Determination of Residues of Chloroneb and a Metabolite by Microcoulometric Gas Chromatography

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A gas chromatographic method utilizes programmed-temperature gas chromatography and the selective microcoulometric detector to measure residues of chloroneb (1,4-dichloro-2,5-dimethoxybenzene) and the metabolite, 2,5-dichloro-4-methoxyphenol, in soil and a variety of plant and animal tissues after they have been separated from the substrate by steam distillation. Further cleanup is not required on most tissues. Distillation is effected from 5*N* H_3PO_4 in the presence of the substrate, and the compounds are continuously extracted from the distillate with hexane. The chromatographic separation is made by programming a column of 10% DC 560 plus 0.2% Epon resin 1001 on High Performance Chromosorb W from 100° to 180° C. Recoveries of about 90% for each compound have been demonstrated on a variety of substrates at levels down to 0.02 p.p.m.

A sensitive, highly selective analytical method has been developed for determining residues of chloroneb (1,4-dichloro-2,5-dimethoxybenzene) (Demosan, E. I. du Pont de Nemours & Co., Inc.), an effective fungicide for the control of seedling diseases in cotton. It has potential use for other crops, such as beans, cucurbits, and sugar beets. 2,5-Dichloro-4-methoxyphenol, a metabolite of chloroneb in animals, can be simultaneously determined by this method. It is based on the gas chromatographic measurement of the two compounds after they have been separated from the substrate by continuous steam distillation and extraction into an organic solvent. A selective microcoulometric detector is used to minimize the possible interferences and permit direct analysis of the concentrated solvent extract of most of the crops investigated without further cleanup. Satisfactory recoveries at the 0.02-p.p.m. level have been demonstrated on a variety of samples. Average recoveries of about 90% have been obtained for both chloroneb and the metabolite.

EXPERIMENTAL

Reagents and Apparatus. Chloroneb and the metabolite, 2,5-dichloro-4-methoxyphenol, standard reference materials available from E. I. du Pont de Nemours & Co., Wilmington, Del.

Phosphoric acid, 5*N* aqueous solution.

Dow Corning Antifoam A, emulsion, Dow Corning Corp., Midland, Mich.

n-Hexane, distilled-in-glass, Burdick & Jackson Laboratories, Inc., Muskegon, Mich.

Distillation-extraction head, construct as shown by Bleidner *et al.* (1954).

Flasks, round-bottomed, glass, 1-liter capacity, 29/42 ⅄; 2-liter capacity, 29/42 ⅄.

MT-220 gas chromatograph, Micro-Tek Instruments, Baton Rouge, La., equipped with Dohrmann Microcoulometric titrating system, consisting of C-200 microcoulometer, T-300S titration cell, and S-100 sample inlet/-combustion unit.

Industrial & Biochemicals Department, Experimental Station, E. I. du Pont de Nemours & Co., Wilmington, Del.

Chromatographic column, 4-foot glass, 1/4-inch O.D., 3/16-inch I.D. containing 10% silicone DC 560 oil plus 0.2% Epon Resin 1001 on 80- to 100-mesh high performance Chromosorb W, acid-washed DMCS-treated (Applied Science Laboratories, Inc., State College, Pa.).

Florisil column (chromatographic tube, 10 × 150 mm., with coarse fritted disk). Fill the chromatographic tube, with gentle tapping, to a depth of 70 mm. with Florisil that has been activated at 130° C. for 4 hours. A glass wool plug may be used at the base of the column to prevent clogging of the fritted disk. Add about 10 mm. of anhydrous sodium sulfate to the top of the column. Wash the column with 25 ml. of *n*-hexane just prior to use.

Gas Chromatographic Calibration. Equilibrate the gas chromatograph as follows: vaporizer temperature, 240° C.; transfer temperature, 260° C.; furnace temperature, 880° C.; column temperature, 200° C.; carrier flow, helium 80 cc. per minute; purge flow, helium 50 cc. per minute; oxygen flow, 50 cc. per minute.

Condition the column by maintaining its temperature at 200° C. for at least 72 hours. Prepare a calibration curve for each compound by chromatographing appropriate-sized aliquots from standard solutions containing both chloroneb and the metabolite at concentrations of 0.4, 1, 3, and 5 μg. per ml. in *n*-hexane and plotting peak heights against micrograms injected. Peak height is used because of the elevated base line obtained at the high chromatographic temperatures of this method, which nullify the advantage of an electromechanical integrator for determining peak area.

Set the column temperature at 100° C. and the sensitivity at 90 ohms using the Hi-Gain mode. Inject the sample, 100 μl. of the 0.4- and 1-μg.-per-ml. standards, lesser volumes of the 3- and 5-μg.-per-ml. standard. Wait 2 minutes and program the column temperature at 10° per minute to 180° C. Hold at this temperature for about 10 minutes to recondition the column. The retention time from start of programming is about 10.7 minutes for chloroneb and about 9.3 minutes for the metabolite. A typical gas chromatographic scan of a standard solution is shown in Figure 1.

Isolation. Place 50 to 100 grams of a representative sample in a 2-liter round-bottomed flask, and add several

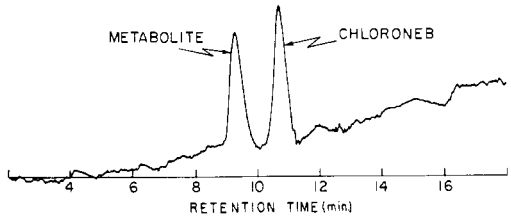

Figure 1. Calibration curve

100 μl. injected
0.055 μg. of chloroneb
0.050 μg. of metabolite

boiling chips, 2 to 3 ml. of Dow Corning Antifoam A, and 1000 ml. of 5*N* H_3PO_4. Fill the U-tube of the distillation-extraction head to the level of the lower arm with distilled water and attach the flask to the lower arm. Add 400 ml. of *n*-hexane and a boiling chip to the 1-liter flask and attach to the upper arm. With a 24-inch water-cooled condenser in position on top of the head, apply heat to both flasks at such a rate that condensed hexane and water pass through the capillary in the form of small sausage-like droplets. Allow the digestion-extraction to continue for at least 12 hours.

At the completion of the digestion-extraction period, wipe the grease from the neck of the 1-liter flask and transfer the hexane quantitatively to a 600-ml. beaker. Wash the flask with several small portions of hexane. Carefully concentrate the solvent at room temperature to about 40 ml. using a well ventilated hood. Quantitatively transfer the concentrated hexane to a 50-ml. beaker and continue to concentrate the solvent to about 4 ml. (Because of the volatility of both chloroneb and the metabolite, it is necessary to use extreme care in the solvent concentration steps. Do not allow the solvent to go to dryness.) Transfer the concentrated extract to a 5-ml. volumetric flask using a finely drawn-out dropper. Assure quantitative transfer by washing with several small portions of hexane. Dilute to volume with hexane and mix thoroughly.

Gas Chromatographic Analyses. Equilibrate the GC instrument, inject an aliquot of the sample, and calculate the micrograms of each compound using the calibration

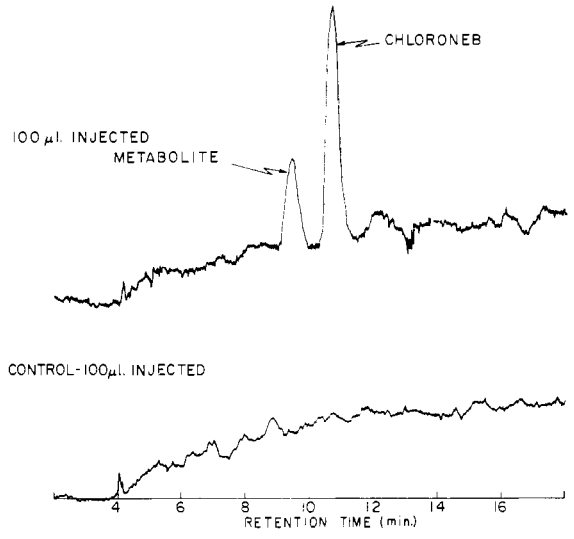

Figure 2. Snap beans fortified with 0.051 p.p.m. chloroneb and 0.022 p.p.m. metabolite

curve prepared as described under Calibration. Correct for aliquot factor and recovery factor for the respective compound to obtain the micrograms of each in the unknown. Calculate the residue of chloroneb and the metabolite in parts per million by dividing the respective micrograms found by the sample weight in grams.

RESULTS AND DISCUSSION

The applicability of the gas chromatographic method for the simultaneous determination of chloroneb (1,4-dichloro-2,5-dimethoxybenzene) and the metabolite (2,5-dichloro-4-methoxyphenol) has been demonstrated on a variety of substances. Recovery of both compounds added to untreated controls averaged about 90% over the range of 0.02 to 25 p.p.m. (Table I). Recovery studies were conducted by adding known amounts of each compound to the untreated controls contained in the digestion flask and the analyses carried out as described in Experimental. Typical gas chromatograms of extracts from a control sample and one fortified with chloroneb and the metabolite are illustrated in Figure 2. The upper curve

Table I. Results of Recovery Studies on Substrates Fortified with Mixtures of Chloroneb and the Metabolite

	Chloroneb				Metabolite			
	Residue level, p.p.m.	No. of detns.	Recovery, % Av.	Recovery, % Range	Residue level, p.p.m.	No. of detns.	Recovery, % Av.	Recovery, % Range
Snap beans (100-gram sample)	0.020–0.52	8	95	81–110	0.023–0.52	8	91	78–110
Bean plant (100-gram sample)	0.020–1.0	7	96	77–106	0.023–3.1	7	91	83–100
Dry green beans (100-gram sample)	0.020–0.050	2	105	100–110	0.050–0.10	2	84	78–89
Southern peas (100-gram sample)	0.020–0.20	4	96	90–105	0.023–0.23	4	85	83–91
Soybeans (100-gram sample)	0.026–0.10	5	89	81–96	0.022–0.10	5	88	75–105
Cotton seed (100-gram sample)	0.020–0.10	6	100	84–114	0.021–0.11	6	85	71–100
Sugar beet roots (100-gram sample)	0.026–0.10	4	91	85–97	0.022–0.11	4	93	80–105
Sugar beet tops (100-gram sample)	0.026–0.10	4	89	83–96	0.022–0.11	4	84	77–91
Soil (50-gram sample)	0.040–2.1	10	90	70–110	0.068–2.3	10	81	70–100
Dog urine (25-gram sample)	0.020–2.0	3	106	105–108	1.4–9.0	3	97	93–102
Dog feces (25-gram sample)	1.0–9.0	4	106	98–110	1.1–25	4	93	91–96
Dog liver (50-gram sample)	0.20–4.1	6	103	87–112	0.11–4.2	6	89	82–100
Dog kidney (50-gram sample)	0.46–2.3	2	92	87–96	0.21–4.2	2	86	85–87
Dog muscle (50-gram sample)	0.052–0.40	4	96	95–97	0.056–0.21	4	87	82–91
Dog blood (50-gram sample)	0.12–0.35	2	101	95–106	0.21–0.52	2	83	81–85

was obtained on the extract of snapbeans fortified with chloroneb at 0.051 p.p.m. and with the metabolite at 0.022 p.p.m., the lower curve represents the control snapbeans.

Phosphoric acid was selected as the digestion medium in this procedure to assist in complete digestion of the sample being analyzed and to repress the ionization of the acidic phenolic metabolite compound for easier steam distillation. Low recoveries can usually be attributed to losses occurring during the solvent concentration step. Extreme care must be taken because of the known volatility of chloroneb and the metabolite. The Kuderna-Danish evaporative concentrator, recommended for concentrating extracts of many volatile pesticides is not suitable for these compounds. Low and inconsistent recoveries were obtained when attempts were made to use this device. The two-stage concentration procedure described under Isolation has proved satisfactory. The initial concentration step, reducing the volume of *n*-hexane from about 400 ml. to 40 ml. should require about 3 hours. Concentration is continued at a slightly slower rate requiring an additional hour to reduce the hexane volume to 4 ml.

Use of the selective microcoulometric detector eliminated nearly all background, and no interference was encountered from untreated control samples. Occasionally, peaks of unknown components have been observed in the chromatograms of untreated control extracts; however, they do not interfere with the determination of either chloroneb or the metabolite as they are completely resolved from the peaks of these compounds. Further cleanup of the extracts from some substrates may be required. Treatment with a Florisil column or extraction with an acetonitrile–hexane solvent pair may both be used successfully for this cleanup. The concentrated hexane extract containing chloroneb and the metabolite can be applied to a Florisil column, the column washed with 25 ml. of *n*-hexane and the compounds eluted with 25 ml. of ethyl acetate. Alternately, chloroneb and the metabolite may be extracted from the concentrated hexane extract with two equal volumes of acetonitrile. In both cases the solvent, ethyl acetate or acetonitrile, must be carefully concentrated back to the original 5-ml. volume. The solvent pair is useful for separating chloroneb and the metabolite from large amounts of oils and fats, but it will not effectively remove polar lipides, plant pigments, and many other interfering compounds.

Cleanup is advisable when analyzing samples of high oil content, such as cotton seed or soybeans, because the oil contained in the original extracts of these samples apparently modifies the liquid phase of the chromatographic column when repeated large-volume injections are made. Increases of up to 1 minute have been observed in the retention time of each residue when multiple injections of high oil content samples have been carried out. Although the retention times remain relative, more frequent peak height calibration is required.

The chromatographic column used for this study was selected because of its ability to resolve adequately chloroneb and the metabolite and at the same time separate them satisfactorily from interfering materials. The response for both compounds is linear throughout the range studied, and each compound is quantitatively chromatographed. This was determined utilizing the absolute measurement characteristics of the microcoulometer permitting quantitative evaluation of the chromatographic system. Chloroneb and the metabolite can also be resolved on a 5% XE 60 column using chromatographic conditions similar to those recommended in the manuscript. The separation of interfering materials is not as complete, however, and only about 80% of the metabolite is titrated in the coulometric cell. This column could be useful for qualitative purposes. The retention time for chloroneb on the XE 60 column is about 6 minutes and for the metabolite about 7 minutes. This is the reverse order from which these two compounds emerge using the recommended 10% DC 560 chromatographic column.

Preliminary studies were made using the electron affinity detector. As these compounds are not strong electron-capturing species, it was still necessary to concentrate the extracting solvent to low volume before chromatographing to obtain the desired sensitivity. In doing so, the higher background arising from other electron-capturing species was also concentrated and complicated the use of this detector.

LITERATURE CITED

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