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Determination of Polychlorinated Biphenyls in Plant Tissue

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Concern about the presence of polychlorinated biphenyls (PCBs) in the environment has stimulated studies of the effects of these chemicals on various living organisms. Although several excellent studies have been published on the effects of PCBs on aquatic and marine plants (e.g. JOHNSON & WALTZ 1976, MAHANTY 1975, WEBER 1979), to our knowledge there are no documented procedures for extraction, cleanup, and quantification of PCBs in plant tissue.

The purpose of this study was to (1) develop an efficient method for processing large numbers of plant samples, (2) combine extraction and cleanup procedures to limit sample handling, (3) prepare large quantities of dried plant tissue to be used as analytical reference material for long term PCB quality control studies, and (4) analyze aquatic plants exposed to PCBs.

METHODS AND MATERIALS

Reagents and Apparatus. All organic solvents were glass distilled from Burdick and Jackson, Muskegon, MI. Sodium sulfate was obtained from Mallinckrodt, Inc., St. Louis, MO. Silica gel was EM-60 (70-230 mesh ASTM) from E. Merck, Darmstadt, Germany. Gas Chrom Q and Apolane-87 were obtained from Applied Science Laboratories, Inc., State College, PA. Aroclor 1242 and 1254 were obtained from Monsanto Chemicals, Inc., St. Louis, MO. All glassware was thoroughly washed with detergent and tap water, rinsed with distilled water, and heated to 500°C overnight.

Samples were analyzed on a gas chromatograph (GC) equipped with a ^{63}Ni electron capture (EC) detector and a 2 mm i.d. x 1.8 m coiled glass column packed with 0.75% (w/w) Apolane-87 on 120-140 mesh Gas Chrom Q. Nitrogen, delivered at 20 mL/min, was used as the carrier gas. After sample injection, the column temperature was held at 165°C for 2 min and then temperature programmed to 240°C at 5°/min.

Reference Material Preparation. Frozen plant material is thawed at room temperature and any water present is discarded. The material is then placed in a hexane-rinsed aluminum foil pan and allowed to dry at room temperature for 3 days. Approximately 400 g of the dried material is transferred to a blender, mixed

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with anhydrous Na_2SO_4 in a 1:1 ratio, and blended at high speed for 12 min to obtain a homogenous powder.

After the homogeneity of the bulk sample is established (see below), the sample is transferred to a Teflon-capped glass container and stored in a refrigerator at 15°C until used.

Determination of PCBs in Plant Material - Sample Preparation. Environmental samples should be collected in glass jars and frozen until analyzed.

The sample is thawed, dried, and partly minced by hand, and a representative subsample (ca. 20 g) is transferred to a tared grinding jar (½ pint) and weighed. An additional subsample is taken for percent water determination. Granular, anhydrous Na_2SO_4 equal to 3 times the sample weight is slowly added with stirring. If the plant sample is moist, the Na_2SO_4 should be added slowly to avoid the formation of hard lumps. The sample is blended at high speed for 3 min or until homogenous.

Fortified Sample Preparation. Reference plant material (ca. 40 g) is transferred to a tared grinding jar, weighed, and then fortified with a mixture of Aroclor 1242 and 1254 in a 1:1 ratio. The fortified samples are homogenized at high speed for 3 min, then extracted as described below.

Preparation of Sulfuric Acid Silica Gel. Silica gel is heated to 130°C for 12 h. A portion (300 g) is placed in a 500-mL Teflon-lined screw-cap bottle and treated with conc. H_2SO_4 added in small portions followed by vigorous mixing until a 40% (w/w) loading is achieved. The sulfuric acid silica gel is mixed for several hours and then stored at ambient temperature.

Extraction. The extraction column, fitted with a Teflon stopcock, is prepared by placing a small wad of glass wool in the bottom of the column, and adding enough anhydrous Na_2SO_4 to form a 2 cm layer over the glass wool. Sulfuric acid silica gel (5 g) is added to the column followed by another 2 cm layer of anhydrous Na_2SO_4. The column is pre-rinsed with 30 mL of hexane, the stopcock is opened slightly, and the sample is transferred to the column in a typical slurry pack fashion. The sample is eluted with a total hexane volume of 200 mL, then rotoevaporated in a room temperature water bath, to approximately 1 mL.

Residue Cleanup. In a silica gel column, fitted with a Teflon stopcock, a small wad of glass wool is placed at the bottom, and above it a 2 cm bed of anhydrous Na_2SO_4. Silica gel (5 g), still hot from the oven, is poured into the column, which is then tapped several times to ensure uniform packing. Anyhdrous Na_2SO_4 (ca. 1 cm) is poured on top of the silica gel bed to exclude moisture. The column is pre-rinsed with 50 mL of eluting solvent, 0.5% benzene in hexane. The plant extract

is quantitatively transferred with a disposable Pasteur pipet from the evaporator receiver to the top of the silica gel column using 2 mL of eluting solvent. The stopcock is opened and the sample is allowed to drain into the Na_2SO_4 bed. Elution with 40 mL of 0.5% benzene in hexane is then begun.

The hexane eluate is treated with a fine-mesh copper wool to remove GC/EC interferences caused by sulfur contaminants. The copper wool is prepared by rolling it into a ball about 1 cm in diameter, soaking it in HNO_3 (2 M) to remove surface oxides, and then rinsing it with distilled water, acetone, and hexane. When the copper wool is placed into the hexane eluate, it is allowed to soak until it turns black. The procedure is repeated until the copper ball remains shiny after saoking for 10 min, indicating that the sulfur contaminants have been removed. A similar method was described by the U.S. Environmental Protection Agency (1977). The hexane eluate is rotoevaporated in a room temperature water bath to about 1 mL and transferred to a Teflon-lined screw-cap culture tube. The sample is adjusted to the proper volume by dilution or nitrogen evaporation, and stored at $4^{o}C$ until analysis.

Identification and Calculation. Quantitation was based on four peaks of Aroclor 1242 that eluted early enough not to overlap with the four peaks chosen for Aroclor 1254. Peaks are identified by absolute retention time and the areas from the sample component are compared with the appropriate standard area (WEBB & MCCALL 1973, MCCOWN & DAVEY 1977).

RESULTS AND DISCUSSION

The importance of PCB contaminants in the food chain cannot be overstated (CHEN et al. 1981, ROSS et al. 1981, OUW et al. 1976, KIMBROUGH 1974), and accurate and precise analytical techniques are essential in the detection of PCBs in plant and animal tissue. The procedure presented here enables the handling of large numbers of samples. Inclusion of the sulfuric acid silica gel adsorbent in the extraction column provides an extract that is extremely clean and free of biogenic material, eliminating the need for purification of the extract by means such as gel permeation chromatography. By using present method, one can handle several grams of biogenic material.

Recovery experiments have been conducted by fortifying plant tissue with a mixture of Aroclor 1242 and 1254. The results (Table 1) revealed that the recoveries of PCB at all fortification levels ranged from 68 to 92% for Aroclor 1242 and 67 to 90% for Aroclor 1254. Within each fortification level the reproducibility was excellent.

Chromatograms from a PCB mixture and from a plant sample collected in the field are shown in Figure 1. On the basis of the chromatograms of the field samples, a mixed Aroclor standard consisting of equal mixture of 1242 and 1254 was chosen for quantitation and fortification in the recovery experiments.

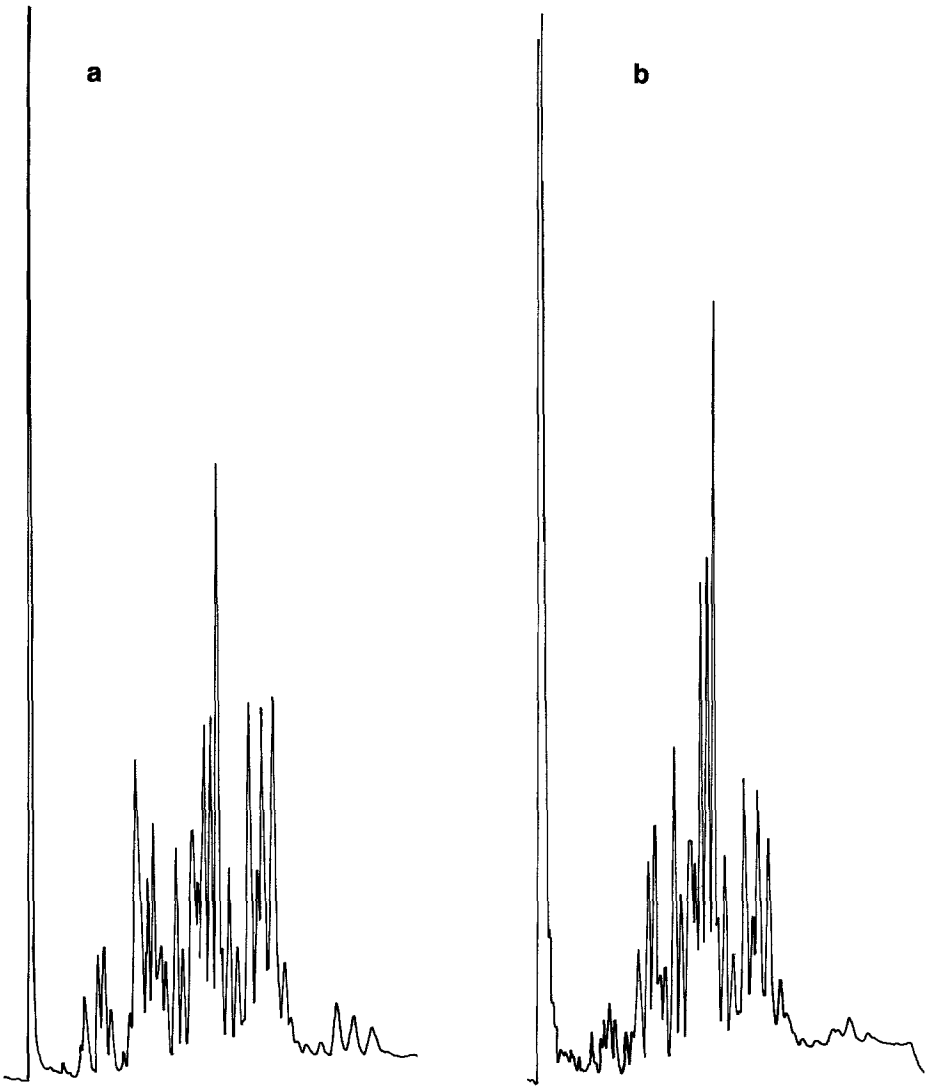

Figure 1. a) Equal mixture of Aroclor 1242 and 1254, 1.5 ng. each.
b) Plant tissue extract.

Table 1. Percent recovery of Aroclor 1242 and Aroclor 1254 from fortified plant samples.

	Fortification level (ppb on a wet basis)					
	10		50		250	
Replicate	1242	1254	1242	1245	1242	1254
1	70	67	71	83	89	88
2	87	73	80	88	91	88
3	81	82	79	84	91	87
4	80	78	83	88	89	87
5	68	74	83	90	91	89
6	68	74	85	88	92	89
$\bar{x}$	76	74	80	87	91	88
σ	8%	5%	5%	3%	1%	0.9%

The present method has been used for analyzing plant tissue in our laboratory throughout 1980 and 1981. In our opinion it offers several advantages: (1) the analyses of samples require little time; (2) sample handling is reduced because the extraction is simple and does not involve transfers; (3) the method is suitable for routine use; (4) the method can be easily expanded to accommodate larger samples; and (5) the method requires little investment in experimental equipment.

References to trade names do not imply Government endorsement of commercial products.

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