Determination of Organotin Residues from Plictran in Fruit Crops by Gas-Liquid Chromatography

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A gas-liquid chromatographic method is described for determining residues of tricyclohexyltin hydroxide and its dicyclohexyl metabolite on strawberries, apples, and grapes treated with plictran miticide. Crop samples were extracted with benzene and derivatized with aqueous hydrobromic acid. A silica gel chromatographic cleanup following derivatization was used when residue levels were less than 1 ppm. Background interferences were minimized through use of a halide-selective Coulson conductivity detector. Recoveries of tricyclohexyltin hydroxide from fortified crop samples were 80–95% at 1 ppm and 78–89% at 0.1 ppm. Conditions are included for gas-liquid chromatography of cyclohexylstannoic acid, a second potential degradation product of plictran.

Plictran miticide, a product of the Dow Chemical Co., contains tricyclohexyltin hydroxide as the active ingredient. It is effective in the control of plant-feeding mites on several crops and is presently registered for use in the United States on apples, pears, citrus, and ornamentals. Its mammalian toxicity is low, with an acute oral LD_{50} of 500–1000 mg/kg for rats, rabbits, guinea pigs, and white mice (Dow Chemical Co., 1968).

Tricyclohexyltin hydroxide is environmentally degraded in steps as follows (Getzendaner and Corbin, 1972)

$$Cy_3SnOH \longrightarrow Cy_2SnO \longrightarrow CySnO_2H \longrightarrow SnO_2$$

tricyclohexyltin hydroxide → dicyclohexyltin oxide → cyclohexylstannoic acid → stannic oxide

(Cy = cyclohexyl)

A tolerance of 2 ppm has been established for the combined residues of the miticide and its two organotin metabolites (expressed as Cy_3SnOH) in or on apples and pears (Federal Register, 1972). Residue methods are currently available to determine total tin, organic and inorganic tin, and each of the cyclohexyltin derivatives. Total tin is determined in the whole sample by wet oxidation, separation of the tin by either distillation as the bromide or extraction from solution as the iodide, and colorimetric measurement after reaction with toluene-3,4-dithiol (Corbin, 1970; Trombetti and Maini, 1970). Total organic tin is determined by applying this procedure to a hexane extract of the sample.

A series of extraction steps can be incorporated to separately analyze the tri-, di-, and monocyclohexyltin compounds (Getzendaner and Corbin, 1972). They are extracted from the aqueous sample with 1% acetic acid and hexane; reextraction of the acetic acid–hexane mixture with dilute HCl removes the $CySnO_2H$, further extraction of the hexane with alcoholic potassium hydroxide removes the Cy_2SnO, and the Cy_3SnOH remains in the hexane layer. If the analytical procedure for total tin is applied to the extracts, a determination of each of the tin derivatives will have been accomplished. The amounts of Cy_2SnO and $CySnO_2H$ determined to remain on the surface of field-treated apples by this method never exceeded 20% of the total organic tin (Getzendaner and Corbin, 1972), and very little translocation of residue from the surface to the interior of apples and pears was noted.

We report here a rapid analytical method for Cy_3SnOH and Cy_2SnO based on gas-liquid chromatography (glc) of their bromide derivatives, Cy_3SnBr and Cy_2SnBr_2, which has been successfully applied to strawberry, apple, and grape samples. Separate conditions are included for glc analysis of $CySnO_2H$ as its bromide derivative, $CySnBr_3$, as this compound was not susceptible to residue determination by the method outlined for Cy_3SnOH and Cy_2SnO.

METHOD

Reagents. Reagent grade 48% hydrobromic acid (Matheson, Coleman and Bell), nanograde benzene, anhydrous sodium sulfate, anhydrous analytical reagent ethyl ether (Mallinckrodt), and reagent grade glacial acetic acid (Allied Chemical) were used as received. Silica gel (Woelm, activity 1, for column chromatography, Waters Associates, Menlo Park, Calif.) was heated at 175° for at least 24 hr prior to storage in a tightly stoppered container; 5 g was weighed into an 8-dram screw-cap vial, 1 ml of 10% aqueous acetic acid was added, and the sealed vial was tumbled slowly for 2 hr for equilibration. The analytical standards, tricyclohexyltin hydroxide, dicyclohexyltin oxide, and cyclohexylstannoic acid, were supplied by the Dow Chemical Co., Midland, Mich. The standards of the bromide derivatives of the three organotin compounds were obtained from Ventron Corp., Beverly, Mass. The glc column silylating mixture was prepared by saturating hexamethyldisilazane (Perco Supplies, San Gabriel, Calif.) with triphenylchlorosilane (Pierce Chemical, Rockford, Ill.).

Apparatus. Crop samples were chopped with a Hobart food cutter with a 5-in. blade operating at 1725 rpm. The centrifuge used in the extraction procedure was an International Model FXD with 250-ml glass bottles. Solutions were concentrated by distilling the solvent through a three-ball Snyder distillation column (Kontes Glass Co., Berkeley, Calif.). The silica gel clean-up procedure utilized a Chromaflex No. 22 chromatographic column (Kontes Glass Co.).

Gas-Liquid Chromatography. A Hewlett-Packard F&M Model 400 gas chromatograph employing a Tracor Coulson conductivity detector was used for all of the analyses. A 60 cm × 4 mm (i.d.) glass column containing 2% OV-225 on A/W DMCS-treated 100–120 mesh Chromosorb G, operated at 200°, was used for the analysis of Cy_3SnBr and Cy_2SnBr_2. The $CySnBr_3$ chromatographed best on a 120 cm × 4 mm (i.d.) glass column of 0.5% OV-225 on 100-mesh glass beads operated at 100°.

Columns were conditioned overnight at 230° and then cooled at 100° prior to silylation. Several 10–20 μl injections of the silylating mixture were made, the column temperature increased 20–30°, and the injections repeated. The entire process was continued until the operating

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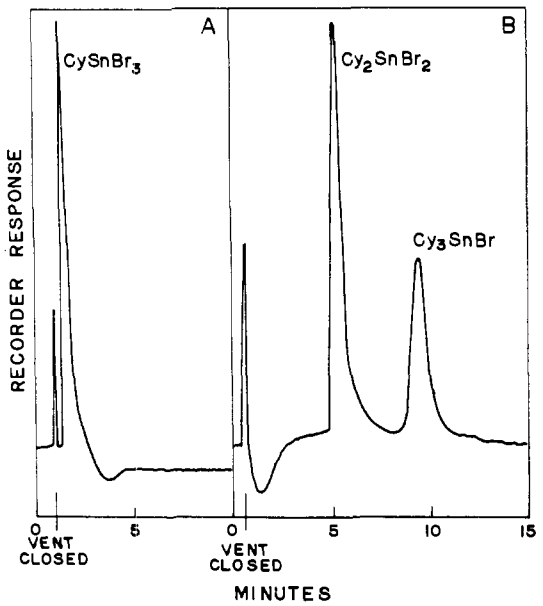

Figure 1. Gas–liquid chromatograms of standards (100 ng each) of (A) $CySnBr_3$ on 0.5% OV-225 at 100° and (B) Cy_3SnBr and Cy_2SnBr_2 on 2% OV-225 at 200°.

temperature of the column was reached, and the column was allowed to remain there for at least 4 hr before use.

The Coulson detector was operated in the reductive mode. Helium carrier gas and hydrogen combustion gas flow rates were 30 and 60 ml/min, respectively. On-column injection without auxiliary forecolumn heating was followed throughout the analysis.

Extraction and Derivatization. The frozen crop sample was chopped to a fine uniform consistency. The chopped sample (50 g) was weighed into a 500-ml flask, enough water (usually 50 ml) was added to just slurry the sample, 30 ml of 48% hydrobromic acid was added, and the mixture was swirled mechanically for 30 min. The agitation was then stopped, 50 ml of benzene was added, and the contents of the flask were swirled for an additional 30 min. The mixture was quantitatively transferred to a 250-ml centrifuge bottle and centrifuged for 10 min at 2000 rpm, the benzene layer was removed by decanting or pipeting, the aqueous layer was reextracted with benzene and centrifuged two additional times, and the aqueous layer was discarded. The combined benzene extracts were washed once with 50 ml of water in a separatory funnel and dried by passing through a 0.5-in. diameter glass column containing 2 in. of anhydrous sodium sulfate into a 300-ml round-bottomed flask. The volume of benzene was reduced to *ca.* 5 ml by distillation, the contents of the flask rinsed quantitatively into a graduated centrifuge tube, and the volume of benzene further reduced under a stream of dry nitrogen at room temperature to *ca.* 1 ml.

The sample was analyzed by glc at this point to determine the extent of crop interferences. If the residue level was low and interferences prohibited the use of the detector at higher sensitivity settings, the silica gel clean-up method outlined below was incorporated; if the residue level was high and interference difficulties were at a minimum, analysis was carried out without further cleanup. Quantitation was achieved by comparing peak areas (planimetry) with those of standard bromide derivatives.

The above procedure, from the extraction through the glc analysis, should be accomplished in rapid succession to avoid possible losses of derivative by hydrolysis. If it is necessary to delay at some point, the benzene extract should be stored in the cold and in contact with a few drops of hydrobromic acid, removing the acid with a water wash and drying, if necessary, upon resuming the procedure.

Table I. Average Percent Recoveries from Samples Fortified with Cy_3SnOH and Cy_2SnO at 0.1 and 1.0 ppm

Sample	No. of samples	1.0 ppm	0.1 ppm
Cy_3SnOH			
Water	2	99	94
Strawberries	12	85	80
Apples	6	95	89
Grapes	4	80	78
Cy_2SnO			
Water	2	94	90
Strawberries	6	80	
Apples	6	91	
Grapes	4	80	

Silica Gel Cleanup. The chromatographic column was prepared by introducing in order a plug of glass wool, 2 g of deactivated silica gel, *ca.* 2 cm of sodium sulfate, and another glass wool plug. The column was prewashed with 25 ml of benzene; when the liquid level just reached the top of the upper glass wool, the derivatized sample was introduced, followed by three 1-ml portions of benzene. With the sample well on the column, 25 ml of benzene was added and the eluate collected and discarded. When the benzene level just reached the glass wool, 50 ml of 10% (v/v) diethyl ether–benzene was added and the eluate collected in a 100-ml round-bottomed flask. The eluate volume was reduced to *ca.* 2 ml by distillation, quantitatively transferred to a graduated tube, and further reduced to 0.1 ml under a stream of dry nitrogen at room temperature. The sample was then analyzed by glc as described previously.

RESULTS AND DISCUSSION

The apparent adsorption or decomposition of the cyclohexyltin bromides on column packing, walls, and fittings gave rise to peak tailing and variable response. Several liquid phase–solid support combinations were tested in an attempt to alleviate this; columns containing cyanosilicone liquid phases, particularly 2% OV-225 on Chromosorb G, gave the best overall performance, providing sufficient resolution at a relatively low elution temperature for analysis of the two compounds of greatest interest, Cy_3SnBr and Cy_2SnBr_2. On-column silylation with triphenylchlorosilane improved the peak shape noticeably, although some tailing was still observed (Figure 1B). The amount of tailing was least with relatively clean samples on a freshly silylated column. The response of the Coulson conductivity detector to Cy_3SnBr and Cy_2SnBr_2 was linear in the range of 50 to 500 ng. The sensitivity of this detector, indicated by the slope of the standard curves, was greater to Cy_2SnBr_2 than to Cy_3SnBr as expected. The microcoulometric detector gave results comparable to those obtained with the Coulson detector and represents a suitable alternative.

Irreversible glc column losses were much greater for $CySnBr_3$ than for Cy_2SnBr_2 and Cy_3SnBr. Of the column packings examined, 0.5% OV-225 on glass beads was the most satisfactory for $CySnBr_3$ (Figure 1A). Even at relatively low column temperatures, however, the retention time was too short to allow analysis of residue samples since the $CySnBr_3$ peak was always obscured by a large postventing response. Since $CySnO_2H$ was found by Getzendaner and Corbin (1972) to be the least significant residue of the three tricyclohexyltin compounds on plictran-treated apples, no further attempt was made to include this chemical in the analytical method.

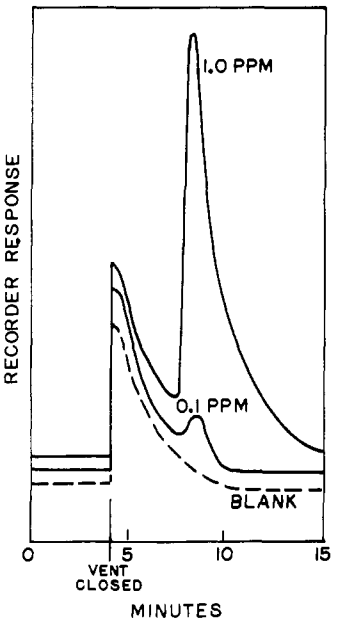

Figure 2. Gas–liquid chromatograms (2% OV-225 at 200°) of Cy_3SnBr equivalent to 1.0 and 0.1 ppm of Cy_3SnOH in fortified strawberries.

Recoveries of Cy_3SnOH and Cy_2SnO from strawberries, apples, and grapes fortified at the 1.0-ppm level were satisfactory (Table I). Analyses at this level were carried out without cleanup of the bromide derivatives. For analysis of Cy_3SnOH at the 0.1-ppm level, however, a silica gel cleanup of the derivatized sample was included. The additional cleanup provided a reduced postventing response and allowed the further concentration of samples necessary for the required sensitivity. In no case were any discrete interference peaks noted in either the cleaned or uncleaned extracts, attesting to the selectivity of the method against normal crop constituents. However, Cy_2SnBr_2 and $CySnBr_3$ do not elute from silica gel and this cleanup results in loss of these compounds from the extract. The practical minimum detectability for Cy_3SnOH and Cy_2SnO, estimated from chromatograms of fortified crop samples such as those depicted in Figure 2, were 0.1 and 1.0 ppm, respectively, in the three crops studied.

That the bromide derivatization is nearly quantitative is evidenced by the high recoveries of Cy_3SnOH and Cy_2SnO from water (Table I). No attempt was made to optimize the reaction time or quantity of hydrobromic acid beyond those used in the present method, since both were convenient for the single-step extraction–derivatization procedure employed. It was noted that some loss in response occurred with benzene solutions of both bromide standards and derivatized samples allowed to stand long periods of time before analysis; treatment with a few drops of hydrobromic acid served to regenerate the expected response, indicating that the loss was due to partial hydrolysis of the derivatives.

No significant difference in extraction efficiency was observed between the benzene slurry method routinely used and blending for variable lengths of time. Chloroform was originally considered as the extraction solvent, but the relatively large postventing response discouraged its use and benzene was selected.

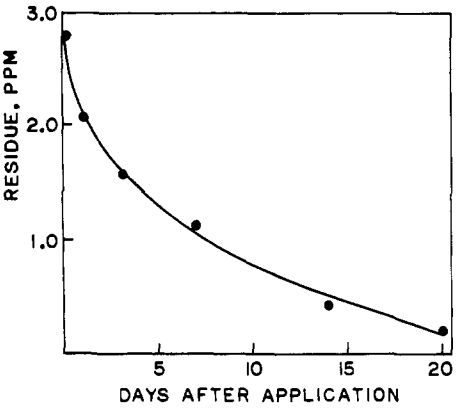

Figure 3. Residue decline curve of Cy_3SnOH on field-treated strawberries.

Earlier attempts at filtering the extraction–derivatization emulsion were discontinued when Cy_3SnBr losses up to 40% were found. This apparently resulted from the use of filter aids (Celite 545 and Hy-flow Supercel). The somewhat more time-consuming but otherwise satisfactory centrifugation procedure was the preferred replacement.

A residue decline curve for Cy_3SnOH on strawberries treated with plictran 50W at the rate of 1 lb/acre active ingredient is shown in Figure 3; no measurable Cy_2SnO was observed in any of the samples. The present method provides for rapid screening for residues of Cy_3SnOH and Cy_2SnO in excess of 1.0 ppm and for more sensitive determination of Cy_3SnOH to 0.1 ppm. The results should closely approximate the organotin residue on many commodities treated with plictran, as our own and previous work (Getzendaner and Corbin, 1972) indicate Cy_3SnOH to be the major organotin residue. The more lengthy but inclusive method based on conversion to inorganic tin (Corbin, 1970; Getzendaner and Corbin, 1972) is recommended when a highly accurate determination of total organotin residue is required.

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LITERATURE CITED

Corbin, H. B., *J. Ass. Offic. Anal. Chem.* **53**, 140 (1970).
Dow Chemical Company, Technical Information Bulletin, Midland, Mich., 1968.
Federal Register **37**, 16803 (1972).
Getzendaner, M. F., Corbin, H. B., *J. Agr. Food Chem.* **20**, 881 (1972).
Trombetti, G., Maini, P., *Pestic. Sci.* **1**, 144 (1970).

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