graphed in the usual manner. Radioactivity was found in the area of the tissue components in these chromatograms. It therefore appears likely that the secondary spots are due to dalapon occluded in the tissue components. In no case were the secondary spots associated with areas containing the reference standards.

In the commercial production of cottonseed oil, the oil is generally removed from the kernel by pressing or solvent extraction The resulting crude oil is then purified by a caustic extraction procedure. Under these conditions, some of the dalapon is carried over with the oil during extraction. The caustic treatment probably then removes the dalapon from the oil during the cleanup procedure. This was checked in the laboratory by adding radioactive dalapon to cottonseed oil and then processing the oil through the caustic cleanup procedure. Radiochemical analysis of the oil indicated that the dalapon could be removed by this procedure.

These studies indicate that dalapon can be absorbed by the cotton plant and will accumulate in the actively growing tissues. The dalapon does not appear to be metabolized to any significant extent in the plant but remains in the tissues in a form which can readily be removed by a simple water extraction. The material extracted from the plant can be identified as dalapon by paper chromatography and chemical analysis. In commercial production dalapon associated with the cottonseed oil will be removed during the cleanup procedure.

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INSECTICIDE STABILITY

Stability of Malathion in Small Package Formulations

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Four methods of analysis for malathion have been compared. A modification of the original Norris spectrophotometric procedure based on measurement of copper dimethyl dithiophosphate is the most reliable for general-purpose pesticides. A survey of 100 pesticide samples containing malathion showed that over one half were unstable upon storage for one year. Reasons for this instability are discussed and the necessity for better packaging is suggested.

MALATHION, *S*-[1,2-bis(ethoxycarbonyl)ethyl]*O,O*-dimethyl phosphorodithioate is frequently used in general-purpose pesticide mixtures because of its low mammalian toxicity and wide insecticidal effectiveness. Although malathion has been marketed since 1952, instability in formulations was not a serious problem until 1957. The sudden appearance of numerous samples, particularly dust formulations, which were seriously below guarantee for malathion was first reported in California (*7*). These deficiencies could be attributed to no single cause, but to a combination of independent factors. The more important factors were: improper methods of formulation and inadequate quality control, the use of inert diluents and carriers which promoted decomposition of malathion, overly severe conditions of storage and a gradual build-up of overage samples on retail dealers' shelves, improper packaging, and inadequate analytical methods.

The present investigation is concerned with an examination of 100 samples collected at random by inspectors of the Department of Agriculture and Markets in the State of New York during 1957 and 1958. These are all small packages, and represent a large number of general-purpose mixtures, so that the results reported here do not necessarily apply to bulk packages for commercial use. Among the uncontrolled variables were age, method of formulation, and composition of both inert and active ingredients, so that the conclusions as to the interrelation among packaging, formulation, and quality are developed from a pragmatic point of view. Because the validity of the chemical methods has been questioned, four analytical procedures were compared in order to establish the soundness of the test methods.

Experimental Procedures

Method 1. Spectrophotometric Method for Copper Dimethyl Dithiophosphate.

REAGENTS. Acetonitrile, anhydrous. Bromobenzene, technical grade, redistilled if not colorless.

Ethyl alcohol, USP anhydrous, or denatured formula 2B.

Alkaline Salt Solution. Dissolve 40 grams of NaCl (technical grade) in 500 ml. of copper-free water. Add 0.10 gram of NaOH or 25 ml. of a 0.1*N* solution. Dilute to 2 liters with copper-free water. Deionized water or water redistilled from glass (*3*) is satisfactory. Do not use distilled water from a block tin condenser because it contains an appreciable amount of copper.

Sodium Methylate Solution. To 78 grams of anhydrous ethyl alcohol add 2.0 grams of copper-free water and 1.3 grams of powdered sodium methylate (Matheson). Shake well to dissolve, and protect from moisture and carbon dioxide. A small amount of carbonate usually settles out, but does no harm.

Hydrochloric acid, 2.8*N*. Dilute 25 ml. of concentrated HCl to 100 ml. with copper-free water.

Copper sulfate pentahydrate, 1% solution.

Malathion Stock Solution, 1 mg. per ml., approximately. Use purified material in sealed ampoules supplied by the American Cyanamid Co. Weigh about 100 mg. by difference into a 100-ml. volumetric flask. Dilute to volume with acetonitrile. Store in a cool dark place.

Malathion Standard Solution, 0.1 mg. per ml., approximately. Dilute a 10-ml. aliquot of the stock solution to 100 ml. with anhydrous ethyl alcohol. Store in a cool dark place for not longer than 1 week.

PROCEDURE. Weigh a sample of 0.2 to 5.0 grams containing about 100 mg. of malathion. Weigh liquid formulations in a small beaker or a weight buret and transfer directly to a 100-ml. volumetric flask. Place dusts and wettable powders in a glass-stoppered flask and add exactly 100 ml. of acetonitrile. Shake well and allow several hours for the solids to settle. Transfer a 10-ml. aliquot to a 100-ml. volumetric flask and dilute to volume with anhydrous ethyl alcohol. Transfer a 10-ml. aliquot of the alcoholic extract into a 125-ml. separatory funnel containing 40 ml. of ice-cold bromobenzene. Add 40 ml. of cooled alkaline salt solution. Shake vigorously for 1 minute, separate, and draw off the lower layer into a 50-ml. glass-stoppered volumetric flask. Wash the aqueous layer once with 5 ml. of bromobenzene by shaking for 15 seconds, adding it to the main solution. Adjust the volume of bromobenzene to exactly 50 ml. Add 1 to 2 grams of anhydrous sodium sulfate, shake well to remove traces of water, and keep the solution in an ice bath.

To a clean, dry 125-ml. separatory funnel add 20 ml. of bromobenzene and 10 ml. of sodium methylate solution. Mix and then add 20 ml. of the cold anhydrous bromobenzene extract. Shake the funnel vigorously for at least 1 minute. Add 30 ml. of cold alkaline salt solution and shake again for 1 minute. Allow the phases to separate, and discard the lower layer. Wash the upper layer with 10 ml. of bromobenzene and 1 ml. of 2.8*N* HCl. Shake 30 seconds, separate, and discard the lower layer. Again shake the aqueous layer with 10 ml. of bromobenzene and repeat if the lower layer is not colorless. To the purified aqueous solutions of dimethyl dithiophosphoric acid add exactly 10 ml. of bromobenzene and 1 ml. of copper sulfate solution. Shake the funnel vigorously for 1 minute, separate, and draw off most of the lower layer into a test tube containing 1 to 2 grams of anhydrous sodium sulfate. Shake well to dry and decant a portion of the solution into a 1-cm. cuvette. Measure the absorbance at 418 mμ against bromobenzene set at 100% transmittance. To construct a calibration line prepare a 50-ml. bromobenzene extract from the ethanolic malathion standard solution in the same way as from the unknown solutions. From this extract take aliquots of 0, 5, 10, 15, and 20 ml. for the hydrolysis with sodium methylate, adjusting the volume of pure bromobenzene in each case to give a total of 40 ml. of bromobenzene.

Method 2. Alcohol–Ferric Oxidation Method. The reagents and procedures are given in the manual of the American Cyanamid Co. (*2*). As copies have been widely distributed, detailed directions are not repeated here.

Method 3. Organic Phosphorus Method. Prepare an acetonitrile–ethyl alcohol solution of malathion exactly as in Method 1. Transfer a 10-ml. aliquot of the second dilution to a 250-ml. Erlenmeyer flask and treat with 2 ml. of 1*N* NaOH. Boil off the solvent on a steam bath in a well ventilated hood. Cool, and add 3 ml. of concentrated H_2SO_4 and 10 ml. of 30% H_2O_2. Digest on a hot plate to fumes of SO_3, adding 1 ml. of $HClO_4$ if the liquid does not clear promptly. Cool, add 25 ml. of water, neutralize with 1 + 1 ammonia, and then make just acid to phenolphthalein with 1*N* H_2SO_4. Transfer to a 100-ml. volumetric flask and dilute to volume. A 5-ml. aliquot from this solution is a convenient amount for the phosphomolybdate determination according to the directions of Pons, Stansbury, and Hoffpauir (*5*).

Method 4. Total Sulfate Method. Oxidize a sample containing about 0.2 gram of malathion with bromine-KBr and determine gravimetrically as barium sulfate (*6*).

Comments on Procedures

The original spectrophotometric determination of malathion in carbon tetrachloride solution was designed for residue analysis (*4*). For composition analysis, nonpolar carbon tetrachloride is much too weak as extractant, and forms unstable solutions of the yellow copper chelate. Carbon tetrachloride

Table I. Malathion Found by Four Methods of Analysis

Method 1, Copper Salt	Method 2, Ferric Oxidation	Method 3, Molybdate	Method 4, Sulfate
50.6	50.3	49.6	55.4
50.1	50.6	57.8	56.0
47.1	48.6	45.1	48.0
13.9	14.4	14.4	14.5
12.2	12.4	12.2	12.6
2.3	2.3	2.3	2.4
1.6	1.7	1.7	1.7
1.7	1.7	1.8	2.0
0.0	(0.1)	4.0	6.0
4.0	(2.2)	4.5	...
6.5	(4.1)	6.2	...
5.1	(4.2)	5.1	7.1
8.2	(1.0)	8.2	8.5
2.6	(1.3)	2.7	4.3

Table II. Decomposition in Storage at Room Temperature of Several Types of Malathion Formulations

Formulation	% Malathion Found after Storage: None	6 months	12 to 15 months	20 months
4% dust, 1760	2.9	2.3	1.7	1.4
4% dust, 1400	2.3	1.2	0.7	0.6
4% dust, 2094	4.5	...	3.9	...
4% dust, 846	2.2	1.5	0.9	0.8
6% dust, 1328	4.8	4.5	...	3.7
12% dust, 1953	8.2	5.8	...	3.5
9% liquid, 1438	9.0	7.5	7.2	6.9
12.5% liquid, 808	12.0	10.8	9.1	8.9
15% liquid, 1955	13.9	12.5	12.2	8.2
24% liquid, 2141	25.9	26.0	24.8	...
50% liquid, 220	47.5	43.7	...	36.8
50% liquid, 1881	47.3	39.5	...	32.8
55% liquid, 1692	53.3	50.8	45.3	...
1% aerosol 2019	1.2	...	1.2	...
2% aerosol 2020	2.7	...	2.5	...
1% dry bait, 84	2.3	2.3	2.3	...
2% dry bait, 76	1.6	1.5	1.5	...
2% dry bait, 08	1.5	1.4	1.4	...
2% dry bait, 925	1.9	1.4	1.2	...

Table III. Changing Quality of Malathion Samples Collected in Spring of 1958

Quality Group	% Samples within Each Group Examined in May 1958	October 1959	March 1959
A. As guaranteed	57	26	17
B. Deficient, up to 20% below guarantee	26	36	36
C. Seriously deficient, more than 20% below guarantee	17	38	47

Table IV. Relation between Formulation and Quality of Stored Malathion Samples

Quality Group	Number of Samples in Each Formulation: Aerosol	Liquid	Dry bait	Dust
A. As guaranteed	5	16	7	9
B. Deficient, up to 20% below guarantee	0	14	9	8
C. Seriously deficient, more than 20% below guarantee	0	8	2	22

Table V. Effect of Packaging on Quality of Malathion Samples

Type of Container	Number of Samples in Each Quality Group: A, as guaranteed	B, deficient	C, seriously deficient
Tin	5	0	0
Glass	21	13	4
Plastic	3	2	2
Cardboard	12	16	22
Total	41	31	28

has been displaced by acetonitrile as extractant and by bromobenzene as stabilizing solvent for copper dithiophosphate. A recent revision of the alcohol–ferric oxidation method (*1*) replaces carbon tetrachloride with cyclohexane as solvent. Cyclohexane is satisfactory for this method, but inconvenient for use with Method 1, because it is lighter than water. Bromobenzene was selected from several stabilizing solvents having a density greater than 1. Chloroform is not a stabilizing solvent and is less satisfactory than carbon tetrachloride (*4*).

While rapid fading of the yellow color within a few minutes in diffuse daylight has not been observed in our laboratory, appreciable changes occur within a half hour. Consistently lower results have been obtained in summer than in winter daylight when less ultraviolet was present. Photosensitivity probably contributes to the lower precision found with this procedure. A series of comparative analyses on 19 samples yielded a standard deviation of ±0.30% by barium sulfate, ±1.3% by organic phosphorus, and ±2.43% by the original long carbon tetrachloride method (*4*). The decomposition of copper dimethyl dithiophosphate in carbon tetrachloride is complicated by a photochemical reaction, and a dark reaction which does not proceed at a measurable rate below 25° C. unless it is initiated by the photochemical reaction. Even a few seconds of illumination with a mercury vapor light is sufficient to start the second reaction, which then proceeds in darkness even at temperatures down to 0° C. Above 25° C. decomposition occurs without photochemical activation. By using low actinic glassware and keeping all solvents in an ice bath, it was possible to prepare a copper chelate in carbon tetrachloride solution with no change in absorbance for 1 hour.

The alcohol–ferric oxidation method is much simpler than the longer first method. However, the stepwise removal of each interfering impurity as originally proposed by Norris, Vail, and Averell (*4*) is the only valid method for many general-purpose mixtures. Carbamates are known to interfere in the short method. Captan, which is very commonly incorporated into malathion formulations, also interferes with Method 2. The last six samples in Table I contain captan. The figures in the third column are obviously low (enclosed in parentheses). The phosphomolybdate and sulfate methods agree well with the copper chelate methods but are less specific. Method 1 has been adopted for all further analyses reported in this paper.

Instability of typical malathion samples is shown in Table II and summarized in Table III. Aerosols and dry baits appear to be stable, although the latter are often below guarantee when first examined. In Tables IV and V the relationship among quality, formulation, and container type is indicated. Quality, formulation, and container type are not independent variables, so that unwarranted conclusions should not be drawn from these data. Because malathion is known to hydrolyze in moist air, the use of vapor-tight containers to maintain product stability is recommended.

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