A number of common insecticides and drugs have been tested for possible interference in this procedure. Of the materials tested, the only substance which interfered in the analysis was dinitro-*o*-cresol (DNOC). This finding is not surprising in view of the similarity between the dinitro-*o*-cresol molecule (which is really a disubstituted *p*-nitrophenol) and that of *p*-nitrophenol. Dinitro-*o*-cresol produces a yellow color in the reaction which has the effect of a high blank value. The magnitude of this interference is not very great even at low *p*-nitrophenol values and is undetectable at levels above 25 γ.

Studies of exposed workers carried out during the 1958 spray season have shown that, when measured by the present method, urinary *p*-nitrophenol excretion is significantly more sensitive than blood cholinesterase in measuring absorption of parathion. This statement is true even under optimal conditions for usage of blood cholinesterase values—when the individual's pre-exposure enzyme level is known. The results of these studies will be reported in detail elsewhere.

The present method for determination of urinary *p*-nitrophenol content can be carried out in about the same period of time as required for blood cholinesterase determination by the Michel procedure (*4*). It is not proposed that measurement of urinary *p*-nitrophenol be substituted for blood cholinesterase determination in all cases involving exposure to parathion or EPN. In some instances, one or the other analysis may be preferable; in other cases, it may be informative to carry out both tests.

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SOIL FUMIGANT DETERMINATION

Extraction and Determination of Ethylene Dibromide in Soils

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A method utilizing vacuum distillation and catalytic oxidation is described for the determination of ethylene dibromide in soils. Recovery is reported in the range of about 82 to 0.2 mg. of ethylene dibromide per 50 grams of soil. Moisture and organic matter affect recovery.

IN STUDIES INVOLVING penetration and phytotoxic effects of ethylene dibromide (EDB) in soils it is necessary that an analytical technique be developed to determine the low concentrations of the fumigant usually encountered in soil studies.

Previous studies were either lengthy and prone to both oxidizing and reducing side reactions (*1*), or lacking in sensitivity at low concentrations (*4*).

This investigation is an attempt to develop a simple but sensitive technique for the determination of ethylene dibromide in soils. This determination is carried out in three stages: recovery of ethylene dibromide from the soil sample, conversion of ethylene dibromide to inorganic bromide, and determination of inorganic bromide.

Reagents and Apparatus

2,4,5,7-Tetrabromofluoroscein, sodium salt (eosin Y), 0.1% solution.

The catalytic oxidation of ethylene dibromide is based on a modification of the method described by Lubatti and Harrison for the determination of methyl bromide (*3*). The combustion or reaction chamber is shown in Figure 1. Two 15-mm. lengths of 20-gage platinum wire are introduced into the stopper, *B*, by means of capillaries, *C* and *C'*; and sealed in airtight with Apiezon W wax. To the lower ends of the thicker platinum wires are connected 16 mm. of coiled 30-gage platinum wire which serves as the oxidizing surface. During the catalytic oxidation, the leads of *C* and *C'* are connected to an electrical circuit through a variable transformer. In this study, 5 volts were sufficient to bring the filament to a bright red glow. The 14/35 taper attached to *B* is used to connect the reaction chamber to the vacuum manifold for the evaluation of the flask. The vacuum system used maintained a pressure of 1 to 5 microns as measured with a McLeod gage.

Procedure

The purity of ethylene dibromide used in the study was determined by adding, with a micropipet, known amounts of ethylene dibromide to flask *A*, then immediately immersing the flask in a dry ice–acetone bath. To the frozen system were then added 2 ml. of 0.8*N* sodium hydroxide and 1.5 ml. of 30% hydrogen peroxide. Stopper *B* was placed on the flask and the system was completely evacuated while still immersed in the freezing bath. The system was then removed from the vacuum manifold, was thawed, and sufficient voltage was placed across the filament to produce a bright glow. The combustion period consisted of alternate closing and opening the circuit at 1-minute intervals, to prevent overheating, for about 15 minutes. Stopper *B* was then removed and rinsed. The solution in flask *A* was then directly titrated with either 0.01*N* or 0.025*N* silver nitrate using a microburet and following the eosin Y method of Kolthoff and Sandell (*2*).

At two concentration levels of 21.7 and 43.4 mg. of ethylene dibromide added to the reaction flask successive determinations indicated a recovery of 96.3, 96.5, and 96.7% and 95.5, 96.6, and 96.7%, respectively. Hence under

Table I. Recovery of EDB from Air-Dried Mineral Soils

Columbia Fine Sandy Loam				Yolo Silty Clay			
EDB dosage, mg.	Glycerol added, ml.	EDB Recovered Mg.	EDB Recovered %	EDB dosage, mg.	Glycerol added, ml.	EDB Recovered Mg.	EDB Recovered %
82.4	20	81.9	99.4	82.4	20	81.1	98.4
	20	80.4	97.6		20	80.7	97.9
	20	81.7	99.2		20	80.7	97.9
	0	70.6	85.7		0	72.0	87.4
	0	70.7	85.8		0	68.5	83.0
2.14	20	2.09	97.7	2.14	20	2.10	98.1
	20	2.04	95.3		20	2.14	100.
	20	2.08	97.2		20	2.07	96.7
0.256	20	0.235	91.8	0.256	20	0.216	84.4
	20	0.226	88.9		20	0.226	88.3
	20	0.226	88.9		20	0.220	85.0
	0	0.197	77.0		0	0.188	73 4
	0	0.196	76.6		0	0.189	73.8

Table II. Recovery of EDB from Air-Dried Organic Soil

(Staten peaty muck)

EDB Dosage, Mg.	EDB Recovered, Mg.	Recovery, %
82.4	79.4	96.4
	79.5	96.5
2.14	1.76	82.2
	1.76	82.2
0.188	0.132	70.2
	0.140	74.4

Table III. Recovery of EDB at Field Capacity

(Yolo silty clay)

EDB Dosage, Mg.	EBD Recovered, Mg.	Recovery, %
82.4	78.8	95.7
	79.1	96.0
	79.3	96.3
3.36	2.99	89.1
	2.95	87.8
	3.02	89.8
0.217	0.178	82.5
	0.171	78.8
	0.171	78.8

experimental conditions the ethylene dibromide was considered 96.4% pure. The above value also includes the efficiency of the oxidation procedure for the analysis of ethylene dibromide.

The extraction of ethylene dibromide from air-dried soils was as follows. Two milliliters of 0.8*N* sodium hydroxide and 2 ml. of 30% hydrogen peroxide were added to flask *A*. The flask was then immersed in a dry ice–acetone bath, connected to the vacuum distillation manifold, and evacuated. Flask *A* remained in the freezing bath and acted as the cold trap throughout the extraction period. In a similarly modified 125-ml. Erlenmeyer flask a given amount of ethylene dibromide was placed at the bottom and 50 grams of sieved, air-dried soil were added. The flask was immersed in a dry ice bath and 20 ml. of technical grade glycerol were added. The sample flask was then connected to the vacuum distillation manifold through a reflux condenser and evacuated while frozen out. The distillation system was then closed from the pumps and the cold bath was removed and replaced by a sand bath maintained at 150° C. for 2 hours. At 20- to 30-minute intervals, a stopcock was closed to isolate the sample and the connecting system between the flasks was open to the pumps to remove noncondensable gases. Care must be exercised when returning the sample to distillation, after pumping out noncondensable gases, to prevent the blowing of the sample into the vacuum lines. After 2 hours of distillation, flask *A* was removed from the system and stoppered with *B*, and the condensate was oxidized and titrated as previously described.

Field capacity samples were prepared by adding adequate water to 50 grams of air-dried soil and allowing the mixture to equilibrate for 24 hours. The ethylene dibromide was then added to the moist soil, and the flask was stoppered and immersed in a dry ice bath. When the contents were frozen, 20 ml. of glycerol were added and the flask was attached to the vacuum manifold through a reflux condenser and evacuated. Because of the greater amount of vapor distilled over to flask *A*, field capacity soils were heated at 150° C. for 3 hours. The remaining procedure was the same as with air-dried soils.

The ethylene dibromide was added to the soils either by micropipets or as an aqueous solution of the fumigant. The actual amount of ethylene dibromide delivered to the soil by both methods was determined by catalytic combustion analysis.

Results and Discussion

Table I shows the recovery of ethylene dibromide from two air-dried mineral soils and the effect of glycerol on the recovery at high and low rates of application. The beneficial effect of glycerol is ascribed not only to its refluxing action during distillation, but also to its displacement of sorbed ethylene dibromide from the soil surface in the air-dried systems. The recovery of ethylene dibromide from an organic soil, Staten peaty muck (40% organic matter), is shown in Table II. At the lower rates, a definite decrease in recovery is noted. This relative inability to recover ethylene dibromide from this organic soil indicates either a rapid interaction between the organic fraction and the fumigant or that a modification of the procedure is necessary in organic systems. The data from soil blanks did not significantly alter the results of the procedure and hence are not reported.

Of interest is the applicability of this determination in soils supplied with adequate moisture for plant growth. Table III shows the determination of ethylene dibromide in soils at field capacity.

These data indicate that moisture does decrease the apparent recovery of ethylene dibromide when compared to soil analyzed in the air-dried state. This decrease is ascribed mainly to the accumulation of moisture in the cold trap during distillation of the wetted sample. Data obtained in this laboratory indicate that the presence of excess water in the reaction chamber definitely decreases the efficiency of the catalytic oxidation reaction. The magnitude of this decrease is in the order of 10 to 15% in the presence of 10 ml. of excess water. The detrimental effect of moisture is probably due to the hindering action of moisture on the vaporization of ethylene dibromide from its liquid and solution phases.

The data of this study indicate that vacuum distillation coupled with the catalytic oxidation reaction shows promise as a method of determining

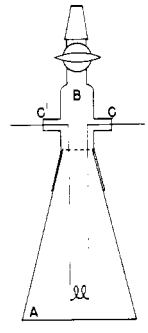

Figure 1. Reaction flask used in catalytic oxidation of ethylene dibromide

A. 125-ml. Erlenmeyer flask with 24/40 taper
B. 4-mm. stopper with 14/35 taper and mounted platinum filament

ethylene dibromide in dry and moist soils at dosages approaching field applications of ethylene dibromide (4 to 6 gallons per acre). At present, this method shows particular usefulness in dry systems where the complicating factor of moisture is minimized.

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INSECTICIDE RESIDUES

Persistence of Dimethoate and Metabolites Following Foliar Application to Plants

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Dimethoate [O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate] is known to be effective as a systemic insecticide following foliar application. Analyses were made of surface and absorbed residues following foliar treatment of corn, cotton, pea, and potato plants with radioactive dimethoate. The insecticide was rapidly absorbed and decomposed both on the surface and inside the foliage by phosphorothionate oxidation and hydrolysis. Only trace amounts of dimethoate and its oxygen analog were present 32 days after treatment. Of the five identified hydrolysis products, the predominant one from near mature peas was phosphoric acid and from the other plants used as seedlings was O,O-dimethyl S-carboxymethyl phosphorothiolate on the surface and O-methyl O-hydrogen S-(N-methylcarbamoylmethyl) phosphorodithioate within the leaf tissue. Limited studies were also made on the persistence of the N-ethyl analog of dimethoate.

DIMETHOATE [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate] is active as a systemic insecticide for grub control in cattle (*6*). This same chemical (designated as Rogor in England and Europe) has been used for insect control on plants for several years (*12, 13*). It displays good systemic activity following foliar application (*2*) and as a side dressing in the soil when formulated on granules (*11*). The oxygen analog of dimethoate [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolate] has been demonstrated in bean and cherry plants after root and foliar dimethoate treatment, respectively (*12, 13*). Studies on the mammalian metabolism of dis methoate were reported in a previou paper (*5*).

The present investigation concerns the persistence and metabolites associated with the use of dimethoate as a systemic insecticide for treating plant foliage.

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Methods

Dimethoate Derivatives Utilized. In addition to dimethyl phosphoric, phosphorothioic, and phosphorodithioic acids, the following compounds related to dimethoate were studied: *O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolate, $(CH_3O)_2P(O)SCH_2C(O)NHCH_3$, the oxygen analog of dimethoate; *O,S*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolate, $(CH_3O)(CH_3S)P(O)SCH_2C(O)NHCH_3$, the *S*-methyl isomer of dimethoate; *O,O*-dimethyl *S*-carboxymethyl phosphorodithioate, $(CH_3O)_2P(S)SCH_2C(O)OH$, the thio-carboxy derivative of dimethoate; *O,O*-dimethyl *S*-carboxymethyl phosphorothiolate, $(CH_3O)_2P(O)SCH_2C(O)OH$, the oxy-carboxy derivative of dimethoate; *O*-methyl *O*-hydrogen *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate, $(CH_3O)(HO)P(S)SCH_2C(O)NHCH_3$, the des-methyl derivative of dimethoate; and *O,O*-dimethyl *S*-(*N*-ethylcarbamoylmethyl) phosphorodithioate, $(CH_3O)_2P(S)SCH_2C(O)NHC_2H_5$, the *N*-ethyl analog of dimethoate which is designated as CL 18,706.

Synthesis. Radioactive dimethoate was prepared, purified, and characterized as previously described (*5*) using isotopic exchange to obtain the phosphorus-32 pentasulfide intermediate (*4*). The radioactive product was identical in infrared spectrum with highly purified dimethoate and had a specific activity of about 25 mc. per gram. Radioactive CL 18,706 was prepared in 60% yield by the same procedure as for dimethoate, except that *N*-ethyl α-chloroacetamide replaced *N*-methyl α-chloroacetamide. The radioactive product was identical in chromatographic properties and infrared spectrum with a highly purified sample of known CL 18,706.

Volatilization. The volatilization rates for radioactive dimethoate from cotton leaves and the surfaces of metal, wood, glass, plastic, rubber, Masonite, and Pressboard were determined. Approximately 5 γ of radioactive dimethoate in 0.1 ml. of acetone were applied to the upper leaf surface of each cotton plant in a spot 0.5 inch in diameter, or to comparable areas of the other surfaces. The treatments were replicated six times and the materials were held in a greenhouse at about 28° C. Radioassays (*7*) were made periodically after treatment.

The volatilization rate for radioactive