Table I. 1,2-Dibromo-Ethane[a] and Total Bromide Residue from Fumigated Apples

Time after Fumigation, Hours	1,2-Dibromo-Ethane Remaining,[b] Mg./Kg.
1	33.0
2	19.2
24	1.71
Days	**Total Bromide[c]**
1	1.23
8	0.53
10	0.47
Nonfumigated	0.40

[a] Ethylene dibromide, 4 mg./liter, 2 hours fumigation at 15.6° C. [b] Av. of two determinations on Winesap apples. [c] Av. of two determinations on McIntosh apples.

from the weighing balance was transferred into a flask and broken by a glass rod introduced through the condenser. After vaporization of the liquid 1,2-dibromo-ethane, the reagent used for absorption and conversion was introduced through the condenser rinsing the glass rod as it was removed. The conversion and coulometric determination were the same as described above.

Results and Discussion

Standard samples of 1,2-dibromo-ethane were used to determine the per cent conversion. In this way, the accuracy of the method was found for the selected range of 0.75 mg. to 30 mg. of 1,2 - dibromo - ethane. In the above range, the recovery was 99.6 to 101%, and for the range 0.75 mg. to 19 mg. of 1,2-dibromo-ethane, the recovery was 99.9 to 101%.

For the determination of 1,2-dibromo-ethane and bromide residue, two varieties of apples were used, Winesap for 1,2-dibromo-ethane disappearance and McIntosh for bromide residue because Winesap variety was not available at the time of the experiment for residue determination.

Each sample of apple consisted of three segments from three different apples, with total weight of about 60 grams. After fumigation, apples were stored at 15.6° C. The rate of 1,2-dibromo-ethane disappearance after fumigation is shown in Table I. After 24 hours at 15.6° C., the remaining 1,2-dibromo-ethane was 1.71 mg. per kg. The increase of inorganic bromide residue due to fumigation after 10 days was very low (0.07 mg. in 1000 grams of apples). By extraction, no volatile halide was found in nonfumigated apples, but 0.40 mg. of bromide residue was present.

Acknowledgment

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INSECTICIDE RESIDUES

Method for the Detection of Microgram Quantities of *O,O*-Dimethyl-*S*-(*N*-methyl-carbamoylmethyl) Phosphorodithioate (Dimethoate) in Milk

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A specific semiquantitative method for the estimation of dimethoate in milk has been developed. It is based on a paper chromatographic separation and is sensitive to 0.01 p.p.m. Dimethoate is extracted from milk by using a diethyl ether–hexane mixture. After removal of the ether, the hexane is extracted with acetonitrile. Residual pigments and lipides are removed by adsorption on a Florisil column. Dimethoate is further separated from interfering extractives by paper chromatography, and detected as a red spot after spraying the dried chromatogram with a solution of 2,6-dibromo-*N*-chloro-p-quinoneimine.

AEROSOL and spray formulations containing *O,O*-dimethyl-*S*-(*N*-methyl-carbamoylmethyl) phosphorodithioate (dimethoate) are effective in the control of biting flies on dairy cattle (*1*, *2*). A method for detection of this insecticide in milk has been developed to determine whether these formulations could be used on lactating cows without subsequent contamination of the milk. Chilwell and Beecham (*3*) have summarized the methods available for the determination of dimethoate residues. These have been investigated and found to lack the desired sensitivity of at least 0.01 p.p.m. A specific method with such a high sensitivity would be most useful in studies of residues in milk.

Dimethoate can be extracted from milk by using either chloroform (*4*) or a diethyl ether–hexane mixture (*5*). The latter is more convenient to use because fewer emulsions are encountered and there is no need to evaporate to dryness before partitioning with acetonitrile. The ether is removed by vacuum distillation, and the insecticide is extracted from the hexane with acetonitrile (*6*). The extract is evaporated and the residue dissolved in chloroform. Following adsorption on a Florisil column (*8*), the column is developed, and the effluent is concentrated and spotted on Whatman No. 1 filter paper impregnated with Carbowax 4000 (*10*). The chromatogram is developed with a hexane–

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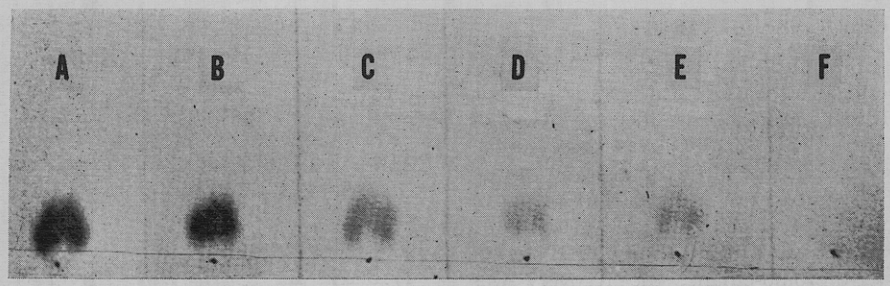

Figure 1. Paper chromatograms of microgram quantities of dimethoate

A = 5.0 μg., *B* = 3.0 μg., *C* = 2.0 μg., *D* = 1.0 μg., *E* = 0.5 μg., *F* = reagent blank

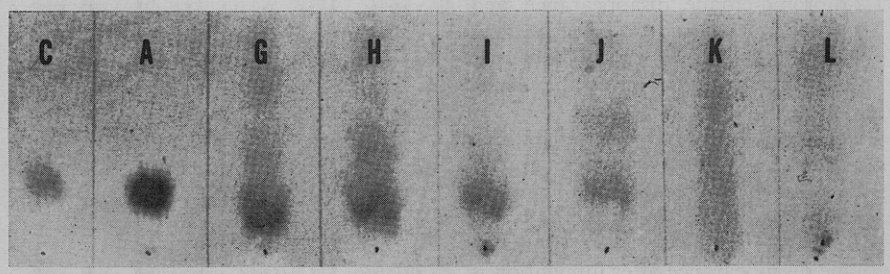

Figure 2. Paper chromatograms of 200-ml. milk samples

G contains 5.0 μg.; *H*, 4.0 μg.; *I*, 3.0 μg.; *J*, 2.0 μg.; *K*, 1.0 μg. *C* and *A* contain 2.0 and 5.0 μg., respectively, of dimethoate without milk, and *L* is milk alone

acetic acid mixture, dried, and sprayed with a solution of 2,6-dibromo-*N*-chloro-*p*-quinoneimine (*7*). Dimethoate appears as a reddish-brown spot a few millimeters from the origin.

Reagents and Apparatus

Ether–*n*-hexane mixture, 2:1 (v./v.).

Acetonitrile, redistilled over concentrated sulfuric acid.

Florisil, 60 to 100 mesh, heated to 300° C. for 2.5 hours and stored in a tightly closed container until used.

DCQ solution. Dissolve 0.5 mg. of 2,6-dibromo-*N*-chloro-*p*-quinoneimine in 100 ml. of spectrograde cyclohexane. Store in a tightly stoppered glass bottle under refrigeration. Prepare a fresh solution every 2 weeks.

Carbowax 4000 solution. Dissolve 5 grams of Carbowax 4000 in 100 ml. of $CHCl_3$.

Mitchell chromatographic assembly (stainless steel tank, etc., for 8 × 8 inch paper) (*9*).

Analytical Procedure

Extract a 200-ml. sample of milk with three 250-ml. aliquots of an ether-hexane mixture. Filter the combined extracts through a cotton plug into a 1-liter boiling flask containing 25 to 50 grams of anhydrous sodium sulfate. Concentrate the extract under vacuum to 25 ml., and filter into a 250-ml. separatory funnel through a No. 40 Whatman folded, filter paper. Rinse the boiling flask with two 25-ml. portions of fresh hexane followed by a 30-ml. portion of acetonitrile and add the washings to the funnel.

Shake the separatory funnel for 1 minute and allow the layers to separate. Transfer the lower acetonitrile layer to a second separatory funnel containing 50 ml. of fresh hexane. Shake this funnel for 1 minute and again allow the layers to separate. Transfer the lower layer to a 125-ml. Erlenmeyer flask and retain the upper hexane layers for further extraction. Rinse the boiling flask with two additional portions of acetonitrile and successively extract the contents of the two funnels with each portion and drain into the same 125-ml. Erlenmeyer flask.

Concentrate the combined acetonitrile extracts to ca. 0.2 ml. under vacuum. Transfer the residue to a 15-ml., graduated, conical centrifuge tube with the aid of three 5-ml. portions of chloroform. Concentrate this solution to about 1-ml. with a stream of dry air. Wash the extract onto a Florisil column with the aid of two 0.5-ml. portions of chloroform.

The Florisil column is prepared as follows: A chromatographic tube 12 × 200 mm. containing a plug of glass wool is wet-packed with Florisil to a height of 175 mm., using chloroform as the liquid medium.

Develop the column with chloroform at the rate of approximately 3 ml. per minute. Discard the first 40 ml. fraction and save the next 130 ml. containing the dimethoate. Concentrate the dimethoate fraction to 2 ml. under vacuum and quantitatively transfer it to a conical centrifuge tube. With the aid of a stream of dry air concentrate to a volume convenient for spotting on a paper chromatogram. Spot on No. 1 Whatman filter paper which has previously been dipped in a 5% solution of Carbowax 4000 in chloroform. (Carbowax 1540 has also been used with equal success.)

Develop the chromatogram for 4 hours by the ascending technique using a solvent mixture containing 95% hexane and 5% acetic acid (v./v.). Either seal the chromatographic tank or place it in a water bath at 15° C. for the entire development period. Dry the chromatogram and spray with DCQ reagent. Allow the chromatogram to dry overnight or heat cautiously at 60° C. for 15 minutes. The insecticide is identified as a reddish-brown spot about 10 mm. from the origin, if Carbowax 4000 is used, or 2.5 cm. from the origin, if Carbowax 1540 is employed.

Results and Discussion

Figure 1 is a photograph of paper chromatograms of known amounts of dimethoate ranging from 0.5 to 5 μg., and shows the variations in spot intensity which are directly proportional to the amounts of dimethoate present. Quantities as small as 0.5 μg. gave a read ly detectable spot, while the reagent blank at the extreme right of the figure shows no coloration.

Milk samples fortified with known amounts of dimethoate were carried through the procedure described and the results are shown in Figure 2. While the spots were slightly less well defined than in Figure 1, they were easily detectable at the 1-μg. level, and readily distinguishable from the milk sample containing no dimethoate at the extreme right of Figure 2. These results were obtained with homogenized, pasteurized market milk. Raw Guernsey milk, which contains larger amounts of pigments, had a slight masking effect, but even under these conditions 2 to 5 μg. were readily detectable. For control purposes in routine analysis, a sample of milk containing no dimethoate and one to which 2 to 5 μg. of the compound have been added are carried through the entire procedure with each series of unknown samples.

Tests with other commonly used organic phosphate insecticides including Diazinon, Guthion, Systox, Trithion, and malathion showed that none of these compounds gave positive chromatograms.

Results obtained using this procedure have been reported elsewhere (*1*, *2*).

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INSECTICIDE RESIDUES

Microdetermination of Thiodan Residues

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Thiodan residue is extracted from plant material with *n*-hexane, and the extract is cleaned up, evaporated to dryness, and reacted with aqueous pyridine–alkali. The reaction, carried out in a single test tube, is sensitive to both isomers of technical Thiodan and to Thiodandiol. The method is sensitive to 5 μg. of Thiodan, requires no special apparatus or reagents, and is adaptable to routine laboratory work when a large number of samples are to be analyzed. It has been used for determining Thiodan residues on sugar beet leaves, alfalfa, and strawberries, and for Thiodan vapor in air.

ANALYTICAL methods suggested for determining Thiodan (6,7,8,9,10,-10 - hexachloro - 1,5,5*a*,6,9,9*a* - hexahydro - 6,9 - methano - 2,4,3 - benzodioxathiepin - 3 - oxide) have been based on chlorine analysis or hydrolysis of Thiodan to Thiodandiol and sulfur dioxide and determination of sulfur dioxide by iodometric or colorimetric procedures (*2*). The chlorine method is not specific, and the sulfur dioxide method will not determine Thiodan degradation products in pesticide residues. Zweig and Archer (*6*) have developed a gas chromatographic technique for the analysis of Thiodan residues, and Zweig, Archer, and Rubenstein (*7*) have combined the gas chromatographic technique with infrared analysis to increase the sensitivity of the method. Thiodan has been found to react with alkali to form a compound that can be evaluated quantitatively with a pyridine–water–sodium hydroxide reagent by a Fujiwara-type reaction (*3*). The method is sensitive to 5 μg. of Thiodan and has been used to measure up to 100 μg. Both isomers of technical Thiodan and Thiodandiol can be measured by this reaction. Thiodan ether will not react. The method requires no unusual apparatus or reagents and is adaptable to the routine laboratory work associated with a large number of samples. Chlorinated solvents, such as carbon tetrachloride and chloroform, must be absent from the air and the apparatus.

Reagents

Thiodan Standard Solution. Dissolve 0.1063 gram (94% Thiodan, Food Machinery & Chemical Corp., Niagara Chemical Division), in 1 liter of distilled *n*-hexane. One milliliter of this solution contains 100 μg. of Thiodan. Dilute an aliquot of this solution with *n*-hexane so that 1 ml. contains 10 μg. of Thiodan.

Cotton, acetone extracted and oven dried.

n-Hexane, redistilled over sodium. Skellysolve B or any other *n*-hexane with a low reagent blank can be used.

Pyridine Solution, 96% in distilled water. Purify the pyridine by refluxing over solid potassium hydroxide for 1 hour (700 ml. of pyridine and 50 grams of KOH), cool, decant into a clean dry flask, and distill. Add 4% of water to the purified pyridine.

Mineral Oil Solution. Dissolve 0.2 ml. of mineral oil (Nujol or equivalent) in 100 ml. of *n*-hexane.

5*N* Sodium Hydroxide Solution. Dissolve 200 grams of reagent-grade sodium hydroxide in distilled water and dilute to 1 liter.

Apparatus

Oil bath (100° ± 2° C.).

Spectrophotometer.

A modified, Smith-Greenberg, all-glass impinger or equivalent for air samples.

Preparation of Standard Curve

Pipet aliquots of the Thiodan standard solution containing 0 to 100 μg. into glass-stoppered test tubes. Dilute to a volume of 10 ml. with distilled *n*-hexane. Add 1 ml. of the mineral oil solution and evaporate to dryness with the aid of a gentle stream of air on top of a steam bath, or in a water bath, at about 50° C. Add 5 ml. of the pyridine-water solution and 2 ml. of 5*N* sodium hydroxide, stopper, mix well, and immerse in an oil bath (100° C.) for 3 minutes. Swirl occasionally while the samples are heating without removing them from the bath. At the end of the heating period, remove the test tubes and cool them in an ice-water bath for 1 minute. Loosen the stoppers in the test tubes to prevent freezing. Decant the pyridine solution through a loosely packed cotton plug, in a small funnel, into 1-cm. cells and determine the absorbance at 525 mμ, with distilled water as a referenee. A cloudy filtrate indicates that the reaction mixture has not been sufficiently cooled before filtration. If the