20 minutes. The supernatant liquid is drawn off and the residue is re-extracted once or twice with 5 ml. of acetone. The combined volume (about 15 ml.) of acetone extract is evaporated in a stream of nitrogen gas to a final volume of 30 to 50 μl. and is transferred by self-filling capillary tubes onto the center of a strip of Whatman No. 4 chromatographic filter paper, 2.5 inches long and 1 inch wide, but at one end leading to a tapering point. Disposable glass capillaries are more convenient than micropipets, give faster transfer, and require no cleaning. One microliter of a 0.5% solution of *N,N'*-dimethyl-*p*-1-naphthylazoaniline (NDN) in benzene is spotted over the extract spot. In tests using carbon-14–DDT, carbon-14–Systox, and Thimet (detected by the sensitive color reaction with 2,6-dibromoquinonechloroimide), in extracts of houseflies and German roaches, it was found that when the NDN was completely extracted from the spot, no insecticide was detectable in the spot, but all the insecticide moved with the dye. The dye is a useful visual indicator of complete extraction. Both dye and insecticides move out of the spot gradually as very diffuse bands, by a slow elution process, probably analogous to a Soxhlet extraction.

Extraction is effected either by an upward-washing or a downward-washing method. The former is more convenient, but requires that the entire sample be spotted on one chromatographic strip. The latter allows the use of several aliquots of the sample on several strips if desired.

Upward Washing. The broad end of the spotted strip is dipped into a 50-ml. beaker containing 5 to 10 ml. of acetonitrile. The ascent of this solvent by capillarity carries insecticide, reference dye (NDN), and some lipoid material to the pointed tip of the strip. The bulk of the lipoid material stays in the original spot. The strip is held upright by clamping between two glass microscope slides (3 × 1 inch). Solvent is transferred from the tip by contact with a paper chromatographic strip to form a spot. This is dried and more solvent transferred to it repeatedly until transfer is complete—as indicated by complete removal of the dye from the tip (*5*).

Downward Washing. The spotted strip is hung in a closed tube in a cup containing acetonitrile, with the tip pointing down and touching a glass rod standing in a receiving cup (Figure 1). After 0.5 hour the solvent is collected from the receiving cup, concentrated to a small volume, and spotted on a paper chromatographic strip.

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Other solvents (chloroform, cyclohexane, and iso-octane) were also tried. They either fail to extract the insecticides completely, or also extract most of the interfering lipoid material. Acetonitrile has been previously used (*1*, *4*) as the eluting solvent to separate insecticides from plant lipoid extractives by an alumina-wax or alumina-polyethylene column without loss of toxicant.

Results and Discussion

This technique has given apparently quantitative extraction of microgram quantities of DDT, DDE, Systox, Isosystox, Thimet, and Sevin. Precise recovery data cannot be presented, because precise quantitative analysis of quantities of the order of 1 γ is difficult. Comparison of the areas under peaks recorded by a scanner for radioactive spots on chromatograms indicates that at least 90% of the insecticide is extracted by acetonitrile, and no residual insecticide (radioactivity) can be detected. With some very polar insecticide metabolites such as the sulfoxide of the oxygen analog of Systox, or naphthoquinones formed by oxidation of 1-naphthol, recoveries seem to be less than quantitative, especially when quantities of less than 1 γ are involved. However, the chromatography and identification of very polar compounds are difficult, and no definite conclusions are possible.

The success of the method rests on the fact that acetonitrile extracts the insecticides, but not the bulk of the tissue extractives. Table I shows data on the amount of tissue extractives before and after purification of extracts from three insect species. The number of insects is that used in experiments on insecticide metabolism. The fresh weight is a few hundred milligrams, and the extracted fat weighs 2 to 4 mg. Acetonitrile extracts from 20 to 30% of this fatty material together with the insecticides, but the acetonitrile-soluble material does not cause much interference on paper chromatograms.

Figure 2 shows an example of acetonitrile purification taken from work on the metabolism of organic phosphates. The reference curve on the left shows the separation of a mixture of carbon-14–labeled Systox, Isosystox, and two possible metabolites, the sulfoxide of Systox and its oxygen analog sulfoxide. The radiogram at the upper right is of the same mixture added to an extract from five male German roaches before chromatography. The radiogram at the lower right shows the effect of acetonitrile purification of the section of the upper strip between dotted lines. The large smeared spot is now clearly resolved into three peaks. The R_f values of these peaks are lower than those of the reference radiogram at the left. This is an effect of the heavy load (about 0.6 mg.) of acetonitrile-soluble roach lipoid not separated from the insecticides by the purification procedure. This effect can be compensated for by adding a set of four dyes to the original spot. According to the unpublished procedure of Gordon (*2*, *3*), the position of the insecticide spots relative to that of the reference dyes—shown as dotted circles in Figure 2—is constant, even when R_f values show large variations.

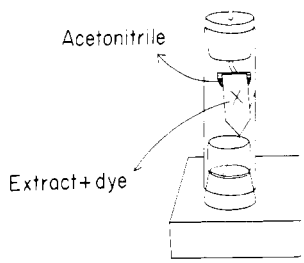

Figure 1. Method of downward-washing purification by acetonitrile extraction on filter paper strip

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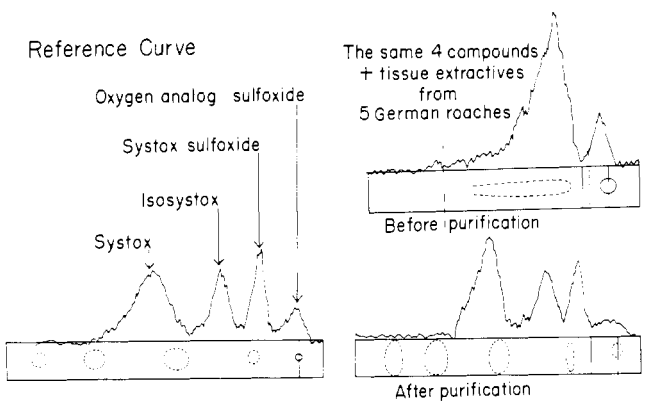

Figure 2. Example of improved paper chromatogram of carbon-14 Systox and related organic phosphates following acetonitrile purification

Table I. Degree of Purification of Tissue Extract from Three Insect Species Following Wash with 100% Acetonitrile for 0.5 Hour

Insects	Tissue Extractive Wt., Mg. Total	Tissue Extractive Wt., Mg. Extracted by solvent	Extractives not Separated from Insecticide, %
5 male German roaches	1.8	0.4	21
	2.4	0.8	33
100 mosquito larvae	2.8	0.6	21
	2.2	0.8	27
25 houseflies	3.8	1.0	26
	4.2	1.2	28

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Received for review June 11, 1959. Accepted October 5, 1959.

Corrections

Mechanism of Liquid Seed Treatment

In the article on "Mechanism of Liquid Seed Treatment" [Olle Lindström, J. AGR. FOOD CHEM. **6,** 283 (1958)] the following corrections should be made:

Page 285, Equation 1. $dn/(dt \times G)$

Page 285, second column, the second and third lines from the bottom of the page should read: accordingly the concentration gradient may be put equal to c_s/l.

In the subcaption to Figure 3: ● P_3B_1, ○ P_3B_2, ◓ P_1B_1

In the first column of page 293 the equation should read:

$$1 - \frac{X_m(E_i)}{X_m(E_1)}$$

where E_i is E_2, E_3, or E_4

Toxicology of Butoxypolypropylene Glycol 800

In the article on "Toxicology of Butoxypolypropylene Glycol 800" [C. P. Carpenter, C. S. Weil, P. E. Palm, M. D. Woodside, and H. F. Smyth, Jr., J. AGR. FOOD CHEM. *7*, 763 (1959)] the next to the last sentence of the abstract should read: It is not stored in the bodies of animals, and 50% or more of a single dose may be found in the feces unchanged.