olites remain, essentially, academic in nature.


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### Literature Cited


(1) Babers, F. H., and Pratt, J. J., Jr., *J. Econ. Entomol.*, **46,** 977–81 (1953).
(2) Babers, F. H., and Roan, C. C., *Ibid.*, **46,** 1105 (1953).
(3) Butts, J. S., Chang, S. C., Christensen, B. E., and Wang, C. H., *Science*, **117,** 699 (1953).
(4) Communicable Disease Center, Technical Development Laboratories, Public Health Service, U. S. Dept. Health, Education, and Welfare, Savannah, Ga., Chem. Memo. 3 (1954).
(5) Davidow, B., *J. Assoc. Offic. Agr. Chemists*, **33,** 130 (1950).
(6) Ferguson, W. C., and Kearns, C. W., *J. Econ. Entomol.*, **42,** 810–17 (1949).
(7) Mattson, A. M., Spillane, J. T., Baker, C., and Pearce, G. W., *Anal. Chem.*, **25,** 1065–70 (1953).
(8) Pearce, G. W., and Jensen, J. A., J. AGR. FOOD CHEM., **1,** 776–8 (1953).
(9) Perry, A. S., Fay, R. W., and Buckner, A. J., *J. Econ. Entomol.*, **46,** 972–6 (1953).
(10) Perry, A. S., and Hoskins, W. M., *Ibid.*, **44,** 850–7 (1951).
(11) Perry, A. S., and Sacktor, B., *Ann. Entomol. Soc. Amer.*, **48** (No. 6), in press.
(12) Sacks, J., "Isotropic Tracers in Biochemistry and Physiology," McGraw-Hill, New York, 1953.
(13) Sternburg, J., and Kearns, C. W., *J. Econ. Entomol.*, **43,** 497–505 (1952).
(14) Sternburg, J., Kearns, C. W., and Bruce, N. W., *Ibid.*, **43,** 214–19 (1950).
(15) Tahori, A., and Hoskins, W. M., *Ibid.*, **46,** 302–6, 829–37 (1953).
(16) Vinson, E. B., and Kearns, C. W., *Ibid.*, **45,** 484–96 (1952).
(17) Winteringham, F. P. W., Loveday, P. M., and Harrison, A., *Nature*, **167,** 106 (1951).



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**PESTICIDE RESIDUES**

# Schradan Residues in Cotton and Cottonseed Products


**R. L. METCALF, T. R. FUKUTO, H. T. REYNOLDS, and R. B. MARCH**

**University of California Citrus Experiment Station, Riverside, Calif.**



**Radioactive phosphorus-32-schradan was sprayed on cotton at the rate of 1.0 pound per acre and after 41 days the extent of contamination of leaves, seeds, raw and refined oils, cake, cottonseed meal, and soapstock was evaluated. Schradan showed a surprising affinity for the oily seeds, and about 8 to 16 p.p.m. was present in the raw oil. Upon refining, this was decreased to 0.02 p.p.m. and the schradan was transferred to the soapstock. Ground cottonseed meal and cake contained 70 to 80 p.p.m. of radioactive phosphorus-32 calculated as schradan, but the very low chloroform–1N sodium hydroxide partition ratios indicated that this material was completely metabolized to acidic products. The experiment demonstrates the value of radiotracer studies in evaluating the behavior of systemic insecticides.**


THE SYSTEMIC INSECTICIDE OCTAMETHYL PYROPHOSPHORAMIDE (OMPA or schradan) has proved effective during the past several seasons in controlling red spider mites and aphids attacking cotton, and the compound has been in semicommercial use for this purpose. The dosages employed have ranged from 0.5 to 1 pound (approximately 0.5 to 1 pint) of technical material per acre applied at 5 to 25 gallons per acre by air or ground equipment. As a consequence of the large scale utilization of cottonseed oil in the preparation of food products, such as butter substitutes and cooking oils, it became of interest to establish the possible levels of contamination of cottonseed, oil, and other products resulting from this use of schradan. The use of a radiotracer seemed ideal for this purpose because of the extreme sensitivity of detection, and the simplicity of assay as compared with other analytical methods (*2, 3*).

### Materials and Methods

Radioactive schradan was prepared from 3.35 grams of $P^{32}OCl_3$ (purchased from Tracerlab, Inc., Boston, Mass.) with an initial activity of 10 millicuries, by the following reactions (*1*).

$$P^{32}OCl_3 + 2(CH_3)_2NH \xrightarrow{\text{ether}} (Me_2N)_2\overset{O}{\overset{\|}{P}}{}^{32}Cl$$

$$(Me_2N)_2\overset{O}{\overset{\|}{P}}{}^{32}Cl + (Me_2N)_2\overset{O}{\overset{\|}{P}}OC_2H_5 \xrightarrow{170°} (Me_2N)_2{}^{32}P(=O)-O-\overset{O}{\overset{\|}{P}}(NMe_2)_2 + C_2H_5Cl$$

The final product was distilled at 121° (<1 mm. of mercury) and 6.3 grams was obtained with a relative activity of 4.7 counts per second per microgram. This product was very pure and behaved as a homogeneous compound in paper chromatography upon ethylene glycol-impregnated paper (*4*). When partitioned between chloroform and 1*N* sodium hydroxide, a ratio of 27.8 to 1 was obtained at 30° C.

For use in the spray application on cotton, the phosphorus-32 isomer was diluted by the addition of 4 grams of schradan prepared by the same process and redistilled out of the reaction vessel so as to recover additional tagged product. The final tracer preparation had a relative activity of 3.6 counts per second per $\gamma$.

The radioactive schradan was applied to a heavy stand of Acala 4-42 variety cotton at the Bowlin Ranch in Indio, Calif., on September 2. The temperature was about 100° F. Nine grams of the $P^{32}$ OMPA were dissolved

in 1.874 ml. of water and three rows of cotton each 86.5 feet long, aggregating 10 feet in width (total $^1/_{50}$ acre), were sprayed with this preparation by rapidly walking along each row with a hand sprayer using a fine mist nozzle at 40 pounds per square inch (*6*). The application was thus at the rate of 1 pound of schradan per acre in 25 gallons of water, a dosage rate commonly employed in ground applications for cotton insect and mite control.

Leaf samples were picked at intervals during the experiment to determine the extent of insecticide residue. Twenty-five leaves were selected at random from each of the treated rows, each lot was homogenized in the Waring Blendor with 200 ml. of chloroform and filtered, and a suitable aliquot was assayed for radioactivity.

By the middle of October, considerable cotton had matured in the treated plots and all this was picked on October 13. Each row was treated as a replicate. The yields were as follows:

| | Cotton, Lb. | Raw Oil, Grams |
|---|---|---|
| Row 1 | 8 | 64.9 |
| Row 2 | 8 | 49.6 |
| Row 3 | 5 | 25.3 |

The cotton was ginned and hulled to remove the meats from the seed coat, and the meats were ground to a coarse meal and placed in a heated press at 240° to 280° F. for approximately 15 minutes until the raw oil had separated. These conditions approximated commercial processes as closely as was practicable with the limited quantities of seed available. Samples of the whole seeds, ground meal, residual cake, lint, and raw oil were then wet-ashed with nitric acid and assayed for total radioactivity, and the radioactive material from water homogenates of these substrates was partitioned between chloroform and 1*N* sodium hydroxide to determine the nature of the radioactivity present—i.e., whether it was unmetabolized schradan, partition coefficient 24 to 1 or greater, or degraded products such as tetramethylamidophosphorodiamidic acid (tetramethyldiamidophosphoric acid), orthophosphoric acid, etc. (*3*). This was carried out as described by Metcalf and March (*5*) by shaking the plant homogenate in a mixture of equal volumes of chloroform and 1*N* sodium hydroxide for 1 minute, centrifuging, and determining the radioactivity in an aliquot of each layer.

The raw oil samples were refined as described by the standard method of the National Cottonseed Products Association (*7*). The free fatty acid content of the raw oil was determined by titration in alcohol with standard sodium hydroxide to the phenolphthalein end point. The oil from this experiment had a free fatty acid (F.F.A.) value of 0.715. Samples of approximately 40 grams of this oil from rows 1 and 2 were accurately weighed in 100-ml. beakers. The weighed samples were placed in a constant temperature water bath at 24° C. and stirred at 250 r.p.m. while 5.7%, the specified quantity, of carbonate-free sodium hydroxide (*7*) was added, using a 9.5% solution (14° B.). The quantities used were:

Row 1 sample 40.0 grams, 2.07 ml. of sodium hydroxide
Row 2 sample 40.9 grams, 2.12 ml. of sodium hydroxide

The samples were stirred for exactly 15 minutes after the addition of the lye, removed immediately to a 65° C. water bath, and stirred for 12 minutes. They were then allowed to settle for 1 hour at 65° and cooled overnight at 24° C. The refined oil was decanted and all sediment centrifuged out. The remaining soapstock was freed from the oil by vacuum filtration and washed three times with petroleum ether. Aliquots of both the refined oil and the soapstock were assayed for radioactivity.

The weighing and wet-ashing procedure gave very dependable results in the evaluation of the radioactivity in the leaves, seeds, crude oil, etc. In the refined oil, however, the radioactive contamination was at such a low level that this procedure was not sufficiently sensitive. Therefore, the activity in the oil was determined by extracting 20- to 40-gram samples with 75 ml. of 6*N* hydrochloric acid for 2 minutes in a separatory funnel, separating the bottom layer over a 1-hour period, and repeating the extraction with three additional 40-ml. portions of acid. The combined extractives were extracted with a few milliliters of Skellysolve B to remove any traces of oil, and the bottom layer was concentrated under vacuum in the Kuderna-Danish evaporative concentrator to a total of about 1 ml., which was washed into a planchet, wet-ashed, and assayed for radioactivity.

The samples for radiological assay were all prepared by wet-ashing with concentrated nitric acid at 300° C. in a muffle furnace. Ashing was done directly in the cupped, stainless steel counting planchet. Counts were made with a standard Geiger-Müller end-window tube and were corrected for background and for progressive decay to give values expressed in terms of micrograms of schradan. Samples showing less than 0.03 count per second

**Table I. Recoveries of Phosphorus-32-Schradan from Fortified Cottonseed Oil**

| P.P.M. | Amount Added | Recovery, % | Method |
|---|---|---|---|
| 1 | 10 γ in 10 g. oil | 103 | Direct wet ash |
| 0.01 | 1 γ to 100 g. oil | 102 | Acid extract |
| 0.1 | 10 γ to 100 g. oil | 83 | Acid extract |
| 0.3 | 30 γ to 100 g. oil | 108 | Acid extract |

**Table II. Phosphorus-32-Schradan in Cotton and Cottonseed Products Treated with 1 Pound per Acre**

| | Row 1 | | Row 2 | | Row 3 | |
|---|---|---|---|---|---|---|
| | Total p.p.m. | Partition ratio, $HCCl_3$/1N NaOH | Total p.p.m. | Partition ratio, $HCCl_3$/1N NaOH | Total p.p.m. | Partition ratio, $HCCl_3$/1N NaOH |
| Leaves, initial | 242 | 27.8 | 617 | 27.8 | ... | ... |
| Leaves, 41 days | 4.25 | 2.1 | 8.25 | 1.7 | ... | ... |
| Seeds, unopened boll | 9.85 | 13.6 | ... | ... | ... | ... |
| Cotton | 4.95 | ... | ... | ... | ... | ... |
| Cottonseed shell | 12.8 | ... | ... | ... | ... | ... |
| Ground meal | 73.7 | 0.025 | ... | ... | ... | ... |
| Cake | 79.5 | 0.007 | 260 | 0.0002 | 263 | 0.000 |
| Raw oil, ashing | 8.0 | >8.0[a] | 16.2 | 29.4 | 8.6 | 57[a] |
| Raw oil, ashing by acid extract | 7.7 | >8.0 | ... | ... | ... | ... |
| Dregs | 0.47 | ... | ... | ... | ... | ... |
| Refined oil, ashing | <0.1 | ... | <0.1 | ... | ... | ... |
| Refined oil, ashing by acid extract | 0.022 | ... | 0.023 | ... | ... | ... |
| Soapstock | 83.5 | 3.5[b] | 73 | 0.18[b] | ... | ... |

[a] Amounts of radioactivity in sodium hydroxide layer were so small that they could not be determined accurately; hence partition coefficients are of uncertain magnitude. However, they show clearly that radioactivity was largely in the chloroform; hence unmetabolizied schradan.

[b] Formations of very permanent emulsions from soapstock made determinations of partition coefficients uncertain.

greater than background were considered as possessing no activity, as this value represents the standard error of the average background. Recovery data establishing the validity of the techniques used is presented in Table I.

Radioautographs of the cross sections of treated bolls were made by fastening sections covered with aluminum foil to "no screen" x-ray film for 3 weeks.

### Discussion

The tabulated results of the experiments reported are presented in Table II. The initial residue on the upper leaves of the treated plants was very high, from 200 to 500 p.p.m. After 41 days this had declined to 4 to 8 p.p.m. and the partition coefficients showed that a considerable portion of the phosphorus-32 was present as metabolized schradan. The cotton contained about the same activity as the leaves. The seeds had higher activity and most of the phosphorus-32 was present as schradan. After grinding, the cottonseed meal had activity of 74 p.p.m.. which, however, was nearly all in metabolized forms. The activity of the cake ranged from 80 to 260 p.p.m., but here also the phosphorus-32 was present as degradation or metabolic products. In the raw oil, the activity ranged from 8 to 16 p.p.m., which partitioned strongly into the chloroform and was apparently almost entirely schradan. After refining, however, only trace amounts of activity remained, some $^1/_{400}$ to $^1/_{800}$ of that initially present. Most of the schradan in the raw oil was evidently carried into the soapstock which contained about 70 to 80 p.p.m., a large portion of which was phosphorus-32-schradan.

The above results were confirmed by a series of radioautographs of treated bolls which showed very high concentrations of radioactivity in the seeds (*6*).


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### Literature Cited


(1) Gardiner, J. E., and Kilby, B. A., *J. Chem. Soc.*, **1950,** pp. 1769–72.
(2) Hall, S. A., Stohlman, J. W., III, and Schecter, M. S., *Anal. Chem.*, **23,** 1866–8 (1951).
(3) Hartley, G. S., Heath, D. F., Hulme, J. M., Pound, D. W., and Whittaker, M., *J. Sci. Food Agr.*, **2,** 303–9 (1951).
(4) March, R. B., Metcalf, R. L., and Fukuto, T. R., J. AGR. FOOD CHEM., **2,** 732 (1954).
(5) Metcalf, R. L., and March, R. B., *J. Econ. Entomol.*, **45,** 988–97 (1952).
(6) Metcalf, R. L., March, R. B., and Fukuto, T. R., *Agr. Chem.*, **9** (3). 33 (1954).
(7) National Cottonseed Products Assoc., "Rules and Standard Methods," 1950–51.



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**PESTICIDE RESIDUES**

# Determination of Residues of *O,O*-Diethyl *O*-(2-Isopropyl-6-methyl-4-pyrimidyl) Phosphorothioate in Milk

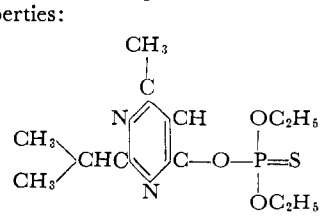

**R. C. BLINN and F. A. GUNTHER**

**University of California Citrus Experiment Station, Riverside, Calif.**



**The promising control of the housefly by treatment with O,O-diethyl O-[2-isopropyl-6-methyl-4-pyrimidyl] phosphorothioate (Diazinon) has necessitated the evaluation of residual amounts of Diazinon in milk when this material is used for fly control in dairy barns. In a modification of the Harris procedure Diazinon is hydrolyzed to 2-isopropyl-6-methyl-4-pyrimidinol; extraneous material is then extractively removed from its strongly acidic and basic aqueous solutions; the pyrimidinol is isolated from a weakly acidic solution by chloroform extraction and is then determined by its absorption at 272 mμ. The milk is processed by freeze-drying, followed by extraction of the residual milk solids with medium-boiling petroleum ether and partition of Diazinon into acetonitrile. Over-all recoveries of 63 to 70% are obtained.**


THE COMPOUND *0,0*-diethyl *0*-[2-isopropyl-6-methyl-4-pyrimidyl] phosphorothioate or Diazinon (also known as isopropylmethylpyrimidyl diethyl thiophosphate, as G-24480, and as Diazinone) has shown encouraging activity against several insects and mites. Carefully purified Diazinon possesses the following properties:



Molecular weight, 304.35
Boiling point, 83–4° C. at 0.002 mm. (*2*)
Vapor tension, $1.4 \times 10^{-4}$ mm. at 20° C.; $1.1 \times 10^{-3}$ mm. at 40° C.; $3.3 \times 10^{-2}$ mm. at 80° C. (*2*)
Molar absorbancy index (95% ethyl alcohol), 4380 at 247.5 mμ (2,2,4-Trimethylpentane), 557 at 284 mμ; 3850 at 246.5 mμ

Detailed ultraviolet absorption characteristics are shown in Figure 3.

The promising control of the housefly, *Musca domestica* L., by treatment with Diazinon has prompted the evaluation of residual amounts of Diazinon in milk when this material is used for fly control in dairy barns. A general chemical method for the microdetermination of Diazinon residues has been proposed by Harris (*5*), who hydrolyzed Diazinon to 2-isopropyl-6-methyl-4-pyrimidinol, extracted interfering substances with ether and petroleum ether from strongly acidic solution and from strongly basic solution, and then extracted the pyrimidinol into chloroform from a weakly acidic solution for eventual ultraviolet determination. It was found necessary to modify this procedure so as to minimize background interference from incidental milk extractives, and also to increase sensitivity.