Table III. Percentage of 2,4-D Metabolite Present in Urine and Organs of Rats Fed 2,4-D, by Countercurrent Extraction Method

Sample	Metabolite, %
Urine (9)[a]	0.25 ± 0.10
Liver extract, 1-mg. dose (2)	3.63
Liver extract, 80-mg. dose (1)	6.10
Blood extract (1)	1.13
Kidney extract (1)	1.33
Lung extract (1)	0.71

[a] Number of samples analyzed.

at high (80-mg.) and low (1-mg.) doses was observed, a study was made on the cellular incorporation of this herbicide at both dose levels. All four fractions—i.e., nuclear, mitochondrial, microsomal, and soluble—contained significant radioactivity. The majority of the radioactivity—i.e., 57% in liver to 86% in lungs (Table IV)—was found in the soluble fraction. An average of 9% in the heart to 32% in the liver was found in the nuclear fraction while the mitochondrial and microsomal fractions had an average ranging from 1.4 to 6.7%. Although the range of per cent radioactivity in various fractions varied greatly among different tissues, the results were quite consistent for each tissue. There was no significant difference in the percentage in the various fractions when different dose levels were administered, or when the animals were sacrificed 3 and 6 hours after dosing. Hence, incorporation of 2,4-D in the cellular components cannot be used to explain the differences in the elimination rates at the two-dose levels. The radioactivity in the soluble fraction from all tissues could be easily extracted by ether, and the radioactivity in the ether extract was shown to be unchanged 2,4-D by paper chromatography. No attempt was made to determine whether or not the ether extract may contain 2,4-D metabolite by countercurrent extraction method. This observation suggests that the 2,4-D molecule in the soluble fraction is not protein- or peptide-bound as found in the plant tissues.

Table IV. Percentage Distribution of C^{14} in Cellular Fractions of Rat Organs after Administration of 2,4-D-1-C^{14}

	Nuclear			Mitochondrial			Microsomal			Soluble		
Organ	A	B	C	A	B	C	A	B	C	A	B	C
Kidney	29	24	21	6	3	2	7	5	12	59	70	65
Liver	27	38	34	11	3	3	4	2	2	58	56	60
Spleen	15	18	16	3	2	1	2	2	2	79	78	80
Brain	30	25	26	5	2	1	2	2	2	63	69	70
Heart	8	9	12	4	2	1	3	2	1	84	86	86
Lungs	10	12	10	2	2	1	1	2	2	88	84	88

A. Average of 3 rats; 1 male fed 1 mg. and 2 females fed 2 mg. each and sacrificed after 3 hours.
B. Average of 2 rats; females fed 80 mg. each and sacrificed after 3 hours.
C. One female fed 80 mg. and sacrificed after 6 hours.

Paper chromatography of the metabolite (pooled sample from countercurrent separation) revealed that it has the same R_f value as 2,4-D in BAW solvent (0.88–0.90), but a slightly higher R_f than 2,4-D in 2-propanol–NH_4OH–H_2O solvent system. (R_f for 2,4-D 0.55 to 0.59, R_f for metabolite 0.67 to 0.69.)

Cellular Incorporation. Since so much difference in the rate of elimination

Acknowledgment

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INSECTICIDE DETERMINATION

Colorimetric Determination of *O,O*-dimethyl(1-hydroxy-2,2,2-trichloroethyl)-phosphonate and Its Higher Homologs

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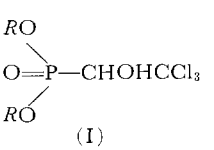
A rapid colorimetric method for the estimation of Dipterex and its higher homologs involves the cleavage of the phosphorus–carbon bond in the α-hydroxyphosphonate derivative, and the determination of the formed dialkyl phosphite by interaction with 3,5-dinitrobenzoic acid in the presence of alkali, whereby a stable violet-blue color develops. As little as 20 p.p.m. of Dipterex can be determined easily. The method allows differentiation between Dipterex and its dehydrochlorinated compound (DDVP).

THE CONDENSATION of chloral with dialkyl phosphites leads to the formation of a series of *O,O*-dialkyl-(1-hydroxy-2,2,2-trichloroethyl)phosphonates (I) (*3, 10*) the lower members of which

$$(RO)_2P(O)H + Cl_3CCHO \rightarrow$$



(I)

(Ia), $R = CH_3$
(Ib), $R = C_2H_5$
(Ic), $R = C_3H_7$-*i*

possess remarkable insecticidal activity. Thus, for example, compound Ia, the dimethyl ester of (2,2,2,-trichloro-1-hydroxyethyl)phosphonic acid (Bayer L 13/59) marketed under the trade name Dipterex, recently attracted much interest as an insecticide effective against DDT-resistant houseflies (*6, 7*). It is widely used in United Arab Republic as

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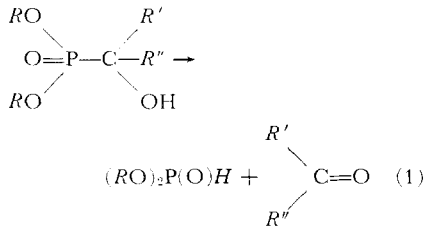
a potent pesticide against the dangerous cotton leaf worm (*Prodenia litura*).

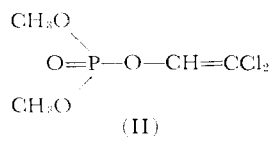
The growing application of Dipterex as an ecto- and endo-parasiticide in both hygienic and veterinary areas (*13*) has created the need for a simple but sensitive analytical method for detecting and estimating its residues in toxicological analysis.

Previous methods for the determination of Dipterex are indirect and lengthy. Thus, based on the fact that dialkyl-α-hydroxyphosphonates are decomposed by heat to the starting materials (*1*) (Reaction 1),



Giang, Barthel, and Hall (*8*) developed a method for the determination of Dipterex (Ia) and its higher homologs (Ib and Ic). The procedure involved the pyrolysis of the compound at 550° C., producing chloroform. This was determined by a modification of the pyridine–alkali–Fujiwara test (*5*), giving a bright red color.

On the basis of the observation of Mattson, Spillane, and Pearce (*11*) that Dipterex undergoes dehydrochlorination in the presence of alkali to yield dimethyl 2,2-dichlorovinyl phosphate (II),

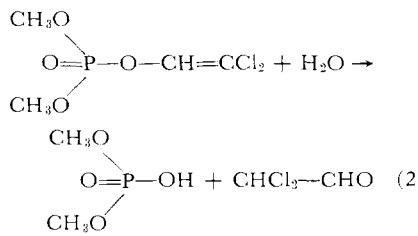


(II)

which condenses with 2,4-dinitrophenyl hydrazine to give the corresponding glyoxal osazone (*2*). Wollenberg (*14*) developed a direct method for the estimation of Dipterex. The compound was made to react with isonicotinic acid hydrazide and the resulting yellow color was determined colorimetrically.

None of these methods appears to be completely satisfactory. Thus, if Dipterex is contaminated with (II)—a very probable contaminant—the two methods will give rise to positive errors. Recently, El-Refai and Giuffrida (*4*) have reported a gas-liquid chromatographic method for separation and simultaneous measurement of very low levels of Dipterex and DDVP.

In the present communication, a new sensitive method for the determination of Dipterex and its higher homologs is reported, a modification of Giang's procedure. Thus, whereas the latter method is based mainly on the determination of chloroform produced on pyrolysis of (I) at 550° C., the present procedure comprises the detection and estimation of dialkyl phosphite formed by the cleavage of the phosphorus–carbon bond in (I) at about 70° C. (Reaction 1). These phosphite esters develop a stable violet-blue color when allowed to react with a solution of 3,5-dinitrobenzoic acid in aqueous sodium bicarbonate (*12*). The proposed method is simple and can be run on a filter paper (spot colorimetry) in routine toxicological analysis. Dehydrochlorinated compounds—e.g., DDVP in the case of (Ia)—do not interfere, since dimethyl phosphoric acid is formed (*9*) (Reaction 2) upon hydrolysis, and the blue color is not developed. On the basis of the proposed color reaction



a differentiation can be made, therefore, between Dipterex (or its higher homologs) and the corresponding dehydrochlorinated compounds.

A number of dinitro compounds—namely, *m*-dinitrobenzene, 1-chloro-2,4-dinitrobenzene, 2,4-dinitrotoluene, 2,4-dinitrophenylhydrazine, 3,5-dinitrosalicylic acid, and picric acid—also were tested. None was superior to 3,5-dinitrobenzoic acid. A freshly prepared sodium bicarbonate solution of the reagent was found essential for the development of the color. When the test was carried out on dried filter paper impregnated with the reagent, the sensitivity increased appreciably. This new test could be used advantageously as a spray reagent for detection of Dipterex and its homologs on paper chromatograms.

Experimental

Apparatus. The color spots were examined by the reflection densitometry technique. An extinction densitometer equipped with a recording integrator (Type ERI 10, Carl Zeiss Jena) operated with filter No. 2 (550 mμ) was used for measuring the reflection of the spots. The apparatus was operated with the slit on mark 5.

Procedure. Strips of Whatman No. 54 filter paper were impregnated homogeneously with a freshly prepared solution of 3,5-dinitrobenzoic acid (1 gram) dissolved in 40 ml. of 5% aqueous sodium bicarbonate, and then dried. The reagent paper then was treated with a solution (0.05 ml.) of known concentration of the test substance in chloroform. Care was taken to ensure round spots of about 5-mm. diameter. After drying, the filter paper strip was heated in an air oven at 70° C. for exactly 2 minutes, and the violet-blue color developed. The area of the reflection wavelength curve was measured either planimetrically or by the square counting method. Allowing the strips to be transparent through the use of anisole, benzyl alcohol, or a mixture of α-bromonaphthalene and liquid paraffin did not improve the results, apparently because the color is unstable in these organic solvents. For the construction of the calibration curves, the averages of 10 areas of spots of the same concentration were plotted as a function of concentration (μg./ml.). Straight lines were obtained.

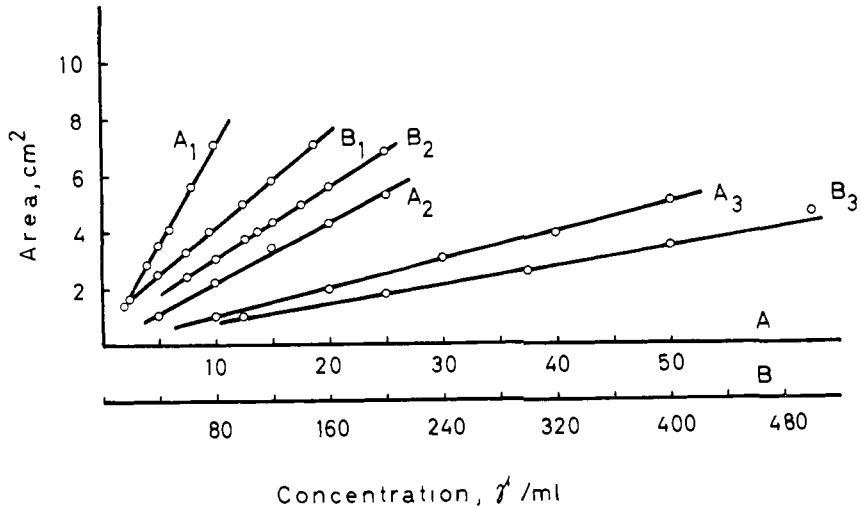


Figure 1. Relation between area under the reflection-wavelength curves and concentration.

A_1. Dimethyl phosphite
A_2. Diethyl phosphite
A_3. Diisopropyl phosphite

B_1. O,O-dimethyl(1-hydroxy-2,2,2-trichloroethyl)phosphonate, Dipterex (Ia)
B_2. O,O-diethyl(1-hydroxy-2,2,2-trichloroethyl)phosphonate (Ib)
B_3. O,O-diisopropyl(1hydroxy-2,2,2-trichloroethyl)phosphonate (Ic)

Results and Discussion

Curves *A*, Figure 1, represent the variation of the area under reflection-wavelength curves and the concentration (μg./ml.) of dimethyl, diethyl, and diisopropyl phosphites. In all cases the relationship is linear. For the same concentration, the area increases in the succession dimethyl > diethyl > diisopropyl phosphite. Concentrations as low as 2, 5, and 10 μ/ml. for the above three esters are determined easily from these calibration curves. The method is, therefore, superior to that described by Saunders and Stark (*12*), who determined colorimetrically a 1 to 10,000 concentration of dialkyl phosphite.

The calibration curves *B* of Figure 1 are for Dipterex and its corresponding ethyl and isopropyl analogs. Again, within the experimental range of concentration studied, Lambert-Beer's law is obeyed. The test is more sensitive for Dipterex (Ia) than its ethyl (Ib) or isopropyl (Ic) analogs, in harmony with the results of the corresponding dialkyl phosphite esters (Figure 1). That the test is less sensitive for Dipterex and its homologs than for the corresponding dialkyl phosphites plausibly can be attributed to incomplete cleavage of the phosphorus-carbon bond to yield the corresponding dialkyl phosphite under the prevailing experimental conditions, or to the possible change of some of the Dipterex into DDVP during the time of analysis. This is, however, to be expected, since total cleavage of α-hydroxyphosphonates is known to take place at temperatures higher than that applied in the present investigation. Attempts to increase the temperature higher than 70° C. resulted in the formation of brown-black spots.

Acknowledgment

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RESIDUE DETERMINATION

A Gas Chromatographic Method for the Determination of Bay 37289, Its Oxygen Analog, and 2,4,5-Trichlorophenol in Crops

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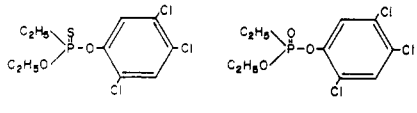
A gas chromatographic method for the determination of residues of Bay 37289 (I), its oxygen analog (II), and the corresponding 2,4,5-trichlorophenol (III) in crops is described. The method is based on a hydrolysis of (I) and (II) to given (III) which, after acetylation, is determined by electron-capture gas chromatography. The method is very satisfactory for measurements at the 0.1-p.p.m. level, and with minor modifications could be used to determine residues of 0.01 p.p.m., if desired.

BAY 37289 (*O*-ethyl *O*-2,4,5-trichlorophenyl ethylphosphonothioate) is an organic phosphorus insecticide being developed by Chemagro Corp. under license. The structural formula is as follows (I):



I

II

The compound has given excellent control of soil insects, particularly wireworms and root maggots (*2–4*). It has also been shown to be effective for the alfalfa weevil (*2*).

One of its suspected metabolites is the oxygen analog (II). This was prepared with good yield and purity by oxidation of I with *m*-chloroperbenzoic acid in chloroform media. Both compounds are amber liquids, insoluble in water, but soluble in most organic solvents. The hydrolysis product of both is the 2,4,5-trichlorophenol (III)—also a possible metabolite in plant tissue.

This gas chromatographic method was developed not only for the parent insecticide but, also, for its oxygen analog and the corresponding 2,4,5-trichlorophenol. The method consists of an initial extraction with acetone–benzene–sodium sulfate followed by a Florisil column chromatography cleanup. Since the oxygen analog (II), unlike the parent compound (I), cannot be directly chromatographed, it is necessary to hydrolyze the compounds to the trichlorophenol. An alkaline wash prior to hydrolysis separates any 2,4,5-trichlorophenol already present from (I) and (II). The phenol is analyzed separately. After hydrolysis and before neutralization, an extraction with chloroform removes all interfering plant extractives not removed