by adding 10  $\mu$ g. of Bay 37289, its oxygen analog, or 2,4,5-trichlorophenol to 100 grams of plant materials unless otherwise specified. The compounds were added at the blending step, and the samples were then processed by the detailed procedure described. All recoveries were run at a 0.1-p.p.m. level. In the case of the phenol, recoveries were run only on nine representative crops. The results are presented in Table I. Recoveries averaged about 90%, making it unnecessary to run recoveries at higher levels. In all crops studied, control values were negligible.

The gas chromatographic response for 2,4,5-trichlorophenol acetate is linear up to 40 nanograms. This is equivalent

## **RESIDUE DETERMINATION**

to approximately 30 nanograms of free phenol or 50 nanograms of Bay 37289 (I) or its oxygen analog (II). Consequently, samples having a response greater than three times that of the phenol standard or five times greater than (I) or (II) standards should be diluted and reinjected.

In order to correct for variations in the response of the electron-capture detector, it is necessary to inject standards with every batch of samples being analyzed. Peak areas for equimolar amounts of (I), (II), and 2,4,5-trichlorophenol are very nearly the same. The sensitivity of the method is considered to be 0.1 p.p.m., but a much greater sensitivity can be obtained if required.

Figures 1 and 2 show typical chromatograms from recovery experiments conducted.

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# A Gas-Liquid Chromatographic Method for the Determination of Trichlorfon in Plant and Animal Tissues

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A sensitive residue method has been developed for the determination of trichlorfon and the possible metabolites, chloral hydrate and trichloroethanol, in plant and animal tissues. Separate extraction and cleanup procedures are necessary for the various types of samples. In all cases, detection and measurement of the compounds are accomplished by electron-capture gas chromatography. The specificity of the method is enhanced by utilizing the high degree of water solubility displayed by all three compounds. Complete specificity is effected by efficient gas-liquid chromatography. The sensitivity of the method is 0.1 p.p.m.

**T**RICHLORFON. 0,0-dimethyl 2,2,2trichloro-1-hydroxyethyl phosphonate, marketed under the trade name Dylox, is used for the control of many insect species which attack vegetable and field crops. It is effective in the control of many pest species of Diptera, Lepidoptera, Hymenoptera, Hemiptera, and Coleoptera (2, 7, 9, 10, 12, 20). Formulations of trichlorfon are sold under the trade name Neguvon for the control of ectoparasites and endoparasites of livestock (3-6, 14, 18, 19, 21). Trichlorfon is also sold under the trade name Dipterex as a sugar bait used for the control of flies (13).

Its structural formula is as follows:

The compound is soluble in alcohols and ketones and slightly soluble in aromatic solvents. It has a solubility of 12% in water at 26° C.

Giang (8) has reported a colorimetric method for the determination of various O,O-dialkyl 1-hydroxyphosphonates, including trichlorfon. This procedure is based on a hydrolysis of the compounds to chloroform and ultimate determination of the chloroform by a modification of the Fujiwara test. This method was not considered adequate because of reported color interferences and inadequate sensitivity.

Early efforts to develop a gas chromatographic method for the determination of trichlorfon residues in plant and in animal tissues centered on the use of a Dohrmann microcoulometric gas chromatograph equipped with a halide sensititration cell (15–17). tive Although such a detection system should provide an ideal basis for analysis, the chromatographic flow and temperature conditions needed to achieve a tolerable level of sensitivity made it difficult to separate frequently occurring contamination peaks (from solvents, etc.) from the trichlorfon peak. These contamination peaks were only partially eliminated by tedious purification of reagents. The extreme chromatographic conditions also made it impossible to separate chloral hydrate and trichloroethanol. These two compounds have been considered as possible metabolites of trichlorfon (1). The findings of Arthur and Casida

concerning the metabolism of trichlorfon in plants and animals indicate that chloral and trichloroethanol are possible breakdown products of the parent compound. The presence of the vinyl derivative (DDVP) was discounted. For this reason, determination of the two trichloro compounds is provided for in the method described.

Since trichlorfon, chloral hydrate, and trichloroethanol have a strong electron affinity, the application of electroncapture gas chromatography was considered. Preliminary experiments indicated that the more efficient electroncapture chromatographic system would make it possible to minimize the control peaks in the trichlorfon area and, at the same time, provide for separation of chloral hydrate and trichloroethanol.

The vapor phase chromatography of trichlorfon is dependent upon its thermal breakdown to form chloral. Decomposition is observed at temperatures near  $100^{\circ}$  C. and is quite rapid at higher temperatures. However, regardless of the temperature of the injection port, the residence time of trichlorfon in the inlet area was so brief that the conversion to chloral was quite erratic. In order

to achieve a maximum conversion of trichlorfon to chloral and also to obtain reproducibility, a plug of glass wool was placed in the injection port end of the column. In addition, an adapter was fixed on the injection syringe to allow the needle to penetrate only one and onefourth inches beyond the septum. The glass wool provides added surface area and the syringe adapter gives the sample a longer residence time in the injection port. These two measures have resulted in good conversion of trichlorfon to chloral. However, improper placement or coating of the glass wool by extracts gives rise to a secondary peak which, as yet, has not been identified. This peak immediately follows the trichlorfon peak. The area of this secondary peak rarely exceeds 5% of the trichlorfon peak area. Proper maintenance of the glass wool will nearly, but not completely prevent its formation.

Since chloral is the compound actually chromatographed when trichlorfon is injected into the instrument, there is no way of determining the separate identities of trichlorfon and chloral hydrate. The response obtained for trichlorfon is 64% of that obtained from an equimolar amount of chloral. The decomposition of trichlorfon is, therefore, less than quantitative. This is not a problem as the amount of decomposition is reproducible.

The column temperature which gives optimum chromatographic results for chloral is  $100^{\circ}$  C. At this temperature, chloral is eluted in about 3 minutes and trichloroethanol in about 45 minutes. Since temperature programing creates a marked baseline shift, samples to be analyzed for trichloroethanol are run separately at  $140^{\circ}$  C. At this temperature, trichloroethanol is eluted in roughly 7.5 minutes.

In most cases, none of the tissue extracts analyzed produced any peaks that interfered seriously with the chloral and trichloroethanol peaks. Occasionally, some moist crops had control peaks that made 0.1 p.p.m. trichloroethanol analyses impossible. To circumvent this problem, an application of the procedure described by Hoff and Feit (11) for the conversion of primary alcohols to their respective acetates was employed. By converting trichloroethanol to trichloroethylacetate, the retention time is changed and the compound is eluted in an area relatively free of interference. The acetylation of trichloroethanol is unaffected by the presence of trichlorfon or chloral hydrate. However, the reaction destroys the trichlorfon peak, because trichlorfon is also acetylated. The acetylated trichlorfon does not decompose to give chloral. Analyses for the trichloroethanol and trichlorfon-chloral hydrate, therefore, must be carried out on separate aliquots of the extract.

The acetylation process requires the

treatment of a portion of the final benzene solution with a sulfuric acid-acetic anhydride mixture. The excess reagent is removed with aqueous sodium bicarbonate. An aliquot of the benzene phase is then injected into the gas chromatograph at a column temperature of  $130^{\circ}$  C. At this temperature, trichloroethylacetate has a retention time of about 5.5 minutes. The acetylation treatment is necessary only for moist crops.

The substrate used in the partition chromatography is XF-1150, an A.G.E. acrylonitrile substituted silicone fluid. This liquid phase was superior to D.C. 200 silicone oil and diisodecyl phthalate.

## **Animal Tissues**

Animal tissues are blended with acetonitrile and then with hexane, and the resulting extracts are combined in a separatory funnel and shaken. Trichlorfon, chloral hydrate, and trichloroethanol partition into the acetonitrile, and the fats and oils remain in the hexane which is discarded.

Following evaporation of the acetonitrile, further cleanup is effected by a heptane-water partition. The remainder of the fats and oils partition into the heptane, while trichlorfon, chloral hydrate, and trichloroethanol are extracted quantitatively into the water. The water then is saturated with sodium chloride and extracted with diethyl ether. Under these conditions the desired compounds pass into the ether. The ether is removed by evaporation, leaving a residue containing the three compounds.

All evaporations are carried out at  $40^{\circ}$  C. in a rotary vacuum apparatus. Even under these conditions, evaporation losses of chloral hydrate and trichloroethanol occur. In order to minimize these losses, water is added to the acetonitrile extract before evaporation. Paraffin oil is added to the ether before evaporation, for the same reason. The water and paraffin oil act as keepers.

## Dry Crops (Less Than 40% Moisture)

Dry crops are extracted by grinding the samples in acetone and then hexane. The combined filtrates are evaporated to dryness. The samples then are treated as in the procedure described for animal tissues, starting at the heptane-water partition step.

## Moist Crops (40% or More Moisture)

Room temperature static dialysis is used for the extraction and major cleanup of moist crops. It is not possible to omit the dialysis step because of difficulties in filtration and subsequent extraction. For dialysis, the sample is macerated in 0.1N H<sub>2</sub>SO<sub>4</sub>, then dialyzed for a minimum of 24 hours. Under these conditions, equilibrium is reached for all three compounds. However, should it be necessary, samples may be dialyzed for at least 72 hours without ill effects. The use of acid prevents dehydrohalogenation of trichlorfon during dialysis.

Following dialysis, an aliquot of the diffusate is extracted with diethyl ether as described in the procedure for animal tissues. The residue is taken up in benzene. An aliquot of the benzene solution is acetylated for trichloroethanol analysis, while the remainder is used directly for trichlorfon-chloral hydrate analysis.

#### Procedure

Apparatus. Dialysis tubing, cellulose, Visking NO JAX,  $1^{7}/_{8}$  S.S., or equivalent.

Gas Chromatograph, F & M Model 700 equipped with an electron-capture detector (F & M Scientific Corp., Avondale, Pa.).

Rotary vacuum evaporator, Swissco.

**Reagents.** All reagents are reagent grade, unless otherwise specified. All solvents should be redistilled from an all-glass apparatus.

Acetic anhydride reagent. To 5 ml. of 99–100% acetic anhydride, add 100  $\mu$ l. of concentrated H<sub>2</sub>SO<sub>4</sub> and mix well. Prepare a fresh solution daily. Chloral hydrate, crystal, U.S.P.

(Fisher Scientific Co.). Trichlorfon, analytical standard

(Chemagro Corp., Kansas City 20, Mo.). Trichloroethanol (Westvaco Chemical Division, Food Machinery and Chemical Corp., New York, N. Y.).

Sample Preparation, Extraction, and Cleanup. Animal Tissues. Grind the entire frozen sample in a Hobart food chopper in the presence of dry ice. Place the chopped sample in frozen storage overnight to allow the dry ice to sublime. Weigh 50 grams (25 grams for fat) of the chopped sample into a Waring Blendor jar. Blend at high speed for 5 minutes with 200 ml. of acetonitrile. (For fat samples, reverse the order of solvents for grinding only. Grind in Skellysolve B first, then acetonitrile.) Filter with suction through an 11-cm. Whatman No. 541 filter paper covered with a 1/8-inch layer of Hyflo Super-Cel. Rinse the blender jar with 200 ml. of acetonitrile and add the rinsings to the filter cake. Transfer the filtrate to a 1000-ml. separatory funnel. Return the filter paper and filter cake to the blender jar, add 200 ml. of Skellvsolve B, and blend at high speed for 3 minutes. Filter as above, then rinse the blender jar with 100 ml. of acetonitrile and add the rinsings to the filter cake. Transfer the filtrate to the same 1000-ml. separatory funnel. Shake the separatory funnel for 30 seconds, then allow the layers to separate. Transfer one half of the lower acetonitrile phase to a 500-ml. round-bottomed flask. Add 50 ml. of distilled water (60 ml. to fat samples) to the flask, and evaporate the sample on the Swissco evaporator at 40° C. until the condensation in the receiver flask ceases. (Some water will remain in the evaporator flask.)

Transfer the sample to a 50-ml. graduated cylinder with distilled water and dilute to 50 ml. Transfer the sample to a 125-ml. separatory funnel. Using the same graduated cylinder for measuring, rinse the boiling flask with 25 ml. of *n*-heptane, then add the heptane to the same 125-ml. separatory funnel. Add a second 25-ml. portion of *n*-heptane to a second 125-ml. separatory funnel. Shake the first separatory funnel for 30 seconds, allow the layers to separate, and draw off the lower aqueous phase into the second 125-ml. separatory funnel. Shake the second separatory funnel for 30 seconds, allow the layers to separate, and draw off the lower aqueous phase into a 250-ml. separatory funnel. Rinse the boiling flask with 50 ml. of distilled water and use the rinse to re-extract both heptane phases in the manner described above. It may be necessary to centrifuge the heptane-water systems for 10 minutes at 800 r.p.m. To the combined aqueous extract (100 ml.) add 31 grams of sodium chloride and shake to dissolve. Add 100 ml. of diethyl ether and shake for 30 seconds. Allow the layers to separate and draw off the lower aqueous phase into a second 250-ml. separatory funnel. Repeat the ether extraction of the aqueous phase, then combine the ether extracts in one 250-ml. separatory funnel. Carefully draw off any water that has collected. Dry the ether by adding 20 grams of anhydrous granular sodium sulfate and shaking the separatory funnel occasionally over a period of 15 minutes. For best results, use sodium sulfate that has been stored at 110° C. Decant the ether through glass wool into a 250-ml. round-bottomed flask. Rinse the sodium sulfate with 25 ml. of ether and add the rinsings to the flask. Add 0.5 ml. of U.S.P. grade paraffin oil to the ether, then evaporate the sample on the Swissco evaporator at 40° C. to a volume of 10 to 15 ml. Remove the remainder of the solvent with an air stream at room temperature. Take up the residue in 4.5 ml. of benzene. Equal sensitivity for fat samples is obtained by taking up the residue in 2.0 ml. of benzene. Analyze the residue as instructed under Chromatographic Analysis.

Dry Crops. Grind the entire sample to a coarse powder in Wiley mill. Weigh 50 grams of the sample into a Waring Blendor jar. Add 200 ml. of acetone and blend at high speed for 5 minutes. Filter with suction through an 11-cm. Whatman No. 42 filter paper. Return the filter cake and the filter paper to the blender jar, add 200 ml. of Skellysolve B, and blend at high speed for 3 minutes. Filter with suction through an 11-cm. Whatman No. 42 filter paper. Rinse the blender jar with 50 ml. of acetone and use this to rinse the filter cake. Transfer the combined filtrates to a 500-ml. roundbottomed flask, and evaporate the sample to a volume of 10 to 15 ml. on the Swissco evaporator at  $40^{\circ}$  C. Remove the remainder of the solvent with an air stream at room temperature.

Transfer the residue to a 125-ml. separatory funnel with 25 ml. of n-

heptane, then continue processing the sample following the procedure described for animal tissues starting at the heptane-water partition step. Dissolve the final extract in 9.5 ml. of benzene. Analyze the residue as instructed under Chromatographic Analysis.

**Moist Crops.** Prepare the sample as described in the animal tissue procedure. Weigh 100 grams of the chopped sample into a Waring Blendor jar. (For convenience, assume the sample to be 80% moisture.) Add 220 ml. of 0.1N H<sub>2</sub>SO<sub>4</sub> and grind at a moderate speed for 5 minutes. Transfer the macerate to an appropriate length of dialysis tubing which has been conditioned by soaking in 0.1N H<sub>2</sub>SO<sub>4</sub> for 30

minutes prior to use, and knotted at one end. Rinse the blender jar with 100 ml. of 0.1N H<sub>2</sub>SO<sub>4</sub>, and add the rinsings to the dialysis tubing. Remove the trapped air from the tubing, and knot the remaining open end. Submerge the sample in a 1-quart jar containing 400 ml. of 0.1N H<sub>2</sub>SO<sub>4</sub>, then cap the jar and allow the sample to stand for at least 24 hours at room temperature. Remove and discard the 0.1N H<sub>2</sub>SO<sub>4</sub> macerate in the tubing. Transfer 200 ml. of the diffusate to a 500-ml. separatory funnel. Add 68 grams of sodium chloride and shake to dissolve. Add 200 ml. of diethyl ether and shake for 30 seconds. Allow the layers to separate and draw off the lower aqueous phase into a second

 Table I. Recovery of Trichlorfon, Chloral Hydrate, and Trichloroethanol from Plant and Animal Tissues

Sample	Compound Added	Added, P.P.M.	Mean Recovery, $\%^a$
	Animal Tissue	s	
Brain	Trichlorfon Chloral hydrate Trichloroethanol	0.1 0.1 0.1	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Fat	Trichlorfon Chloral hydrate Trichloroethanol	0.1 0.1 0.1	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Heart	Trichlorfon Chloral hydrate Trichloroethanol	0.1 0.1 0.1	$\begin{array}{rrrr} 70 \ \pm \ 10 \ (3) \\ 77 \ \pm \ 5 \ (3) \\ 65 \ \pm \ 9 \ (3) \end{array}$
Kidney	Trichlorfon Chloral hydrate Trichloroethanol	0.1 0.1 0.1	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Liver	Trichlorfon Chloral hydrate Trichloroethanol	0.1 0.1 0.1	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Steak	Trichlorfon Chloral hydrate Trichloroethanol	0.1 0.1 0.1	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
	DRY CROPS		
Dry cowpeas	Trichlorfon Chloral hydrate Trichloroethanol	$\begin{array}{c} 0.1\\ 0.1\\ 0.1 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Dry lima beans	Trichlorfon Chloral hydrate Trichloroethanol	0.1 0.1 0.1	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Mustard seed	Trichlorfon Chloral hydrate Trichloroethanol	0.1 0.1 0.1	$\begin{array}{r} 96 \ \pm \ 4 \ (10) \\ 63 \ \pm \ 13 \ (10) \\ 53 \ \pm \ 5 \ (10) \end{array}$
Sunflower seed	Trichlorfon Chloral hydrate Trichloroethanol	0.1 0.1 0.1	$\begin{array}{c} 72 \ \pm \ 13 \ (6) \\ 67 \ \pm \ \ 6 \ (7) \\ 44 \ \pm \ \ 3 \ (5) \end{array}$
	Moist Crops	5	
Cowpeas	Trichlorfon Chloral hydrate Trichloroethanol	0.1 0.1 0.1	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Cowpea vines	Trichlorfon Chloral hydrate Trichloroethanol	$\begin{array}{c} 0.1\\ 0.1\\ 0.1\\ 0.1 \end{array}$	$\begin{array}{r} 86 \ \pm \ 7 \ (5) \\ 84 \ \pm \ 10 \ (7) \\ 66 \ \pm \ 13 \ (6) \end{array}$
Lima beans	Trichlorfon Chloral hydrate Trichloroethanol	0.1 0.1 0.1	$\begin{array}{rrrr} 96 \ \pm \ 3 \ (5) \\ 89 \ \pm \ 8 \ (4) \\ 73 \ \pm \ 10 \ (7) \end{array}$
Lima bean vines	Trichlorfon Chloral hydrate Trichloroethanol	$\begin{array}{c} 0.1\\ 0.1\\ 0.1\\ 0.1 \end{array}$	$\begin{array}{rrrr} 78 \ \pm \ 8 \ (8) \\ 74 \ \pm \ 9 \ (5) \\ 92 \ \pm \ 12 \ (4) \end{array}$
Pumpkin	Trichlorfon Chloral hydrate Trichloroethanol	$\begin{array}{c} 0.1\\ 0.1\\ 0.1\\ 0.1 \end{array}$	$\begin{array}{r} 89  \pm  9  (11) \\ 84  \pm  13  (10) \\ 76  \pm  10  (10) \end{array}$
Spinach	Trichlorfon Chloral hydrate Trichloroethanol	0.1 0.1 0.1	$\begin{array}{c} 82 \pm 12 \; (11) \\ 66 \pm 4 \; (7) \\ 69 \pm 12 \; (10) \end{array}$

<sup>a</sup> Value in parenthesis indicates the number of determinations.

500-ml. separatory funnel. Re-extract the aqueous phase with another 200 ml. of ether. Combine the ether extracts in one 500-ml. separatory funnel. Carefully draw off any water that has col-lected. Add 30 grams of granular anhydrous sodium sulfate, and dry the ether by shaking the separatory funnel occasionally over a period of 15 minutes. Decant the ether through glass wool into a 500-ml. round-bottomed flask. Rinse the sodium sulfate with 25 ml. of ether, and add the rinsings to the flask. Add 0.5 ml. of the paraffin oil to the ether, and evaporate the sample to a volume of 15 to 20 ml. on the Swissco evaporator at 40° C. Remove the remainder of the ether with an air stream at room temperature. Dissolve the residue in 4.5 ml. of benzene, and transfer as much of the benzene solution as possible to a screwcap culture tube. Transfer 1 ml. of

CONTROL

+OI PPM NEGUVON

CONTROL

6

MINUTES

CONTROL

+O.I P.P.M. TRICHLOROETHANOL

CONTROL

8

4

6

MINUTES

Α

8

10

TRICHLOROETHANO.

12

NEGUVON

C

2

benzene to a second culture tube, and add 10 µl. of the acetic anhydride reagent. Mix the sample well. Cap the tube tightly and place it in a  $70^{\circ}$  C. water bath to a depth of about 2 inches for 30 minutes. Remove the tube and place it in an ice bath for 1 minute. Add 1 ml. of a 5% w./v. solution of sodium bicarbonate, and mix by agita-tion for 3 minutes. Centrifuge the sample for 5 minutes at 1000 r.p.m. At this point, the treated extract is ready for trichloroethanol analysis, and the untreated extract is ready for trichlorfonchloral hydrate analysis as instructed under Chromatographic Analysis. One milliliter of an appropriate trichloro-ethanol standard solution should be

column with 60- to 80-mesh, acid-washed, Chromosorb W containing 20% by weight of XF-1150. Loosely place a 10-mg. plug of glass wool one-half inch inside the injection port end of the column. Adjust the 95% argon, 5% methane tank pressure to 20 p.s.i.g. Set the carrier and purge flow rates at 25 and 15 ml. per minute, respectively. Set the detector pulse interval at 15 microseconds, the electrometer attenuation at  $100 \times 2$ , and the recorder chart speed at 0.5 inch per minute. Adjust the temperature settings for the various compounds as follows:





12

10

- Recovery of Dylox from lima beans Ε.
- F. . Recovery of trichloroethanol from lima beans

Inject 2  $\mu$ l. of the benzene solution using a syringe fitted with an adapter that allows the needle to penetrate only  $1^{1}/_{4}$  inches beyond the septum. The adapter is unnecessary for trichloroethanol or its acetate. Inject 2  $\mu$ l. of a  $0.5 \ \mu g$ . per ml. standard before and after each set of samples in order to determine the exact retention time and provide for quantitative measurement of each sample.

**Calculations.** Since 2  $\mu$ l. of sample extract represents 0.010 gram of original sample, the standard corresponds to 0.1 p.p.m. The response for chloral hy-drate is linear up to 6 nanograms, and for trichlorfon, to 12 nanograms. Trichloroethanol and its acetate give a linear response for amounts up to 30 nanograms. Therefore, the method is satisfactory for a range of 0.1 to 1.2 p.p.m. for trichlorfon and 0.1 to 3.0 p.p.m. for trichloroethanol. For higher levels, dilutions have to be made. The parts per million of any sample is calculated as follows:

(area of sample)(0.1)(dilution factor)  $P.p.m. = \frac{1}{(average area of standards)}$ 

#### Discussion

Recovery Experiments. Recoverv experiments were conducted on all of the tissues and crops listed in Table I by adding known amounts of the compound at the blending step, and processing the sample by the appropriate procedure. The results of these experiments are listed in Table I. All recoveries were conducted at a 0.1 p.p.m. level. Chloral hydrate, and especially trichloroethanol, are quite volatile. Recovery of these compounds is, therefore, not as good as for trichlorfon. Typical control and

recovery chromatograms for trichlorfon and trichloroethanol analyses at 0.1 p.p.m. are shown in Figure 1.

The conversion of trichloroethanol to the acetate by the acetylation procedure in the presence of plant tissue extracts is quantitative through 1.0 p.p.m. Since there was no need to go beyond this level, experiments at still higher levels were not attempted.

Control Values. Untreated samples were analyzed for apparent trichlorfon and trichloroethanol peaks. The number of control samples analyzed ranged from three for each animal tissue to ten for each moist crop. In all cases, control values were far less than 0.1 p.p.m. for both compounds. Control values were not calculated for chloral hydrate because it has the same retention time as trichlorfon. Chlorinated hydrocarbon insecticides do not interfere in the method because of their nonpolar nature.

Sensitivity. The method is capable of measuring 0.1-p.p.m. residues of all three compounds with good precision. At this level, relatively little interference from control peaks is encountered. The procedure is inherently capable of considerably greater sensitivity, if required.

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# **TOXICITY STUDIES**

# **Toxicity of Metal Complexes of Octamethylpyrophosphoramide** in Water and Dimethylsulfoxide

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The LD<sub>50</sub> values for metal complexes of octamethylpyrophosphoramide (OMPA) toward mice have been determined. When water is the solvent, all OMPA complexes except that of Co(II) are more toxic than the combined toxicity of the metal salt and OMPA. When dimethylsulfoxide (DMSO) is the solvent, the toxicity of [Fe(OMPA)<sub>3</sub>][FeCl<sub>4</sub>]<sub>3</sub> is extremely high  $(LD_{50} = 4.8)$ . The toxicity of the other OMPA complexes in DMSO is either the same as or less than the combined toxicity of the metal salt and OMPA.

CTAMETHYLPYROPH OSPHOR A MIDE (OMPA) acts as a typical organophosphorus poison by inhibiting the action of acetylcholinesterases (2). The <sup>1</sup> Present address, Department of Chem-try, Vanderbilt University, Nashville, istry,

Tenn. 37203

active metabolite of OMPA is thought to be either





or

A mechanism has been proposed that