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*).

Acknowledgment

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PESTICIDE RESIDUES

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

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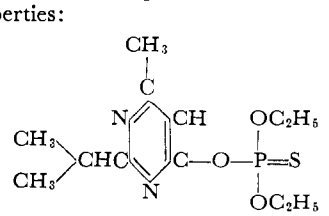
The promising control of the housefly by treatment with O,O-diethyl O-[2-isopropyl-6-methyl-4-pyrimidyl] phosphorothioate (Diazinon) has necessitated the evaluation of residual amounts of Diazinon in milk when this material is used for fly control in dairy barns. In a modification of the Harris procedure 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, 1.4×10^{-4} mm. at 20° C.; 1.1×10^{-3} mm. at 40° C.; 3.3×10^{-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.

To minimize the formation of refractory emulsions during initial extractions, the whole milk was freeze-dried. The over-all efficiency of transfer of insecticide from milk to organic solvent was not increased substantially over that obtained from ordinary liquid-liquid extractions (*7*).

Processing of Milk

Sources of Milk. Fresh whole milk was collected from three dairies. Milk from dairies X and Z was pasteurized and homogenized; that from dairy Y was raw and not homogenized. Samples were immediately refrigerated, and aliquots were fortified with purified Diazinon (see section on reagents) for subsequent evaluation of efficiency of recovery. All milk samples were processed within 2 days after receipt.

Special Apparatus. A unitized lyophil type of freeze-drying apparatus as shown in Figure 1 was used for this study. The dry ice reservoir holds 10 pounds of dry ice; the freezing flasks are of 800-ml. capacity. Other types of freeze-drying apparatus have been used successfully for similar problems (*1*).

Any type of vacuum pump capable of maintaining a vacuum of 2 mm. or better is satisfactory.

Any type of manometer capable of indicating a vacuum of 2 mm. or better is satisfactory.

Procedure. Fill the inner dry ice reservoir of the freeze-drying apparatus with isopropyl alcohol and crushed dry ice. Place 100 to 150 ml. of milk in each of the 800-ml. freezing flasks and rotate in a dry ice–isopropyl alcohol bath to freeze the milk onto the sides of the flasks. Continue cooling until the frozen milk has "cracked" away from the glass. Place the flasks in position on the freeze-drying apparatus, using generous amounts of a high-vacuum, silicone-type lubricant. Evacuate by means of the vacuum pump and maintain a vacuum of 2 mm. or less until the coating of frost and ice formed on the outsides of the flasks (see Figure 1) has melted and the flasks have warmed to room temperature. Additional dry ice should be added to the reservoir as needed. The freeze-drying operation usually requires about 8 hours for completion and consumes about 30 pounds of dry ice.

To the resulting dried milk powder in each flask add 200 ml. of petroleum ether (boiling point 60° to 80° C.) and about 30 grams of pea-sized gravel (previously exhaustively extracted with petroleum ether), then cap the flask with a standard-taper cap and shake vigorously until no lumps remain in the milk powder. Shake the flask mechanically for 1 hour or let sit for 24 hours with occasional shaking before filtering the stripping solution through a coarse-porosity fritted-glass filter. Combine the filtrates from laboratory replicates.

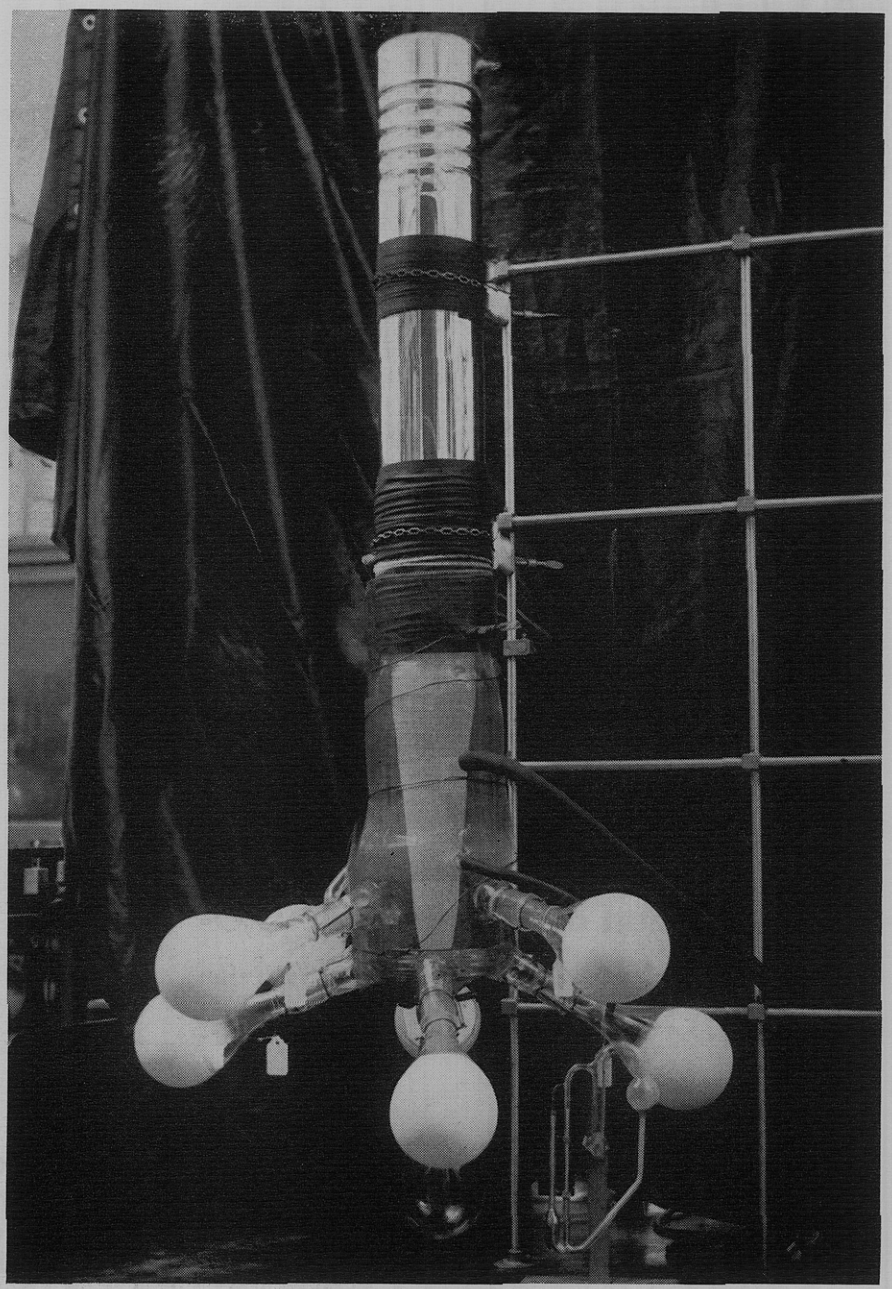


Figure 1. Freeze-drying apparatus

Determination of Diazinon

Reagents. All chemicals are analytical reagent grade, except where specifically indicated.

Purified Diazinon, (boiling point 83–84° C./0.002 mm.) was obtained from the Geigy Co., Inc., Bayonne, N. J. Recent evidence indicates that this material is not stable to some conditions of storage, especially in hydroxyl-containing solvents. Recoveries reported in this paper are based on standards of purified 2-isopropyl-6-methyl-4-pyrimidinol. Solutions of Diazinon in petroleum ether were standardized directly before use by this procedure, omitting the acetonitrile partition step. These solutions, as well as the sample of purified Diazinon, gave decreasing recoveries of apparent Diazinon with age, even when stored under refrigeration.

Petroleum ether, boiling point 60° to 80° C.

Acetonitrile.

Light petroleum ether, boiling point 30° to 60° C.

Wash solution. Add 1 ml. of 25% acetic acid solution to 1000 ml. of water.

Methanol.

Potassium hydroxide, pellets.

Hydrochloric acid, 3*N* solution.

Ethyl ether, U.S.P. grade will do.

Potassium hydroxide, 5% solution.

Chloroform. Distill a commercial grade through an all-glass apparatus before use.

Acetic acid, 25% by volume solution.

Ethyl alcohol, 95%.

2-Isopropyl-6-methyl-4-pyrimidinol (melting point 174.5 −175.5° C.) was

obtained from the Geigy Co., Inc., Bayonne, N. J.

Special Apparatus. Any type of spectrophotometer that responds satisfactorily at 272 mμ may be used.

Kuderna—Danish Evaporative Concentrator (*3*, *4*).

Procedure

Partition Distribution of Diazinon into Acetonitrile. Concentrate a measured volume of the filtered petroleum ether stripping solution in a Kuderna-Danish evaporative concentrator to slightly less than 100 ml. through a three-ball Snyder column. Transfer the concentrate to a 500-ml. separatory funnel and dilute to 100 ml. with petroleum ether, then extract with four 35-ml. portions of acetonitrile, discarding the petroleum ether phase. Transfer the combined acetonitrile extracts to a 1000-ml. separatory funnel, I, using 250 ml. of wash solution and 100 ml. of light petroleum ether (boiling point 30° to 60° C.). Shake this mixture for 30 seconds and pass the aqueous layer into another separatory funnel, II, containing 100 ml. of light petroleum ether. Shake this mixture for 30 seconds and discard the aqueous layer. Extract the petroleum ether solution in I with three 25-ml. portions of wash solution, using each of these in turn to extract the petroleum ether solution in II before discarding. Combine the final petroleum ether solutions in a standard-taper 500-ml. Erlenmeyer flask.

Hydrolysis of Diazinon. Evaporatively concentrate this petroleum ether solution just to dryness through a three-ball Snyder column. Add 25 ml. of methanol and 1.0 gram of potassium hydroxide pellets, and evaporatively concentrate just to dryness through a three-ball Snyder column. Add 10 ml. of water and 6 ml. of 3*N* hydrochloric acid solution. Test this solution with indicator paper to assure its strong acidity. Vigorously shake the solution for 15 seconds with each of two 100-ml. portions of ethyl ether, removing most of the ether layer through a suction stick. Transfer the aqueous solution to a 500-ml. separatory funnel with the aid of 35 ml. of water, then extract it with two 25-ml. portions of chloroform, discarding the chloroform layers. Add 6 ml. of 5% potassium hydroxide solution to the aqueous solution, again testing the solution with indicator paper to assure its strong alkalinity. Vigorously shake this aqueous solution for 15 seconds with each of two 50-ml. portions of chloroform, discarding the chloroform layers. Add 1.5 ml. of 25% acetic acid solution and test with indicator paper to assure a pH of approximately 3.5 (see Figure 2). Vigorously shake this solution for 15 seconds with each of three 100-ml. portions and one 25-ml. portion of chloroform. Separate and pass each portion of chloroform extract successively through a Gooch crucible holder containing a wad of glass wool and 10 grams of anhydrous sodium sulfate, into a Kuderna-Danish evaporative concentrator.

Ultraviolet Determination. Evaporatively concentrate the chloroform solution almost to dryness through a three-ball Snyder column. Transfer the residue with the aid of 5 ml. of light petroleum ether into a 10-ml. volumetric flask. Add a boiling chip and evaporate to dryness, leaving the ground-glass stopper loosely in place. It is important that the stopper be kept in place to minimize losses from volatility of the pyrimidinol derivative. Remove the last traces of solvent vapors with gentle suction. Add 5 ml. of 95% ethyl alcohol and evaporate to about 2 ml., again leaving the ground-glass stopper loosely in place. After cooling, dilute to volume with 95% ethyl alcohol and determine the absorbance at 272 mμ, setting the instrument with a control solution prepared from untreated milk which has been subjected to the same analytical procedures as the sample. A standard calibration curve may be prepared from purified 2-isopropyl-6-methyl-4-pyrimidinol (melting point 174.5–175.5° C.).

Comments on Procedures

Partition Distribution of Diazinon into Acetonitrile. The freeze-drying of 400 ml. of fresh milk afforded 46 grams of dried milk powder. When this powder was stripped with petroleum ether, 8.7 grams of fats and other soluble material were coextracted with the Diazinon. With a final determination of fewer than 100 γ of Diazinon, this amount of material presented a large potential source of interference. Jones and Riddick (*6*) described a partition distribution procedure for separating organic insecticides from plant and animal tissues by use of acetonitrile and *n*-hexane. Their procedure was adapted to the separation of Diazinon from milk extractives, thus reducing the extraneous extracted material from 8.7 grams to 0.4 gram. Partition coefficient data for Diazinon into acetonitrile from petroleum ether are presented in Table I.

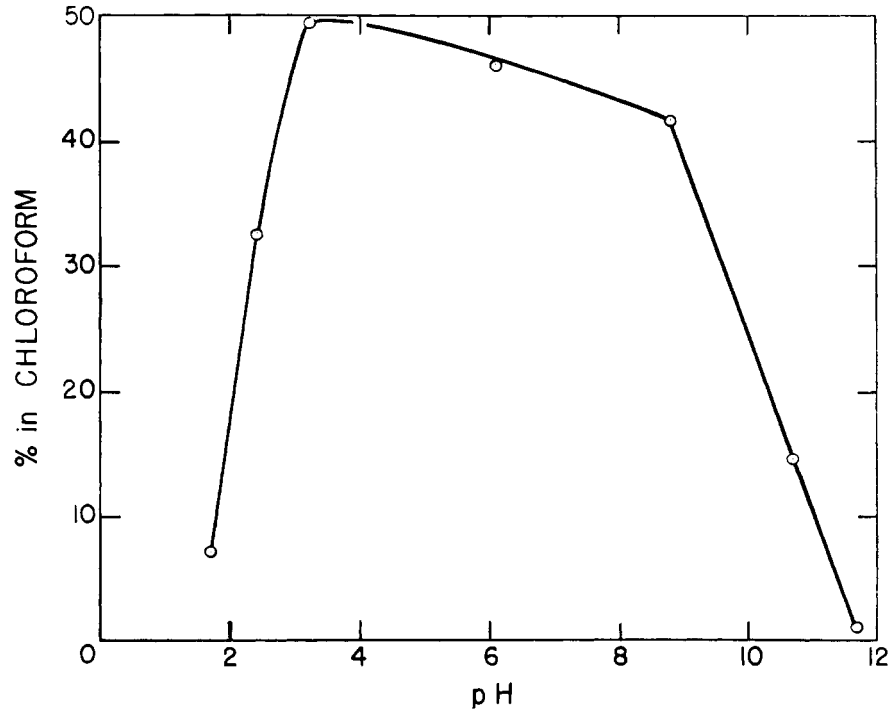
Table I. Percentage Partition of Diazinon into Acetonitrile from 1.00 Volume of Petroleum Ether

Comparative Volume Acetonitrile	Diazinon, % in Acetonitrile
1.00	82
0.50	60
0.35	51
0.25	39

In the procedure as presented, a 94% recovery of Diazinon resulted from the initial partition into acetonitrile with the total of four extractions into acetonitrile (97% recovery) followed by dilution with water and stepwise extraction back into petroleum ether (97% recovery), while only 4.6% of material extractable from milk remained.

Hydrolysis of Diazinon. The Harris (*5*) hydrolysis procedure for Diazinon was modified to take advantage of the stability of the 2-isopropyl-6-methyl-4-pyrimidinol. By using rather rigorous

Figure 2. pH of aqueous solution vs. partition of 2-isopropyl-6-methyl-4-pyrimidinol into chloroform



conditions, the time of hydrolysis was shortened and the methanol was eliminated by evaporation, so that it did not affect the partition distributions encountered in subsequent steps.

The hydrolysis product of Diazinon, 2-isopropyl-6-methyl-4-pyrimidinol, is capable of salt formation with both strong acids and strong bases. Thus, when in strongly acidic or alkaline solutions, the partition distribution of the pyrimidinol into chloroform was negligible; but as the solutions approached neutrality, the partition into chloroform increased. This behavior is graphically illustrated in Figure 2. Ethyl ether, which is a poor solvent for the pyrimidinol salt, was used to extract the bulk of extraneous matter from the acidic solution prior to the chloroform extractions. The losses from all the extraction steps amounted to less than 2%.

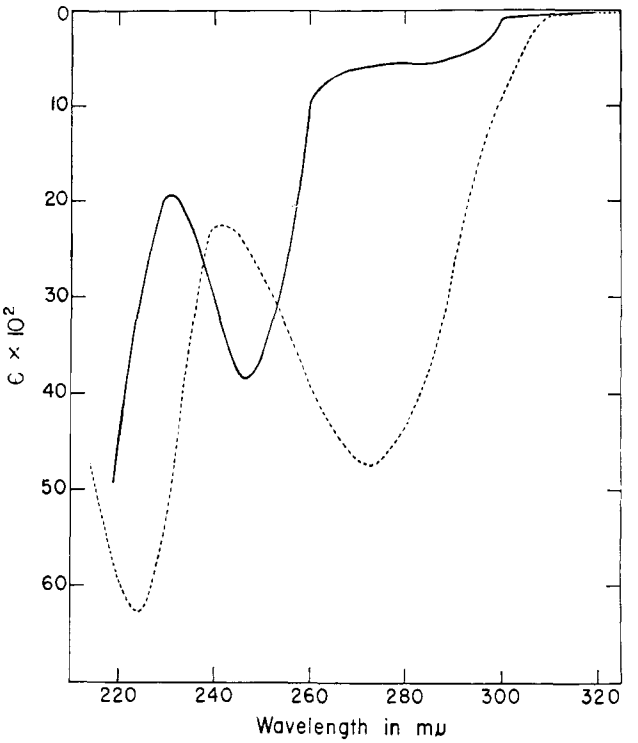


Figure 3. Ultraviolet absorption in 95% ethyl alcohol

—— Diazinon
--- 2-Isopropyl-6-methyl-4-pyrimidinol

Ultraviolet Determination. The ultraviolet absorption characteristics of Diazinon and 2-isopropyl-6-methyl-4-pyrimidinol in 95% ethyl alcohol are reproduced in Figure 3, the latter compound having a molar absorbancy index of 4800 at 272 mμ. A calibration curve for this pyrimidinol prepared by the present procedure conforms to Beer's law at 272 mμ from 20 to 600 γ of Diazinon. Dilutions also follow Beer's law, thus permitting the determination of higher concentrations of Diazinon. The analytical procedure has an over-all efficiency of from 95 to 105%, based upon recovery of 2-isopropyl-6-methyl-4-pyrimidinol in the presence of milk extractives (see Table II).

Interferences. The main sources of interference were from traces of aromatic substances in the light petroleum ether used to transfer the pyrimidinol residue into the 10-ml. volumetric flasks and from chloroform and water incompletely removed from the final ethyl alcohol solution. These are minimized by the evaporative concentration of the final ethyl alcohol solution. It is recommended that glassware be soaked in sulfuric acid–dichromate cleaning solution, rinsed with water, acetone, and distilled water, and then dried above 100° C. Ground-glass surfaces should be lightly greased with a silicon-type lubricant, as other types may contain aromatic substances.

Some naturally occurring substances containing a pyrimidine moiety may interfere if not minimized during the partition of Diazinon into acetonitrile from the petroleum ether solution. This interference, encountered during the preliminary stages of this study, was probably due to vitamin B_1.

Table II. Recovery of Diazinon from Fortified Milk Samples

Dairy	Milk	Diazinon Added		Diazinon Recovered		
		γ	P.p.m.	γ	P.p.m.	%
X	Pasteurized	0	0.00	0	0.00	...
		70	0.18	50	0.13	71
		231[a]	0.58	220	0.55	95
Y	Raw	0	0.00	0	0.00	...
		13	0.03	9	0.02	69
		35	0.08	22	0.05	63
		40[a]	0.10	42	0.11	105
Z	Pasteurized	0	0.00	0	0.00	...
		40	0.10	25	0.06	63
		80	0.20	55	0.14	69

[a] Fortified during stripping operation.

Discussion of Results

Results of the chemical assay of 400-ml. samples of fresh milk fortified and unfortified with purified Diazinon are shown in Table II. With the samples of milk from all three dairies (X, Y, and Z), the control (unfortified) samples exhibited 0.00 p.p.m. interference, and the over-all recoveries varied from 63 to 70%. Aliquot samples of freeze-dried milk from dairies X and Y were also fortified during the stripping operation to afford recoveries of from 96 to 105%. Therefore, the major losses of this method occurred during the freeze-drying process. Whether these losses are attributable to volatility or to occlusion within the particles of dried milk powder is not known. Raw and pasteurized milk behaved similarly in this method.

Acknowledgment

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