

Medical Research and Development Command Contract No. DADA 17-73-C-3132, TRW Systems Group, Redondo Beach, Calif., 1973, p 3.
 Stojanovic, B. J., Kennedy, M. V., Shuman, F. L., Jr., *J. Environ. Qual.* 1, 54 (1972).
 Thompson, J. F., Ed., "Analysis of Pesticide Residues in Human and Environmental Samples", Primate and Pesticides Effects Laboratory, Environmental Protection Agency, Perrine, Fla., 1972, Section 4A.
 Thronsberry, W. L., Jr., *Anal. Chem.* 43, 452 (1971).
 Woodland, R. G., Hall, M. C., Russell, R. R., *J. Air Pollut. Control. Assoc.* 15, 56 (1965).

Zweig, G., Sherma, J., Ed., *Anal. Methods Pestic. Plant Growth Regul.* 6, 692 (1972).

Received for review August 1, 1975. Resubmitted July 2, 1976. Accepted August 13, 1976. This study was supported by Agricultural Research Service, U.S. Department of Agriculture, Cooperative Agreement No. 12-14-7001-108. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA or the Mississippi Agricultural and Forestry Experiment Station and does not imply its approval to the exclusion of other products that may also be suitable.

Fate of Polybrominated Biphenyls (PBB's) in Soils. Persistence and Plant Uptake

Lee W. Jacobs,* Sheng-Fu Chou, and James M. Tiedje

The flame retardant, PBB, which contaminated animal feeds in Michigan, was found to be comprised of 2,2',4,4',5,5'-hexabromobiphenyl as the major component, two isomers of pentabromobiphenyl, three additional isomers of hexabromobiphenyl, and two isomers of heptabromobiphenyl. The PBB's were extremely persistent with only one pentabromobiphenyl isomer showing any significant disappearance after 24 weeks of incubation in soils. Orchard grass and carrots grown in soil contaminated with PBB's showed none or only very minor uptake of PBB's, respectively. PBB's were detected in soils from a field which had received manure from a PBB-contaminated dairy herd 10 months earlier.

In 1973 an industrial flame retardant (fireMaster BP6, manufactured by Michigan Chemical Corporation, St. Louis, Mich.) was accidentally added to livestock feed in place of magnesium oxide (Carter, 1976; Isleib and Whitehead, 1975; Robertson and Chynoweth, 1975). The addition of this chemical, which is a mixture of several polybrominated biphenyl (PBB) isomers, into livestock rations had a catastrophic impact on Michigan's livestock industry and resulted in more than 23 000 cattle, 4000 swine, 1.5 million chickens, and tons of eggs, milk, butter, cheese, and feed having to be destroyed (D. R. Isleib, personal communication). This incident has been termed the most costly and disastrous accidental contamination ever to occur in United States agriculture (Isleib and Whitehead, 1975).

Attention immediately focused on the effects of PBB on animals and humans. Several reports (Fries et al., 1973; Jackson and Halbert, 1974; Babish et al., 1975; Gutenmann and Lisk, 1975) regarding uptake of PBB by and its effect on animals have been published. Information on the contamination of other ecosystem components such as plants, soil, water, and air is needed to better evaluate the fate of PBB's inadvertently added to soils by contaminated manure, milk, carcasses, etc.

We report here on the chemical characterization of fireMaster BP6, persistence of PBB in soils, and uptake of PBB by plants.

EXPERIMENTAL PROCEDURES

Soil Incubations. The susceptibility of PBB's to biodegradation was evaluated using Brookston loam and Spinks sandy loam soils which were freshly collected from the University Experimental Farms. Twenty-five grams of soil which had passed through a 2-mm sieve was placed

in a 125-ml Erlenmeyer flask. One milliliter containing 100 μ g of PBB in acetone was distributed in microdrops over the soil surface. The soil in each flask was then moistened with 5 ml of basal salt solution. Half of the flasks received additional organic matter in the basal salts in an attempt to stimulate cometabolism. This amendment per flask was 0.2% glucose, 0.2% peptone, 0.2% sodium acetate, and 0.05% *m*-bromobenzoic acid per gram fresh weight of soil. The flasks were closed with a rubber stopper and incubated in the dark at 28 °C, except for one set of Brookston soils with the organic amendment which was incubated at room temperature in an anaerobic glove box ($E_h < 0.05$ mV). Three flasks were analyzed for each treatment after 0, 6, 12, and 24 weeks of incubation.

Plant Uptake. A greenhouse experiment was established using a Spinks loamy sand (characterized by Filonov et al., 1976) amended with varying levels of fireMaster BP6 and cropped with two plant species. Five concentrations of PBB (0, 0.1, 1.0, 10, and 100 ppm) with three replicates of each concentration were used for each plant species: Nordstern orchard grass (*Dactylis glomerata* L.) and Spartan Delite carrots (*Daucus carota*). The soil was prepared by mixing powdered PBB with air-dried soil at a PBB concentration of 100 ppm and diluting this mixture with additional soil to achieve the desired concentrations.

Four kilograms of the PBB-amended soil was placed in a tin container lined with a polyethylene bag for each replicate and treated with nutrient solutions initially and during growth to satisfy the needs of each crop. The soil was watered daily to 10% moisture. The orchard grass was grown for about 5 weeks before the first harvest and then allowed to regrow for three additional harvests. Grass was cut about 2.5 cm above the soil for each harvest. The carrots were harvested after 11 weeks growth when roots were about "finger" size. Carrot tops were separated from the roots, and the roots washed thoroughly to remove all soil particles. All plant tissue was refrigerated following harvest until analyzed for PBB.

Departments of Crop and Soil Sciences and of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824.

Table I. Recovery of Six Major PBB Isomers after Incubation of 4 ppm in Brookston Soil

Incubation time, weeks	Recovery of PBB, % of original amendment ^a					
	5 Br (I)	5 Br (II)	6 Br (I)	6 Br (II)	6 Br (III)	7 Br
Glassware	70.3 ^a	88.0 ^a	85.7 ^a	83.1 ^a	71.0 ^a	73.1 ^a
0	74.4 ^a	87.0 ^a	83.2 ^a	82.5 ^a	74.2 ^a	76.7 ^a
6	70.0 ^a	69.3 ^b	82.0 ^a	76.2 ^{ab}	74.5 ^a	75.7 ^a
12	72.0 ^a	65.6 ^b	67.2 ^b	71.0 ^{bc}	69.8 ^a	78.0 ^a
24	67.5 ^a	65.0 ^b	66.7 ^b	69.3 ^c	70.8 ^a	76.3 ^a

^a Superscripts a, b, and c = ranges of equivalence within columns at *P* (0.05).

Analyses. PBB was extracted from soil with three 30-ml portions of benzene-2-propanol (2:1, v/v). For the first extraction, the solvent-soil mixture was allowed to stand in the flask overnight and then was shaken on a rotary shaker at 250 rpm for 30 min. After decanting the solvent, the second and third extractions were made by shaking solvent and soil at 250 rpm for 30 min prior to decanting. The extracts were combined and two 20-ml portions of water were added to remove the 2-propanol. Anhydrous Na₂SO₄ was added to the solvent to remove remaining water. Soil samples from a contaminated farm were extracted in the same manner except that the combined concentrated extract was passed through a Florisil column (10 g, 60/100 mesh) to remove interfering soil organic matter.

Fresh plant tissue and carrot roots were cut into small pieces and extracted with benzene-2-propanol (2:1, v/v) in a food blender using a glass mixing container. Three successive 80-ml portions of benzene-2-propanol were used to extract each plant sample. The three extracts were combined in a 500-ml separatory funnel and washed with three 20-ml portions of water to remove the 2-propanol. The remaining benzene was dried with anhydrous Na₂SO₄, passed through a Florisil column to remove interfering components, and concentrated to 10 ml on hot sand (using a round-bottomed flask with a three-ball Snyder column).

Concentrated extracts from the above samples were analyzed on a Beckman GC-5 gas chromatograph equipped with an electron capture detector and a 2% Dexsil 300 GC column; the column and conditions have been described by Filonow et al. (1976). A glass column, 1.83 m × 3 mm i.d., containing 2% SE-30 on 100/120 mesh Gas-Chrom Q and operated at 265 °C with a carrier flow of 40 ml/min, was used on occasion as it was faster and provided slightly better separations (used for Figure 1). A stainless steel column of 3% SE-30 on 100/120 mesh Chromosorb W, 1.83 m × 2.3 mm i.d., was used in a Perkin-Elmer 900 gas chromatograph for detection by flame ionization; the column was programmed from 150 to 260 °C at 8 °C/min. For the samples from the contaminated farm, the identification of the peaks as PBB's was confirmed by observing their sensitivity to uv according to the method of Erney (1975). For plant tissue the detection limit was 10 ppb (fresh weight) and for the contaminated farm soil 0.1 ppb (dry weight).

The mass spectra were obtained with an LKB 9000 gas chromatograph-mass spectrometer with an ionizing voltage of 70 eV. The GC column was 3% SP2100 on Supelcoport, 1.83 m × 3 mm i.d., and programmed from 200 to 300 °C at 5 °C/min. The ¹³C NMR was done on a Varian CFT-20 instrument with CDCl₃ as the solvent; the sample analyzed was the 6 Br (I) peak purified from fireMaster BP6 (Filonow et al., 1976).

RESULTS AND DISCUSSION

The fireMaster BP6 product (lot no. 6244A) was found to be a mixture of at least 18 different components which could be separated and detected by flame ionization gas

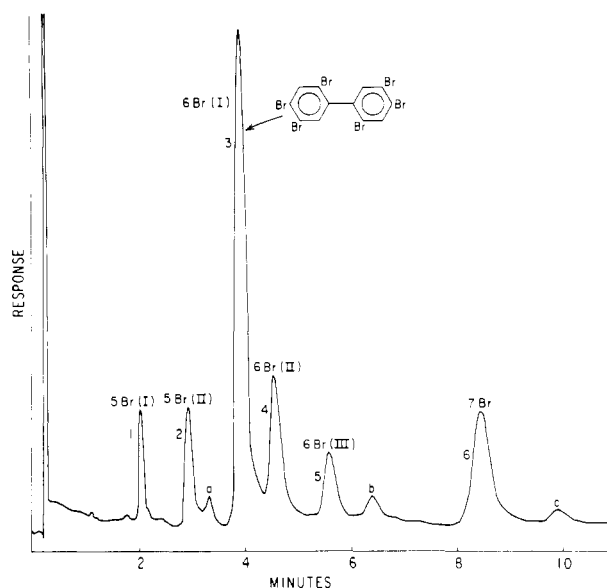


Figure 1. Gas chromatogram showing the six major components of PBB (peaks 1 to 6) and their identity, and the three minor components (peaks a to c). The major component has the isomeric structure shown.

chromatography on a 3% SE-30 column. However, 98% of the product is accounted for by the six major peaks shown in Figure 1 (peaks 1 to 6). The quantity of each component varies somewhat with each batch, but the hexabromobiphenyl isomer I (peak 3) was always the largest and a heptabromobiphenyl isomer (peak 6) the second largest in the samples we have examined.

The peaks were identified from GC-mass spectra (Figure 2) to be isomers of pentabromobiphenyl (*M* + 544), hexabromobiphenyl (*M* + 622) and heptabromobiphenyl (*M* + 700). The major component, 6 Br (I), was identified by ¹³C NMR to be 2,2',4,4',5,5'-hexabromobiphenyl (Figure 1) based on the following evidence: δ_c (ppm from Me₄Si) C-1, 140.3, biphenyl linkage; C-2, 122.4, 2 Br; C-3, 136.7, 2 H; C-4, 125.9, 2 Br; C-5, 123.8, 2 Br; C-6, 134.8, 2 H. The hydrogen shifts were confirmed by a gated decoupling. The three minor components which are shown in the GC trace in Figure 1 but which were not quantitated in these studies were identified by GC-mass spectrometry as follows: (a) hexabromobiphenyl; (b) heptabromobiphenyl; and (c) unknown but suspected to be octabromobiphenyl.

The recovery of PBB components after incubation in Brookston soil is shown in Table I. The data clearly show that the PBB persisted for 0.5 year in soil with only slight disappearance of three of the components and no disappearance of the other three. The same observation of persistence holds for incubations with added available organic matter, under anaerobic conditions, and for Spinks soil as is summarized in Table II. The fact that Spinks soil is more coarse textured and contains less organic matter may account for the inactivity of this soil for PBB's. The persistence of these PBB's is consistent with the

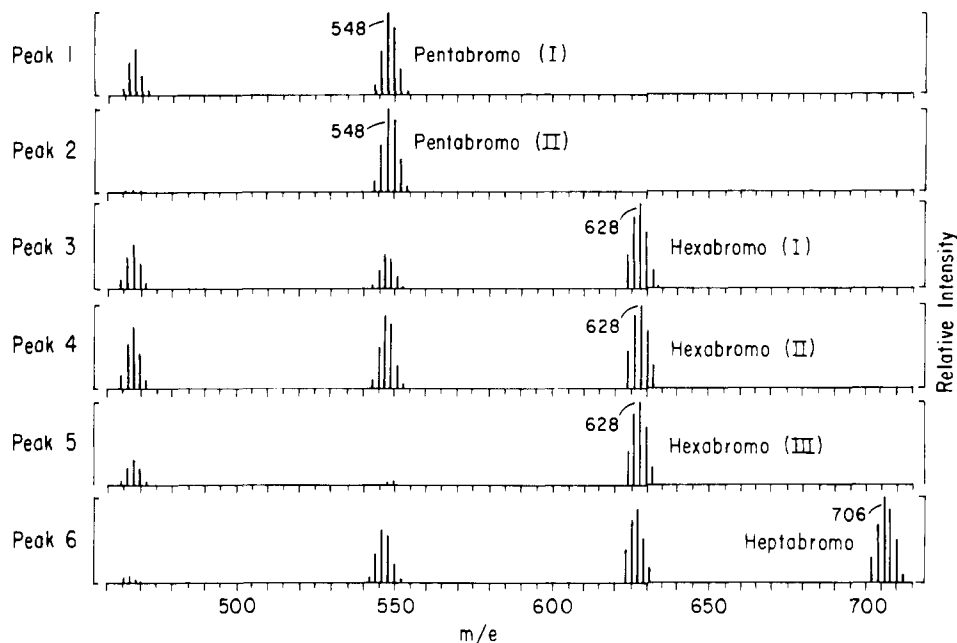


Figure 2. Mass spectra of the six major components of PBB; m/e of base peak is identified.

Table II. F Ratios for Comparison of Recoveries of 6 PBB Isomers at Zero Time and after 24 Weeks of Incubation^a

Treatment	5 Br (I)	5 Br (II)	6 Br (I)	6 Br (II)	6 Br (III)	7 Br
Brookston soil	(60.26)***	(58.33)***	(43.87)***	(31.37)***	(0.21)	(0.01)
Brookston, anaerobic + OM ^b	(4.18)	(50.93)***	(1.16)	(2.56)	(24.25)***	(6.75)*
Brookston + OM ^b	(2.24)	(44.25)***	(0.07)	(6.18)*	(48.96)***	(0.52)
Spinks soil	(1.47)	(0.02)	(0.43)	(0.09)	(0.10)	(6.13)*

^a *, disappearance significant at P (0.10); **, disappearance significant at P (0.05); ***, disappearance significant at P (0.01). ^b Amendment of organic matter.

evidence reported for PCB's which shows that the more heavily chlorinated members (penta or greater) are resistant to degradation though many lesser chlorinated components are metabolized (Ahmed and Focht, 1973; Baxter et al., 1975; Tucker et al., 1975).

A difficulty in these studies is the lack of precision due to variability in quantity recovered among replicates. This does not appear to be due to technique since ratios between peaks vary in a random manner among the extracted solutions. Data in Table I also show that a substantial loss occurred in the glassware in the absence of soil. These analytical difficulties are apparent in other reports (Babish et al., 1975; Gutenmann and Lisk, 1975). Given these difficulties, one can only interpret the highly significant and consistent trends in the data. Only the disappearance of the second pentabromobiphenyl isomer (5 Br (II)) in the Brookston soil (Table II) fulfills this condition. Whether this disappearance is due to microbial degradation, sorption, or masking was not resolved although some biodegradation seems possible since only a specific isomer showed a decrease. In addition, there is precedence for microbial metabolism of certain dichloro-substituted aromatic rings of PCB's (Ahmed and Focht, 1973; Baxter et al., 1975).

Therefore, certain dibromo-substituted aromatic rings of pentabromobiphenyls could be similarly attacked since biphenyl oxidizing bacteria require an adjacent 2-3 position to form the first biphenyl metabolite, *cis*-2,3-dihydro-2,3-dihydroxybiphenyl (Gibson et al., 1973). Because of the inherent analytical imprecision and the very low levels of metabolism (if any), confirmation of biodegradation in soil will require the use of ¹⁴C-labeled PBB's.

The greenhouse experiment was designed to favor the greatest uptake of the PBB's. A grass forage (orchard grass) was selected to obtain an extensive root mass within the pot and four successive crops harvested to encourage maximum PBB uptake. The soil was a coarse-textured, low organic matter Spinks loamy sand with low sorptive capacity (Filonow et al., 1976). Carrots were selected as a root crop since they have an outstanding ability to absorb pesticide residues from soil (Iwata et al., 1974).

No PBB's were detected in any of the orchard grass cuttings or carrot tops. Detectable quantities of PBB were found in carrot roots grown in soil amended with 10 and 100 ppm of PBB, but the concentrations found (20 to 40 ppb) were too low to quantitate with precision. Because PBB uptake was nondetectable (orchard grass) or very minor (carrots) with heavily contaminated soils (10 and 100 ppm), we conclude that plant uptake of PBB's will not be a problem on Michigan farms where PBB-contaminated livestock were located or where PBB-contaminated manure was spread on the soils. Soils on most of these farms are expected to have PBB concentrations much less than 0.1 ppm.

Manured soil from a farm with a dairy herd having a moderate level of contamination (0.2 to 0.3 ppm in the milk) was examined to obtain an estimate of concentration and persistence of PBB's under actual field conditions. Manure was spread at an approximate rate of 18 metric tons/ha on alfalfa sod in December or January, 1974/1975. The following October, soil samples were taken at three different locations in the field. The samples were of a loam on a north facing slope, a sandy loam in a low, central area, and a clay loam on a south facing slope. The organic matter contents ranged from 2.2 to 2.8% and the pH from

6.9 to 7.0. The major isomer, 6 Br(I), was found in concentrations of 4.1, 5.7, and 4.4 ppb, respectively. Peaks corresponding in retention time to all six PBB components were also found. Additional evidence for the identity of these peaks as PBB's was the disappearance of the hexa- and heptabromobiphenyl peaks and the appearance of peaks with shorter retention time after exposure to uv light, as described in the confirmatory procedure of Erney (1975).

Since we do not have previous samples from this farm, we could not determine quantitative changes in PBB or changes in peak ratios with time; however, these analyses clearly show that PBB's can be detected in manured soils nearly a year after application. The quantities found are very small and appear to pose no future hazard. Since PBB's are sensitive to light (Ruzo and Zabik, 1975), surface applied manure may allow some photodegradation.

We conclude that the potential hazards from PBB-contaminated soils are low since PBB's are not taken up by plants or leached to ground water (Filonow et al., 1976) at concentrations expected to be present, and they are probably not volatilized due to their low vapor pressure (5.2×10^{-8} mmHg at 25 °C, calculated from Michigan Chemical Corp. Product Bulletin); however, they may remain in the soils for many years because of their resistance to degradation. Only low levels of contamination are expected in soils on most of the exposed farms because of dilution, as we found for the one farm examined. In certain rare and localized situations where high level contamination may have occurred, potential concern could arise from erosion of contaminated soils or manures into streams and the accumulation of PBB's in terrestrial and aquatic food chains.

ACKNOWLEDGMENT

The authors thank Robert Berger, Department of Chemistry, for the ^{13}C NMR analysis, Charles Sweeley, Department of Biochemistry, for use of the mass spec-

trometry facilities, Bill Thayer for assistance with the study of the contaminated farm, and Marilyn Maternoski for her technical assistance.

LITERATURE CITED

- Ahmed, M., Focht, D. D., *Bull. Environ. Contam. Toxicol.* **10**, 70 (1973).
 Babish, J. G., Gutenmann, W. H., Stoewsand, G. S., *J. Agric. Food Chem.* **23**, 879 (1975).
 Baxter, R. A., Gilbert, P. E., Lidgett, R. A., Mainprize, J. H., Vodden, H. A., *Sci. Total Environ.* **4**, 53 (1975).
 Carter, L. J., *Science* **192**, 240 (1976).
 Erney, D. R., *J. Assoc. Off. Anal. Chem.* **58**, 1202 (1975).
 Filonow, A. B., Jacobs, L. W., Mortland, M. M., *J. Agric. Food Chem.*, following paper in this issue (1976).
 Fries, G. F., Smith, L. W., Cecil, H. C., Bitman, J., Lillie, J. R., 165th National Meeting of the American Chemical Society, Dallas, Tex., April 11, 1973.
 Gibson, D. T., Roberts, R. L., Wells, M. C., Kopal, V. M., *Biochem. Biophys. Res. Commun.* **50**, 211 (1973).
 Gutenmann, W. H., Lisk, D. J., *J. Agric. Food Chem.* **23**, 1005 (1975).
 Isleib, D. R., Whitehead, G. L., IXth Annual Conference on Trace Substances in Environmental Health, University of Missouri, Columbia, Mo., June 10, 1975.
 Iwata, Y., Gunther, F. A., Westlake, W. E., *Bull. Environ. Contam. Toxicol.* **11**, 523 (1974).
 Jackson, T. F., Halbert, F. L., *J. Am. Vet. Med. Assoc.* **165**, 437 (1974).
 Robertson, L. W., Chynoweth, D. P., *Environment* **17**, 25 (1975).
 Ruzo, L. O., Zabik, M. J., *Bull. Environ. Contam. Toxicol.* **13**, 181 (1975).
 Tucker, E. S., Saeger, V. W., Hicks, O., *Bull. Environ. Contam. Toxicol.* **14**, 705 (1975).

Received for review April 26, 1976. Accepted July 30, 1976. Published as Michigan Agricultural Experiment Station Journal Article No. 7691. This study was supported in part by Biomedical Sciences Support Grants from the National Institutes of Health, Department of Health, Education and Welfare.

Fate of Polybrominated Biphenyls (PBB's) in Soils. Retention of Hexabromobiphenyl in Four Michigan Soils

Alexander B. Filonow,¹ Lee W. Jacobs,* and Max M. Mortland

The adsorption of 2,2',4,4',5,5'-hexabromobiphenyl (HBB) by four Michigan soils conformed well to a Freundlich adsorption isotherm. Adsorption of HBB increased with increasing soil organic carbon (correlation, $r = 0.87$ to $r = 0.96$). Leaching studies using the same soils amended with 100 ppm of HBB showed that less than 0.6% of the HBB was lost from each soil with leachate quantities equivalent to 20 times the average annual rainfall in Michigan. Results suggest that PBB, which is present in some Michigan farm soils due to applications of PBB-contaminated manure, should not leach below the depth of incorporation.

Following widespread contamination of Michigan livestock by PBB's, concern developed for potential movement of PBB's from burial sites or from surface soils if the latter were found to be significantly contaminated.

Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan 48824.

¹Present address: Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824.

To acquire information regarding the retention and movement of PBB in contaminated soils, adsorption and leaching studies were conducted in the laboratory with four Michigan soils using 2,2',4,4',5,5'-hexabromobiphenyl (HBB). We report here on the results of these studies.

EXPERIMENTAL SECTION

Reagents. The 2,2',4,4',5,5'-hexabromobiphenyl (HBB) was chromatographically separated and purified (>95%) from fireMaster BP6 (lot no. 6244A), a mixture of polybrominated biphenyls (PBB). The identifications of the