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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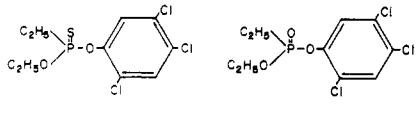
A gas chromatographic method for the determination of residues of Bay 37289 (I), its oxygen analog (II), and the corresponding 2,4,5-trichlorophenol (III) in crops is described. The method is based on a hydrolysis of (I) and (II) to given (III) which, after acetylation, is determined by electron-capture 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

by the Florisil column. After neutralization and buffering to pH 7, the phenol is extracted with hexane and acetylated (*1*). Detection is achieved by the electron-capture detector.

Method

Reagents and Equipment. Acetone, chloroform, *n*-hexane, redistilled in all-glass system.

Acetic anhydride reagent. To 1.0 ml. of acetic anhydride (99–100%) add 10 μl. of concentrated sulfuric acid. This should be done just prior to use of the acetic anhydride.

Alcoholic potassium hydroxide, 2*N* in absolute methanol.

Florisil, 60 to 100 mesh, activated in an oven overnight at 130° C. Floridin Co., Tallahassee, Fla.

Hyflo Super-Cel (Johns Manville).

Phosphate buffer (pH 7). To 500 ml. of 1*M* KH_2PO_4, add 296 ml. of 1*M* NaOH and dilute to 1 liter with distilled water. Check pH and adjust, if necessary.

Superbrite beads, Type 130, Minnesota Mining and Manufacturing Co., St. Paul, Minn., or equivalent.

Waring Blendor, or equivalent.

Centrifuge, International Model BE-50 or equivalent.

Chromatographic tubes, borosilicate glass, 20 × 400 mm. with 300-ml. reservoir and Ultramax stopcock.

Food chopper, Hobart.

Gas chromatograph, F and M Model 700 equipped with pulsed-type electron-capture detector or equivalent.

Microliter syringe, 701N, 705N, or equivalent.

Rotary vacuum evaporator, Swissco or equivalent (all-glass system).

Tubes, centrifuge, glass-stoppered, 13 or 15 ml.

Water bath, 40° C., 60° C.

Wiley mill (3-mm. screen size) A. H. Thomas Co., or equivalent.

Standards of (I), (II), and (III) are prepared as follows:

Weigh out 100.0 mg. of purified standards. Transfer the samples to 100-ml. volumetric flasks with hexane. Dilute to volume with hexane and mix well. These are the stock solutions. For standards (I) and (II), pipet 1.0 ml. of the stock solutions into separate 100-ml. volumetric flasks, and dilute to volume with hexane. These solutions contain 10 μg. per ml. of the compounds. For standard (III), pipet 250 μl. of the stock solution into a 100-ml. volumetric flask, and dilute to volume with hexane.

Preparation and Extraction of Sample. Grind the sample in the Hobart Food Chopper with an equal weight of dry ice. Mix thoroughly. Place the sample in frozen storage overnight to allow the dry ice to sublime. Dry samples are ground to a coarse powder in a Wiley mill. Weigh 100 grams of the ground sample into a Waring Blendor. (For tobacco samples, use 50 grams.) Add 300 ml. of acetone and grind at high speed for 5 minutes. Add 200 ml. of benzene, and while grinding at low speed, add 150 grams of powdered anhydrous sodium sulfate. As soon as all the sodium sulfate has been added, grind at high speed for another 5 minutes. Filter under vacuum through Whatman No. 42 filter paper using a 1000-ml. suction flask. (For strawberry samples place a 1-inch layer of Hyflo Super-Cel on the filter paper.) Rinse the blender with 50 ml. of benzene, and use this to wash the filter cake. Filter the extracts through a fluted No. 12 Whatman filter paper containing 15.0 grams of granular anhydrous sodium sulfate. Rinse the sodium sulfate with 20 ml. more of benzene. Collect the filtrate in a 1-liter round-bottomed flask. At this point the extract should be clear and free of water. Evaporate the solvent in the Swissco evaporator at 40° C.

Column Chromatography. Dissolve the residue in 20 ml. of chloroform. Prepare the Florisil chromatographic column as follows: Tamp a plug of borosilicate glass wool into the bottom of a glass chromatographic tube. Pour in a one-inch layer of Superbrite glass beads. Add 10 grams of dry activated Florisil (60 to 100 mesh) and then 10 grams of granular anhydrous sodium sulfate. Tap the column to obtain good packing. Wash the column with 100 ml. of chloroform and discard the wash. Pour the concentrated extract into the column, and allow it to percolate through the column at a rate of approximately 2 to 3 drops per second. Rinse the round-bottomed flask with a total of 20 ml. of chloroform and use the rinse to wash down the sides of the reservoir. Elute with an additional 250 ml. of chloroform added in several portions. Collect the eluate in a 500-ml. round-bottomed flask. This chromatographic step may be omitted for potato samples.

Alkaline Wash. Transfer the eluate to a 500-ml. separatory funnel and extract with 100 ml. of 0.1*N* NaOH. Allow the chloroform phase to clarify, and then drain it into a 500-ml. round-bottomed flask. Evaporate the chloroform in a Swissco evaporator at 40° C. Proceed to the hydrolysis step with this fraction. Next, approximately neutralize the 0.1*N* NaOH solution containing the free phenol by adding 10 ml. of 1*N* HCl, and complete the adjustment to pH 7 by adding 20 ml. of pH 7 phosphate buffer. Add 50 ml. of hexane, shake the separatory funnel for 30 seconds to extract the phenol. Drain the lower phase into a 250-ml. separatory funnel. Repeat the extraction with another 50 ml. of hexane. Combine the hexane phases, add 30 grams of anhydrous sodium sulfate. Shake the separatory funnel, allow the sample to dry for 15 minutes. Filter through an 11-cm. Whatman No. 41 filter paper into a 250-ml. round-bottomed flask. Rinse the sodium sulfate with 20 ml. of hexane and add the rinsings to the flask. Evaporate the hexane in a Swissco evaporator at 40° C. to near dryness. Complete the evaporation with an air jet at room temperature and proceed to acetylation.

Hydrolysis. To the residue from above and appropriate standards of (I) and (II) (1 ml. of the 10 μg. per ml. standard evaporated to dryness), add 20 ml. of 2*N* methanolic KOH. Mix well and place in water bath for one hour at 60° C. After hydrolysis, cool the flask and transfer the samples to a 125-ml. separatory funnel using 20 ml. of distilled water. Rinse the round-bottomed flask with two 10-ml. portions of chloroform, and add these to the alkaline aqueous hydrolyzate. Shake vigorously for 5 minutes. A slight emulsion may form. Centrifuge the samples for 10 minutes at 800 r.p.m. After this period, the aqueous phase should be clear. Discard the organic phase. Add 40 ml. of 1*N* HCl and 20 ml. of phosphate buffer to the aqueous extract. Transfer the sample to a 250-ml. separatory funnel containing 50 ml. of hexane. Shake vigorously for 5 minutes to extract the phenol. Drain the aqueous phase into a second 250-ml. separatory funnel and repeat the extraction with another 50 ml. of hexane. Combine the hexane extracts. Add 30 grams of anhydrous sodium sulfate and allow the sample to stand for 15 minutes. Shake twice during this period. Decant the hexane through an 11-cm. Whatman No. 41 filter paper into a 250-ml. round-bottomed flask. Rinse the sodium sulfate with 20 ml. of hexane, and add rinsings to the flask. Evaporate the hexane in a Swissco evaporator at 40° C. almost to dryness. Complete the evaporation with an air jet at room temperature.

Acetylation. Dissolve the samples obtained from the hydrolysis and alkaline wash procedures above in separate 4.0-ml. aliquots of hexane. Pipet 2.0-ml. aliquots of each into 13- or 15-ml. glass-stoppered tubes. Also, pipet 2 ml. of the 2.5 μg. per ml. 2,4,5-trichlorophenol standard (III) into a separate tube. Add 50 μl. of acetic anhydride reagent to each sample. Mix well. Stopper the tube tightly and place in a 40° C. water bath for 30 minutes. Cool the tube and add 8.0 ml. of 10% sodium bicarbonate. Shake vigorously until evolution of carbon dioxide is no longer evident. Centrifuge for 5.0 minutes. The hexane phase should be clear. Inject 4 μl. of the hexane phase into the gas chromatograph. Measure the area produced on the strip chart recorder with a polar planimeter. Compare the area of sample with the area of a standard.

Gas Chromatography. The operating conditions for the gas chromatographic analysis are as follows:

Column. 4-foot, 3-mm. i.d. borosilicate glass column of 5% QF-1 on Chromosorb W A/W (60 to 80 mesh).

Carrier gas. 5% methane in argon, 37.5 ml. per minute.

Purge gas. 5% methane in argon, 5.0 ml. per minute.

Temperatures. Column: 140° C. Injection port: 215° C. Detector cell: 200° C.

Electrometer range setting. 100. Attenuation. 2.

Pulse interval. 15 μsec.

Recorder chart speed. 1/2 inch per minute.

The retention time of 2,4,5-trichlorophenol acetate at the above conditions is 3.0 minutes.

A 10-μg. standard added to a 100-gram sample is equivalent to 0.1 p.p.m. Final dilution of sample of 4.0 ml. so that a 4-μl. aliquot contains 10 nanograms. Calculations are as follows:

$$\text{P.p.m.} = \frac{\text{area (sample)}}{\text{area (standard)}} \times \frac{\text{attenuation (sample)}}{\text{attenuation (standard)}} \times \frac{\text{micrograms standard}}{\text{sample weight in grams}} \times \frac{\text{final volume, ml.}}{\mu\text{l. injected}}$$

If the procedure is followed exactly as described and the attenuation settings for both standard and sample are the same, the equation above is reduced to:

$$\text{P.p.m.} = \frac{\text{area (sample)}}{\text{area (standard)}} \times 0.1$$

All results for Bay 37289 and its oxygen analog were expressed as p.p.m. of parent compound. The phenol is calculated separately.

Discussion

This gas chromatographic method is not only specific but also rapid and provides for determination of Bay 37289 and its suspected metabolites. Unlike the parent compound (I), the oxygen analog (II) cannot be chromatographed directly. It was, therefore, necessary to hydrolyze the compounds. Hydrolysis permitted a single method for both (I) and (II). The phenol is acetylated to obtain satisfactory resolution and separation of chromatographic peaks. The alkaline wash is incorporated in the method to separate the 2,4,5-trichlorophenol from both (I) and (II). The phenol is analyzed separately.

The Florisil column cleanup was very useful for eliminating late eluting peaks that would otherwise have lengthened analysis time per sample. This column cleanup, though, was not needed in the case of potato samples. The chloroform-water extraction prior to neutralization gave an excellent cleanup for all crops analyzed.

Recovery experiments were conducted

Table I. Recovery of Bay 37289, Its Oxygen Analog, and 2,4,5-Trichlorophenol from Crops

Crop	Control, P.P.M.	Added, P.P.M.	Bay 37289	Recovery of Oxygen Analog, %	Phenol
Alfalfa	<0.01	0.1	85.3	86.4	70.0
Asparagus	<0.01	0.1	89.4	89.4	...
Beans (green)	<0.01	0.1	92.0	87.0	94.4
Bean vines (green)	<0.01	0.1	77.0	80.5	99.0
Broccoli	<0.01	0.1	95.0	86.0	...
Brussels sprouts	<0.01	0.1	95.6	97.8	87.2
Cabbage	<0.01	0.1	87.2	86.5	...
Carrot (roots)	<0.01	0.1	83.3	78.5	100.0
Carrot (tops)	<0.01	0.1	92.3	83.0	80.6
Cauliflower	<0.01	0.1	94.0	93.8	96.8
Onions (dry)	<0.01	0.1	97.5	97.5	...
Potatoes	<0.01	0.1	98.0	100.0	...
Radish (tops)	<0.01	0.1	89.0	93.0	...
Strawberries	<0.01	0.1	104.0	100.0	...
Sugar beet (roots)	<0.01	0.1	100.0	98.0	...
Sugar beet (tops)	<0.01	0.1	95.0	75.7	81.0
Tobacco[a]	<0.01	0.1	88.5	80.0	...
Turnip (roots)	<0.01	0.1	92.0	94.0	...
Turnip (tops)	<0.01	0.1	100.0	97.8	94.2

[a] Fifty-gram sample. The sample size for all others was 100 grams.

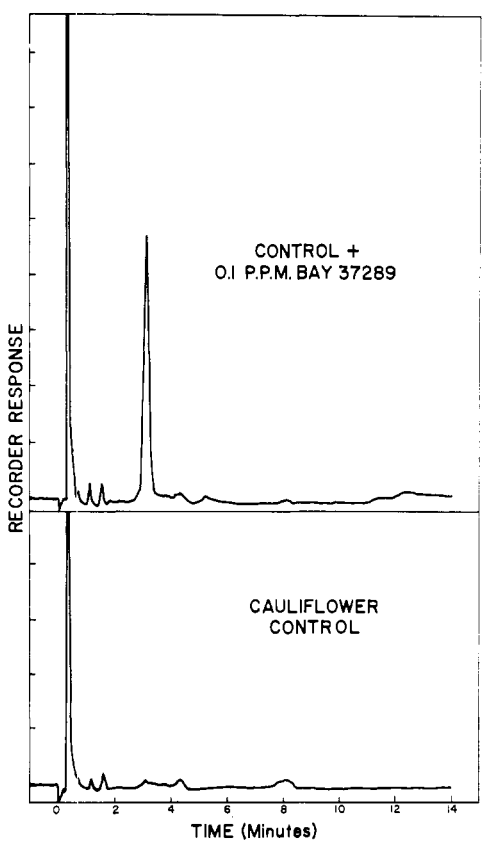


Figure 1. Chromatograms for recovery of Bay 37289 from cauliflower

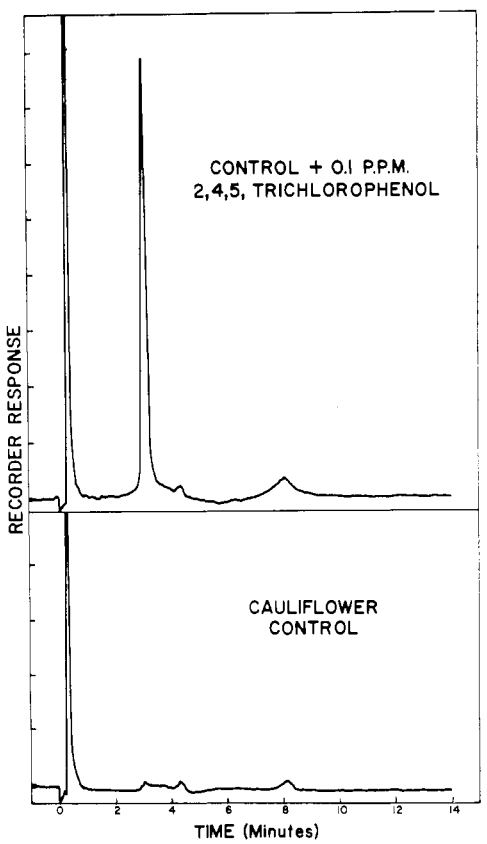


Figure 2. Chromatograms for recovery of 2,4,5-trichlorophenol from cauliflower

by adding 10 μg. 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 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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RESIDUE DETERMINATION

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

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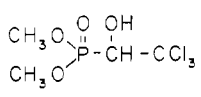
A sensitive residue method has been developed for the determination of trichlorfon and the possible metabolites, chloral hydrate and trichloroethanol, in plant and animal tissues. Separate extraction and cleanup procedures are necessary for the various types of samples. In all cases, detection and measurement 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.

TRICHLORFON, *O,O*-dimethyl 2,2,2-trichloro-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 sensitive titration cell (*15–17*). 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 electron-capture gas chromatography was considered. Preliminary experiments indicated that the more efficient electron-capture 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° 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