Study of Systemic Insecticides

location and amounts of residue in plant tissues determined with aid of radio-phosphorus tracers

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Radioautograph of a chromatogram of labeled technical demeton. The lower spot is demeton, the middle spot is isodemeton, and the top spot is impurities.

The systemic insecticides — schradan — *octamethyl pyrophosphoramide* and demeton or Systox—*O,O-diethyl O-ethyl-beta-mercaptoethyl thionphosphate*—and their derivatives have shown unusual promise for the control of mites and aphids and other sucking insects of a variety of agricultural crops.

These materials are freely transported throughout living plants and concentrate in certain rapidly growing tissues. Therefore it is necessary to have unusually detailed knowledge of possible undesirable residues in edible produce. Moreover it is apparent from investigations conducted with schradan and demeton that they may be readily transformed by processes within plants and animals into secondary metabolites—products of metabolism—with properties markedly different from those of the original material.

Several methods for the quantitative estimation of schradan and demeton residues have proved inadequate because they lack the specificity and sensitivity desired in residue analysis.

All the systemic compounds currently in use are organic phosphorus compounds with a common mode of toxic action—the blocking of the function of cholinesterase and other esterase enzymes. This is accomplished by the binding of the central phosphorus atom firmly to the surface of the esterase enzyme in a manner which blocks the access of the normal enzyme substrate, such as acetyl choline. Any toxic metabolite formed will contain a phosphorus atom and therefore the use of isotopes—containing radio-phosphorus—as easily identified tracers is especially informative in the study of systemic insecticides.

Radio-phosphorus is nearly ideal as a tracer because it is identified or labeled by its high intensity beta-radiation, its short half-life—unit of decay—of 14.3 days which prevents long-term contamination, and because of the relative ease of synthesis and the low cost of the radioisotope.

Synthesis of schradan or demeton with highly active radio-phosphorus will provide tracers that have activity—easily detectable with an end window Geiger tube—for four half-lives or longer. Because of the quick decay of the radiophosphorus and the fact that its beta-radiation will penetrate only the outer layers of the skin, relatively simple precautions will suffice in the handling of these tracers in the small quantities employed.

The radiotracers may be utilized for a ready quantitative estimation of the maximal amounts of toxicant which may be present in plant or animal products. For this purpose they may be formulated in exactly the same manner as the ordinary technical—commercial—insecticide, or they may be diluted with non-radio-phosphorus-labeled material. The low rates of application commonly used for the systemic materials—0.2 to one pound per acre—permit extensive investigations with approximately six to 10 grams of tracer insecticides. This amount when formulated is adequate to treat from 0.01 to 0.05 acre of cotton or other field crops, or 5–20 small fruit trees such

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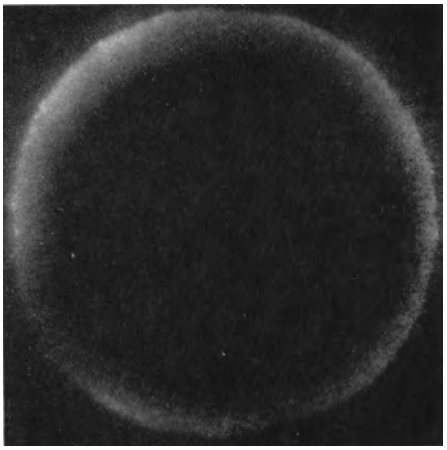

Radioautograph of cross section of orange after treatment with radio-phosphorus labeled isodemeton-demeton.

Spraying Valencia orange tree with a formulation of radio-phosphorus labeled isodemeton-demeton.

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as apples, oranges, pears or walnuts. The actual amount needed depends, of course, on the size of plants and desired dosage.

The precision and sensitivity of analysis by the radio-phosphorus are astonishing. One microgram or less of tracer can be readily and reliably detected by the use of an end window Geiger tube and radio-assay. If 1,000 grams of produce are employed per replicate, a sensitivity of 0.001 ppm—parts per million—is obtainable. This is far better than the sensitivity of normal colorimetric procedures for residue analysis which rarely are reliable to 0.1 ppm.

The utility of radiotracers of demeton and schradan for residue analysis has been demonstrated in the laboratory by several experiments.

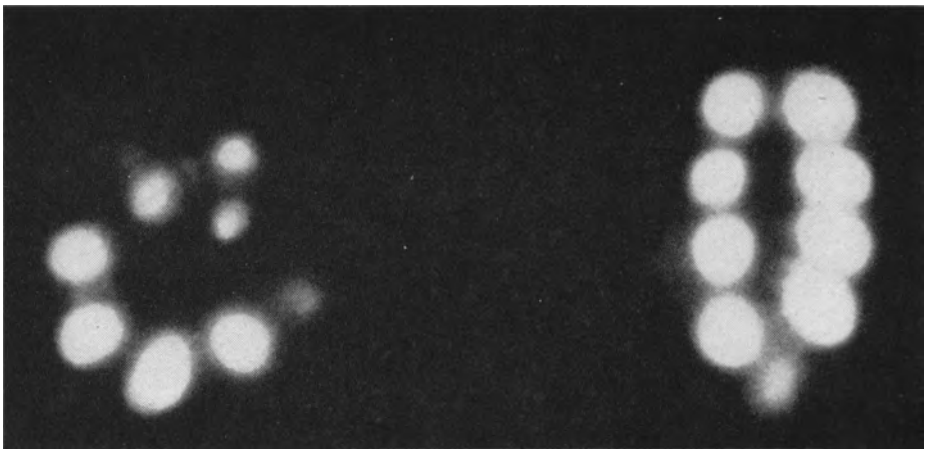
A formulated material prepared with radio-phosphorus demeton was dispersed in water at the rate of 0.25 pound toxicant to 100 gallons of water. The material was sprayed on 10 young walnut trees, five young Valenica orange trees, and five young apple trees. Fruit and foliage were wetted thoroughly to give a final rate of application equivalent to 0.5 pound per acre on mature trees. Replicates of 10 oranges and apples and 20 walnuts were harvested in two and four weeks after spraying. The average residues obtained at the four weeks' harvest calculated as demeton were: apples 0.007 ppm, orange juice 0.02 ppm, and walnut meats 0.009 ppm. Similar studies conducted on potatoes sprayed with the same formulation showed harvest residues of 0.05 to 0.07 ppm in the tubers.

In another experiment, three rows of young cotton plants, aggregating 0.01 acre were sprayed with radio-phosphorus-schradan at the rate of one pound per acre in 25 gallons of water. After 41 days all the cotton was picked, each row serving as a replicate, and a total of 21 pounds was obtained. This was ginned, and the raw oil extracted and refined by standard methods. The raw oil contained an average of 10.9 ppm radioactivity calculated as schradan, while the cottonseed cake contained 167 ppm. After refining, the content of the oil was reduced to 0.02 ppm. By partition technics it was demonstrated that radioactivity in the raw oil was largely intact schradan but that the radioactivity in the cottonseed cake was in the form of nontoxic metabolized products.

The use of radioautographs made by placing leaves and tissue sections in contact with X ray film for several weeks provides additional information as to the localization of radioactivity. Where schradan labeled with radio-phosphorus was sprayed on cotton plants, radioautographs showed the residue to be concentrated in the seeds.

In the residue analysis of these experiments, only the total radioactivity present was measured and assumed to represent the maximum amount of toxicant which could be present.

The investigations with radiotracers in the study of systemic insecticides have indicated the promise this technic shows for the basic studies of the complex biochemical behavior of these materials and—also—for routine analytical determinations of the amounts of residues in treated produce.



Radioautograph of immature cotton bolls 41 days after treatment with radio-phosphorus labeled schradan. *Left*, Cross section. *Right*, Longitudinal section.

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Spraying cotton with radio-phosphorus labeled schradan.