ately after the deposits from the third application were dry, and then after 7, 14, and 21 days. The last sampling coincided with harvest.

The samples were collected as follows. Two pears within reach of the ground were taken from each quadrant of three trees for a total of 24 fruits per sample. Samples were taken in triplicate each time. Two control samples were taken for each set of samples from trees that were in the same orchard but had been sprayed with 1/4 pint of Systox and 1 pound of 50% DDT. The initial samples were placed in polyethylene bags, but paper sacks were used for subsequent samples.

All samples were processed immediately by grinding, mincing 2-pound subsamples with petroleum ether (boiling range 60° to 80° C.), equilibrating, and filtering in the usual manner (*2*). Stripping solutions were stored at 10° C. awaiting analysis. Polyethylene bags from the initial samples were rinsed with solvent, a proper aliquot of which was added to the stripping solution from the corresponding initial subsample; in this manner loose initial deposits were recovered.

From Figure 5 it can be seen that the sulfide has a residue half-life value (*2*) of 11 days, while the sulfone has a residue half-life value of 7 days.

Because these half-life values are expressed in terms of micrograms of the compound in question per gram of Bartlett pear, they incorporate both growth-dilution and decomposition-attenuation factors. In Table V are also recorded the average grams per fruit from each 24-fruit sample replicated five times; these values may be used to calculate and plot the comparative magnitudes of the growth dilution of the residues. Thus, assuming uninterrupted and regular growth of the pears, the growth-dilution half-life value of the sulfide is about 40 days, as contrasted with the above actual half-life value of 11 days.

The chromatographic procedure would adapt itself to either of the total methods (*2*, *4*) mentioned previously. When coupled with a suitable total method for the unchromatographed stripping solution, the sulfoxide may be implied by subtraction.

Acknowledgment

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Received for review April 6, 1956. Accepted August 23, 1956. Paper No. 927, University of California Citrus Experiment Station, Riverside, Calif. Irrespective of the information contained in this report, the pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use.

PESTICIDE RESIDUES

Determination of Ethylene Dibromide in Fumigated Fruit

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Ethylene dibromide is recovered from fumigated fruit by steam distillation and extraction with benzene. It is decomposed with sodium hydroxide in ethyl alcohol–benzene solution, and the liberated bromide is oxidized to bromate, which is determined iodometrically.

For fumigating fruits for the destruction of the larvae and eggs of the fruit fly, a method for the determination of ethylene dibromide in air (*2*) involved absorption in ethyl alcohol and decomposition with sodium hydroxide to yield one mole of inorganic bromide from each mole of ethylene dibromide. The liberated bromide was estimated by the Volhard thiocyanate method.

A number of methods for determining ethylene dibromide in fumigated fruit have been published, but a reasonably rapid and accurate method has not been available. Sinclair and Crandall (*3*) determined total bromide iodometrically after hydrolysis, ashing, and oxidation to bromine. Recoveries of ethylene dibromide added to orange peel or pulp varied from 93 to 105%. However, inorganic bromide liberated in the fruit after fumigation was also included. Tanada, Matsumoto, and Scheuer (*4*) avoided such interference by distilling the ethylene dibromide into ethyl alcohol, refluxing the ethanolic solution with potassium iodide, and titrating the liberated iodine. However, their recoveries of ethylene dibromide added to a number of fruits were low (70 to 79%).

The method described in this paper involves refluxing the sample with water, using a special reflux head in which benzene extracts the ethylene dibromide from the condensate before it is returned to the flask. After decomposition with sodium hydroxide in ethyl alcohol–benzene, the solution is evaporated to dryness. Besides sodium hydroxide and bromide, the residue probably contains traces of sodium salts of acids derived from volatile esters, but these do not interfere if the residue is adequately heated. The Volhard thiocyanate method is not sufficiently sensitive for analysis of fumigated fruit, and instead the bromide is oxidized to bromate with sodium hypochlorite. Excess hypochlorite is reduced with sodium formate and the bromate is determined iodometrically. This procedure is based on that of Alicino, Crickenberger, and Reynolds (*1*), and has the advantage that six equivalents of iodine are liberated for each mole

of bromide. It is also specific for bromide, and any chloride formed from organic chlorine compounds in spray residues will not interfere.

Reagents

Benzene, thiophene-free.

Dow Corning Antifoam A, 0.5 gram suspended in 500 ml. of benzene. The suspension is shaken before use.

Ethyl alcohol, redistilled.

Sodium hydroxide, 2*N*.

Sodium dihydrogen phosphate, 20 grams of $NaH_2PO_4.2H_2O$ in 100 ml. of solution.

Sodium hypochlorite. A commercial solution is analyzed for available chlorine and diluted to 10% (w./v.) sodium hypochlorite.

Sodium formate, 50 grams in 100 ml. of solution.

Potassium iodide, 25 grams in 100 ml. of solution.

Ammonium molybdate, 5 grams in 100 ml. of solution.

Sulfuric acid, 9*N*.

Starch, 1 gram soluble starch and 20 grams of sodium chloride in 100 ml. of solution.

Sodium thiosulfate, 0.005*N*.

Procedure

The sample (100 to 150 grams) is weighed into a 500-ml. flask with a B34 socket, and 100 ml. of water and 10 ml. of benzene containing Antifoam A are added. (Ŧ 19/22, 24/25, and 34/28 joints can be used instead of B19, B24, and B34.) The apparatus is set up as in Figure 1 and water is poured down the condenser so that it rises 8 to 10 cm. in the collection tube. The solution is kept boiling at a brisk rate for 90 minutes. Most of the benzene collects above the aqueous layer in the collection tube within 5 minutes.

The benzene layer is carefully separated from the aqueous layer and run into a 150-ml. conical flask with a B24 socket. The condenser and collection tube are washed down with 2 ml. of benzene, which is added to the flask. After addition of 25 ml. of ethyl alcohol and 1 ml. of aqueous sodium hydroxide, the flask is boiled under reflux for 15 minutes. The condenser is then removed and the solvents are evaporated to dryness on the hot plate. The flask is gently heated over a Bunsen burner in order to remove any traces of solvents. The heating is continued until no further decrepitation occurs and the residue is dry. Slight charring usually occurs in this stage.

The flask is allowed to cool, and 20 ml. of water are added, followed by 10 ml. of sodium dihydrogen phosphate and 5 ml. of sodium hypochlorite solution. The mixture is brought to the boil and 5 ml. of sodium formate solution are added. The solution is then cooled to room temperature, and 1 ml. of potassium iodide, 1 drop of ammonium molybdate, and 10 ml. of 9*N* sulfuric acid are added in succession. The liberated iodine is titrated with thiosulfate, using 5 drops of starch solution as indicator.

A blank titer, which should be negligible with pure reagents, can be obtained by adding to a 150-ml. flask 20 ml. of water, 1 ml. of sodium hydroxide, 10 ml. of sodium dihydrogen phosphate, and 5 ml. of sodium hypochlorite solution, and proceeding as in the determination.

Mg. of ethylene dibromide = $31.31 \times 0.005 \times (T_D - T_B)$ where T_D and T_B are the titers obtained for the determination and blank, respectively.

Table I. Recovery of Ethylene Dibromide

Fruit	Added, Mg.	Recovery, %
Orange	0.100	103, 103, 103
	0.500	99.8, 99.8, 99.8
	2.00	99.0, 99.0, 99.0
	10.00	98.9, 98.9, 99.1
	15.00	99.0, 99.3, 99.4
Apple	5.00	99.4, 99.8, 99.8
Banana	5.00	99.8, 99.8, 99.8
Grapefruit	5.00	100.0, 100.0, 100.2
Lemon	5.00	99.4, 99.8, 100.0
Papaw	5.00	100.2, 100.6, 100.6
Pineapple	5.00	100.0, 100.2, 100.2
Tomato	5.00	100.0, 100.6, 101.2

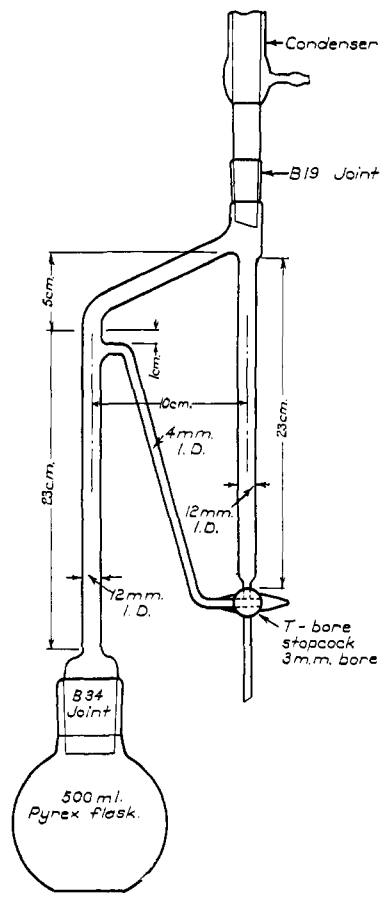

Figure 1. Apparatus for continuous distillation and extraction of ethylene dibromide

Recovery Tests

Amounts of ethylene dibromide varying from 0.1 to 15 mg. were dissolved in benzene and added to the samples of fruit. The determinations were made as described (Table I).

For 0.5 to 15 mg. of ethylene dibromide recoveries ranged from 99 to 101%. For smaller quantities higher recoveries were obtained.

These recovery tests do not give a complete measure of the adequacy of the method for fumigated fruit, as the ethylene dibromide added at the beginning of the determination has not penetrated appreciably into the fruit and is more readily distilled. Unfortunately, the recovery of ethylene dibromide absorbed in fumigation cannot be estimated directly, but the adequacy of the 90-minute reflux was tested by continuing the boiling for further periods after addition of fresh benzene to the collection tube. A number of samples were refluxed for a total period of 4 hours. From fumigated samples of oranges containing approximately 5 mg. of ethylene dibromide the quantity recovered on further boiling was less than 0.01 mg.

The possibility of loss of ethylene dibromide from fumigated fruit by incomplete condensation was tested by attaching a U-tube containing benzene to the top of the condenser. All the ethylene dibromide (about 13 mg.) was found in the collection tube and none in the U-tube. This result is obtained only when the benzene is added initially to the flask (instead of the collection tube); this may indicate that the presence of benzene in the condenser is necessary to trap all the ethylene dibromide.

Hydrolysis of ethylene dibromide in the boiling acid medium before distillation was tested by adding about 10 mg. in a sealed tube to the flask containing pieces of fruit, breaking the tube, and refluxing for 60 minutes, during which benzene was absent from the flask and collection tube. A U-tube containing benzene was attached to the top of the condenser to trap any ethylene dibromide that might escape. Subsequently benzene was added to the collection tube and the determination was made in the usual way. Destruction of ethylene dibromide was shown to be negligible.

Sampling

Where the average concentration of ethylene dibromide in whole fruit is required, careful attention must be paid to sampling. The concentration in the rind of fumigated oranges is about 20 times the concentration in the flesh. It is therefore necessary to ensure that the sample for analysis (which may include one segment from each of a num-

ber of fruits) contains rind and flesh in the same proportion as the whole oranges. The authors' samples are composed of eighth segments, each of which is obtained by making two longitudinal cuts at right angles, followed by an equatorial cut. All oranges are oriented similarly for corresponding cuts, and the personal bias is corrected by selecting left and right pieces alternately for individual oranges.

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STARCH GRANULE FORMATION

Development of Starch Granules in Corn Endosperm

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With the aid of the electron microscope, starch granules are observed in corn endosperm as early as 4 days after pollination. Granules seem to originate in amyloplasts, which often rupture as the granules mature. Central cavities are rarely observed in undried granules and lamellae are seen in less than 15% of the granules. Granules, approximately 15 cells from the aleurone, are largest and the size decreases toward either the center or periphery.

STARCH GRANULES have been examined extensively with the light microscope, and their origin and development have been described (*1, 3, 4, 6, 11, 14*). Most investigators believe that starch granules originate in the amyloplasts (*4, 6, 9–11, 15, 16, 22, 25*). Weier (*22*) and Guilliermond (*10*) consider that amyloplasts are derived from the chrondriosomes and Badenhuizen (*4*) considers that the chrondriosomes are filiform. Duvick (*7*) and Shaw (*20*) conclude that amyloplasts contain phosphorylase, an enzyme needed for the synthesis of starch.

Duvick (*6*) describes large-knobbed filaments (plastids) which give rise to starch granules. The largest knob in any given cell is the first to form starch, but not all such knobs form starch.

Numerous previous investigators consider starch granule formation to begin some 10 to 15 days after pollination (*4, 6, 11, 12, 14, 25*).

Badenhuizen (*4*) assumes that starch chains of various lengths are present in the amyloplast at the time of starch granule formation. When a given number of chains of sufficient length are available, they precipitate, thus forming the young granule. The region of the amyloplast, immediately surrounding the granule, is thus depleted of carbohydrates. As the supply of carbohydrate material to the amyloplast is not constant, the whole amyloplast may become exhausted. This deficiency is overcome, possibly, by diffusion. Badenhuizen concludes that these processes could explain the origin of lamellae.

Another less satisfactory theory considered by several workers (*2, 13*) is that a starch coacervate crystallizes *in toto* to produce a granule. Rhythmic crystallization in the droplet could lead to lamellae.

Reichert (*17*) describes fissures or central cavities in mature corn starch granules and such cavities were observed in ultrathin sections by Whistler and Turner (*23*).

Methods

Corn kernels were obtained from common yellow field corn, single-cross Wf-9x38-11, grown on the Purdue University Agronomy Farm and pollinated by hand.

The methods of collecting, sorting, transporting, subdividing, and killing were those used by Sass (*19*). Kernels were collected every other day starting at 4 days after pollination and ending at 72 days.

Samples were removed from the killing solution and placed in an osmium tetroxide solution for 6 hours. This solution consisted of 1% osmium tetroxide in a veronal-acetate buffer at pH 7.4. After removal from the osmium tetroxide solution, samples were washed in cold distilled water for 1 hour.

For dehydration, samples were stored successively for at least 8 hours in 10, 25, 50, 75%, and anhydrous dioxane, with three changes of the latter.

Samples were impregnated and embedded in methacrylate (20% methyl methacrylate in *n*-butyl methacrylate, containing 0.4%, 2,4-dichlorobenzoyl peroxide as a catalyst). For impregnation, samples were stored successively for 8-hour periods in 10, 25, 50, and 75% solutions of methacrylate in dioxane and finally in 100% methacrylate, with three changes of the latter. After the final 8-hour storage, the mixture was polymerized in No. 0 gelatin capsules at 45° to 48° C. for 48 hours.

Sections were made with a wedge-